DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
51

CHOLECYSTOKININ (CCK) — INDUCED ANXIETY IN RATS: INFLUENCE OF ENVIRONMENTAL STIMULI AND INVOLVEMENT OF ENDOPIOID MECHANISMS AND SEROTONIN

SULEV KÕKS

TARTU 1999

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

51

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
51

CHOLECYSTOKININ (CCK) — INDUCED ANXIETY IN RATS: INFLUENCE OF ENVIRONMENTAL STIMULI AND INVOLVEMENT OF ENDOPIOID MECHANISMS AND SEROTONIN

SULEV KÕKS

TARTU UNIVERSITY
PRESS

Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia.

Dissertation is accepted for the commencement of the degree of Doctor Philosophy (in Molecular Biomedicine) on April 23rd, 1999 by the Council of the Institute of Molecular and Cell Biology, University of Tartu.

Opponent: Professor Pekka V. Rauhala (Institute of Biomedicine, Department of Pharmacology and Toxicology, University of Helsinki).

Commencement: June 8, 1999.

Publication of this dissertation is granted by the University of Tartu.

Tartu Ülikooli Kirjastuse trükikoda
Tiigi 78, Tartu 50410
Tellimus nr. 361

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I Kõks S., Vasar E., Soosaar A., Lang A., Volke V., Võikar V., Bourin M., Männistö P. T. Relation of exploratory behavior of rats in elevated plus-maze to brain receptor properties and serum growth hormone levels. Eur Neuropsychopharmacol 1997; 7: 289–294.

II Kõks S., Soosaar A., Võikar V., Volke V., Ustav M., Männistö P. T., Bourin M., Vasar E. Opioid antagonist naloxone potentiates anxiogenic-like action of cholecystokinin agonists in elevated plus-maze. Neuropeptides 1998; 32: 235–240.

III Kõks S., Bourin M., Võikar V., Soosaar A., Vasar E. Role of CCK in anti-exploratory action of paroxetine, 5-HT reuptake inhibitor. Int J Neuropsychopharmacol 1999; 2 (in press).

IV Kõks S., Soosaar A., Võikar V., Bourin M., Vasar E. BOC-CCK-4, CCKB receptor agonist, antagonizes anxiolytic-like action of morphine in elevated plus-maze. Neuropeptides 1999; 33 (in press).

V Kõks S., Männistö P. T., Bourin M., Shlik J., Vasar V., Vasar E. Cholecystokinin (CCK)-induced anxiety in rats: relevance of pre-experimental stress and seasonal variations. J Psychiatry Neurosci (re-submitted).

ABBREVIATIONS

ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
BOC-CCK-4	N-tert-butoxy-carbonyl-cholecystokinin tetrapeptide
CCK	cholecystokinin
CCK_A	cholecystokinin A receptor subtype
CCK_B	cholecystokinin B receptor subtype
CCK-4	cholecystokinin tetrapeptide
CCK-8	cholecystokinin octapeptide
CCK-8s	sulphated cholecystokinin octapeptide
CCK-8us	unsulphated cholecystokinin octapeptide
CCK-LI	cholecystokinin-like immunoreactivity
CNS	central nervous system
CRH	corticotropin releasing hormone
[^{3}H]pCCK-8	[propionyl-^{3}H]-propionylated-CCK-8-sulphated
GABA	gamma-amino butyric acid
GH	growth hormone
5-HIAA	5-hydroxyindole acetic acid
HPA	hypothalamic-pituitary-adrenal
HSD	honest significant difference
5-HT	serotonin (5-hydroxytryptamine)
i.p.	intraperitoneally
NIH	National Institutes of Health
NIDDK	National Institute of Diabetes and Digestive and Kidney Disorders
8-OHDPAT	8-hydroxy-2-(di-n-propyl amino) tetralin
PRL	prolactin
SSRI	selective serotonin re-uptake inhibitor
s.c.	subcutaneously
TSH	thyrotropin

1. INTRODUCTION

Cholecystokinin (CCK) belongs to the family of brain-gut peptides. It was originally discovered in the gut and shown to influence pancreatic secretion and contraction of gall bladder. CCK is present in different biologically active molecular forms cleaved from a 115-amino-acid precursor molecule (pre-pro-CCK), including CCK-58, CCK-39, CCK-33, CCK-22, sulphated CCK-8 and CCK-7, unsulphated CCK-8 and CCK-7, CCK-5 and CCK-4 (Rehfeld and Nielsen, 1995). The C-terminal pentapeptide conserves the structural homology of these CCK sequences and also homology with peptide gastrin (Mutt, 1980). Full biological activity requires the octapeptide to be sulphated at the tyrosine in the seventh position from the C-terminus (Jensen *et al.*, 1982). Also, α-amidation of the C-terminus is essential for biological activity. The shortest biologically active form is C-terminal tetrapeptide, although it has much lower potency (Knight *et al.*, 1984).

CCK interacts with two receptor subtypes named according to their main distribution: CCK_A (peripheral type) receptors, mainly located in the gastro-intestinal tract, and CCK_B (brain type) receptors that are abundantly found in the central nervous system (CNS) (Moran *et al.*, 1986). These receptor subtypes can be described by pharmacological means on the basis of their affinity for the fragments of CCK. CCK_A receptors are highly selective for sulphated analogues of CCK (Wank *et al.*, 1988; Wank *et al.*, 1990). CCK_B receptors have high affinity not only for sulphated fragments but also for gastrin and nonsulphated analogues of CCK (Saito *et al.*, 1981; Saito *et al.*, 1997). Therefore, CCK_B receptors are also called CCK_B/gastrin receptors (Wank, 1995). For CCK_A receptors the minimal sequence for high affinity binding is CCK-8. CCK-4 is the shortest form required for binding to the CCK_B receptors (Saito *et al.*, 1981; Durieux *et al.*, 1988).

The biggest problem in the studies on neuropeptides is their unstability and fast degradation by the peptidases. Selective, non-peptide antagonists with different chemical structures have been developed for overcoming this problem (Woodruff and Hughes, 1991). For CCK_A receptors the compound of particular interest is devazepide (L-364,718 or MK-329) (Chang *et al.*, 1986; Chang and Lotti, 1986). Devazepide interacts with CCK_A receptors at low nanomolar concentration and it has also high selectivity for CCK_A receptors. The selective antagonist for CCK_B receptors is L-365,260 (Lotti and Chang, 1989). Both compounds are benzodiazepine derivatives with relatively long lasting efficacy *in vitro* and *in vivo*, and with oral bioavailability (Chang and Lotti, 1986; Woodruff and Hughes, 1991).

CCK_B receptors in CNS are responsible for the modulation of various physiological processes including pain processing, learning and memory,

motivation and anxiety (Singh *et al.*, 1991; Costall *et al.*, 1991; Shlik *et al.*, 1997). The first observation that administration of CCK-4 to healthy subjects can produce anxiety, dyspnea, and depersonalisation was made in the end of sixties (Rehfeld, 1992). In 1979 two papers described the first hints about anxiogenic-like behavioural action of CCK (Della-Fera and Baile, 1979; Ishibashi *et al.*, 1979). Della-Fera and Baile studied the satiating effect of CCK-peptides and they found that intracerebroventricular injection of pentagastrin (CCK-5) induced foot stamping and vocalisations in sheep (Della-Fera and Baile, 1979). In 1984 Bradwejn and de Montigny demonstrated in the electrophysiological experiment that the anxiolytic drugs block the excitatory effect of CCK (Bradwejn and de Montigny, 1984). After that several human studies have revealed the implication of CCK-system in the neurobiology of anxiety and panic attacks (Bradwejn *et al.*, 1991; Abelson and Nesse, 1994). Also the results obtained from the animal experiments have shown that the manipulations with CCK receptors can influence anxiety in the different behavioural paradigms. CCK agonists inhibit exploratory behaviour of mice and rats in the elevated plus-maze (Harro *et al.*, 1990; Harro and Vasar, 1991) and decrease the time spent in the aversive light compartment of the light/dark compartment test (Singh *et al.*, 1991; Chopin and Briley, 1993). Moreover, CCK_B antagonists have been shown to possess the anxiolytic-like properties in the ethological models of anxiety (Hughes *et al.*, 1990).

The aim of the present study was the further clarification of the background of CCK-induced anxiety in rats. At first, the influence of pre-experimental stress on the exploratory behaviour of rats and the anxiogenic-like action of CCK agonist was studied. Moreover, we explored also the seasonal variations in the exploratory behaviour of rats and the role of CCK in those behavioural changes. In the second part of the experiment the individual differences in the exploratory behaviour in the elevated plus-maze and the role of CCK-ergic neurotransmission in these differences were studied. The third aim of the study was to investigate the interaction of CCK and endopioid mechanisms in the generation of anxiety in rats. Finally, as serotonin (5-hydroxytryptamine, 5-HT) has been shown to be an important mediator influencing the behaviour under aversive circumstances, the 5-HT and CCK interaction in the regulation of exploratory behaviour was studied.

2. REVIEW OF LITERATURE

2.1. CCK in the Central Nervous System (CNS)

CCK was first described in the mammalian CNS as gastrin-like immunoreactivity in 1975 (Vanderhaeghen *et al.*, 1975). It is now generally believed that CCK is the most abundant neuropeptide in CNS (Rehfeld and Nielsen, 1995). Of the multiple available fragments, the sulphated octapeptide of CCK is the predominant derivative (Dockray and Taylor, 1976). Radioimmunological studies have shown the localisation of CCK-like immunoreactivity (CCK-LI) in high amounts throughout the human brain with the highest levels (300–1200 pmol CCK-LI/g wet weight) in the neocortex (in the frontal and occipital regions) (Lindefors *et al.*, 1993), hippocampus, and subiculum (Lindefors *et al.*, 1991). The intermediate amounts (100–500 pmol CCK-LI/g wet weight) have been found in several subcortical structures, including the caudate nucleus, putamen, nucleus accumbens, septum, ventromedial thalamus, periaqueductal grey, and substantia nigra (Emson *et al.*, 1982; Taquet *et al.*, 1988). Low amounts (<100 pmol CCK-LI/g wet weight) have been established in the globus pallidus, lateral thalamic nuclei, mesencephalic, and metencephalic nuclei (Lindefors *et al.*, 1993). Structures containing few or no CCK neurones are the cerebellum, corpus callosum, internal capsule, anterior and posterior commissures (Rehfeld *et al.*, 1992; Rehfeld and Nielsen, 1995). In the cortex, CCK is present mainly in thin nerve terminals throughout all the cortical layers, slightly less in the molecular than in deeper layers (Rehfeld, 1978). Therefore the staining of CCK nerves produces general light staining of the entire cortex (Larsson and Rehfeld, 1979). Most of CCK positive neurones belong to the nonpyramidal types such as multipolar or bipolar, but some small pyramidal cells are also present (Sakamoto *et al.*, 1984). Considerable evidence suggests that CCK functions as a neurotransmitter (Crawley and Corwin, 1994; Vanderhaeghen and Crawley, 1985) and iontophoretic application of the peptide to the nervous cells produces the excitatory effect (Boden and Hill, 1988; Liu *et al.*, 1994). CCK is localised in the neurones, concentrated in the nerve terminals, and synthesised *de novo* in the nerve cells. The neuronal CCK is released by depolarisation, its inactivation occurs by enzymatic degradation and reuptake from the synaptic cleft. The effect of CCK could be interfered by means of suitable receptor antagonists (Rehfeld and Nielsen, 1995).

CCK is co-localised with several other neurotransmitters. Co-localisation with dopamine (Hökfelt *et al.*, 1980), substance P (Skirboll *et al.*, 1982), enkephalin (Gall *et al.*, 1987), GABA (Hendry *et al.*, 1984), and the corticotropin-releasing factor (Mezey *et al.*, 1985) is established. This widespread co-localisation with the other neurotransmitters can be an anatomical support for

the regulatory role of CCK in various brain functions. The application of modern techniques has identified two receptor subtypes to CCK: CCK_A (peripheral) and CCK_B (central) receptors. CCK_A receptors are found mostly in the gastrointestinal tract and CCK_B receptors are located mainly in the brain (Moran *et al.*, 1986). Nevertheless, there are some exceptions to that rule since CCK_A receptors are also found in the discrete regions of brain (area postrema, nucleus tractus solitarius, interpeduncular nucleus, and posterior hypothalamus) (Hill *et al.*, 1992). At the same time, CCK_B receptors are established in the peripheral organs (de Weerth *et al.*, 1998). The highest densities of CCK_B receptors in the brain are in the cerebral cortex, limbic system (the olfactory tubercles, hippocampus, nucleus accumbens, and amygdala), striatum, hypothalamus, ventral tegmentum, and dorsal raphe nuclei (Innis and Snyder, 1980; Honda *et al.*, 1993). The weak to moderate signals of radioactive ligands have been detected in the cerebellum and spinal cord (Saito *et al.*, 1980; Gaudreau *et al.*, 1983; Moran *et al.*, 1986).

Table 1. Characteristics of cholecystokinin receptors (Shlik *et al.*, 1997)

Nomenclature	CCK_A receptor	CCK_B receptor
Alternative names	Peripheral subtype	Central subtype CCK_B/gastrin receptor
Potency order of CCK agonists	Caerulein>CCK-8s>>gastrin=CCK-4	Caerulein>CCK-8s>gastrin=CCK-4
Agonists	Caerulein CCK-8s A71623 A70874 JMV-180	Caerulein CCK-8s CCK-8us CCK-4 Pentagastrin BC264
Antagonists	Proglumide Devazepide Lorglumide Lintitript (SR27897)	Proglumide L-365,260 L-740,093 LY288513 CI-988
Effector	G-protein q/11	G-protein q/11
Gene	*CCKA*	*CCKB*
Structural information	428-amino-acid sequence human P32238 7TM 444-amino-acid sequence rat P30551 7TM	447-amino-acid sequence human P32239 7TM 452-amino-acid sequence rat P30553 7TM
Location in chromosomes	Human chromosome 4 Mouse chromosome 5	Human chromosome 11 Mouse chromosome 7
Distribution	Gall bladder, pancreas, pylorus, spinal cord, vagus nerve, limited brain areas	Throughout the brain, stomach, vagus nerve, kidneys
Functions	Mediates actions of CCK on gall bladder contraction, secretion of pancreatic enzymes, gastric emptying, inhibits feeding and respiration, potentiates dopamine-mediated behaviours and dopamine release in shell of nucleus accumbens	Mediates actions of CCK on increases in neuronal firing rates, nociception, anxiety, respiration, inhibits dopamine-mediated behaviours and dopamine release

Despite the pharmacological evidence for the existence of the third and even the fourth receptor subtype, modern technology of cloning has supported the existence and distribution of only these two receptor subtypes (Wank *et al.*, 1992; Wank *et al.*, 1992; Pisegna *et al.*, 1992; Lee *et al.*, 1993). *In situ* hybridisation technique has revealed a regional distribution of CCK receptor mRNA that generally parallels the distribution of radioligand binding sites (Hill and Woodruff, 1990; Honda *et al.*, 1993; Wank *et al.*, 1994). Only in the cerebellum, despite the positive hybridisation signal, the autoradiographic labelling of CCK receptors is not observed (Jagerschmidt *et al.*, 1994).

2.2. CCK and anxiety

The ability of CCK to induce anxiety was first described by Jens Rehfeld during his military service in 1969. He injected tetragastrin (CCK-4) to the volunteers in order to investigate insulin secretion. He noticed that immediately after the injection of 70 μg of CCK-4 the soldiers became quiet and looked fearful (Rehfeld, 1992). In 1978, Rehfeld studied the effect of CCK-4 on himself and his colleague Thue Schwartz. Intravenous injection of CCK-4 provoked a fast-onset anxiety reaction that was accompanied by palpitations, sweating, and faintfulness (Rehfeld, 1992). Early electrophysiological studies revealed that CCK-8 can produce marked excitation of the cortical and hippocampal neurones — an effect that can be antagonised by benzodiazepine anxiolytics (Bradwejn and de Montigny, 1984; Bradwejn and de Montigny, 1985). Then intensive research towards the understanding of CCK functions in the mechanisms of anxiety started. The data obtained from the human studies established the panicogenic properties of CCK-4 (de Montigny, 1989; Bradwejn *et al.*, 1990). This effect of CCK-4 is clearly mediated via the CCK_B receptor subtype (Bradwejn *et al.*, 1994; Lines *et al.*, 1995). Moreover, patients suffering from the panic disorder are more susceptible to the emotional action of CCK-4 compared to healthy volunteers (Bradwejn *et al.*, 1991; van Megen *et al.*, 1994). Despite the string similarity of CCK-4-induced panic-like attacks to the natural ones, the CCK_B antagonists have not yet been proven to be the effective drugs for the treatment of anxiety disorders (Kramer *et al.*, 1995).

Studies on rats have also revealed the anxiogenic-like effect of CCK agonists in the ethological and conditioned models (Harro *et al.*, 1990; Harro and Vasar, 1991; Rex *et al.*, 1994). Different anxiogenic manipulations and stressful events increased the levels of CCK-4 in a number of brain areas of rat, including the frontal cortex (Pavlasevic *et al.*, 1993). Harro and co-workers (1990) described that diazepam withdrawal anxiety was associated with the increased density of CCK receptors in the rat frontal cortex and hippocampus. The anxiogenic β-carboline FG-7142 also upregulated the number of CCK

binding sites in the frontal cortex (Harro *et al.*, 1990) and increased the content of prepro-CCK mRNA in the limbic structures (Pratt and Brett, 1995). Similarly, the social isolation induced anxiogenic-like action and elevated the number of CCK receptors in the frontal cortex (Vasar *et al.*, 1993).

Several early studies have found that CCK_B receptor antagonist L-365,260 (1 and 10 μg/kg) and CI-988 (0.01 and 1.0 mg/kg) display the anxiolytic-like action in the rat plus-maze paradigm (Hughes *et al.*, 1990; Ravard *et al.*, 1990). The dose-effect curve for L-365,260 was bell-shaped with loss of activity at doses above 10 μg/kg (Chopin and Briley, 1993). However, some studies have described the anxiolytic-like effect of CCK_A antagonist devazepide (Hendrie *et al.*, 1993b). Although early studies with the selective and non-peptide CCK antagonists revealed the anxiolytic potential, the further experiments failed to show consistent anxiolytic-like activity of CCK_B antagonists (Dawson *et al.*, 1995; Johnson and Rodgers, 1996). Lack of anxiolytic-like profile of the CCK_B antagonists seems to be related to the experimental paradigm. In the conditioned models (fear-potentiated startle, punished drinking, etc) different CCK_B antagonists (L-365,260; CI-988; L-740093) are not effective against the behavioural suppression (Dawson *et al.*, 1994; Rodgers *et al.*, 1997). However, CCK antagonists display the anxiolytic-like profile in the ethological or unconditioned models (the elevated plus-maze and the light/dark compartment test) (Costall *et al.*, 1991; Chopin and Briley, 1993). These tests are based on the natural neophobia of animals towards the unfamiliar environment, which invariably include areas of relative safety. These tests are generally believed to be more sensitive for non-benzodiazepine anxiolytic drugs (including CCK antagonists) (Rodgers *et al.*, 1997). Moreover, the activation of peptide neurotransmission occurs only in the case of bursting or high-frequency neuronal activity. Therefore, the peptide antagonists itself should not necessarily show any effect under normal tonic conditions (Harro *et al.*, 1995b).

The systemically administered CCK produces a large and immediate increase in plasma levels of the adrenocorticotropic hormone (ACTH) and corticosterone in rat and man (Spath-Schwalbe *et al.*, 1988; Kamilaris *et al.*, 1992; Biro *et al.*, 1993). CCK stimulates the release of corticotropin-releasing hormone (CRH) from the cultured hypothalamic neurones and also increases the release of ACTH in vitro (Reisine and Jensen, 1986). The possible site for the interaction between CCK and CRH seems to be in the paraventricular nucleus, where the co-localisation of two neuropeptides is established (Mezey *et al.*, 1985). These findings suggest that CCK can act at the circumventricular sites of the hypothalamus and activate the hypothalamic-pituitary-adrenal (HPA) axis. In humans, the administration of pentagastrin (CCK-5) is accompanied by the two-fold elevation of the ACTH levels (Abelson *et al.*, 1994). Also, studies with animals confirmed the raised plasma corticosterone levels in rats after exposure to the elevated plus-maze or to the cat odour (File *et al.*, 1993;

File *et al.*, 1994). On the other hand, it has been shown that several psychiatric disorders exhibit seasonal variability. Many reports have confirmed increase in the incidence of panic attacks and incidence of suicides in summer (Maes *et al.*, 1993; Marriott *et al.*, 1994). Moreover, circannual endogenous rhythm in the function of 5-HT activity exists (Egrise *et al.*, 1986; Nagayama and Lu, 1998). Therefore, the possible seasonal variation in the exploratory activity of the rats and its relation to the changes in the CCK-ergic neuronal circuits was studied.

2.3. CCK-opioid interactions

At the beginning of the eighties two studies described the ability of CCK-8 to antagonise opioid-induced analgesia (Faris *et al.*, 1983; Itoh *et al.*, 1985). Since then there has been a considerable interest in exploring the possibility that CCK may act as the endogenous antagonist of opioid peptides (Faris, 1985a; Faris, 1985b). In addition, the distribution of CCK peptides in the brain matches that of opioid peptides — enkephalin, β-endorphin, and dynorphin (Stengaard-Pedersen and Larsson, 1981; Baber *et al.*, 1989; Skinner *et al.*, 1997). Despite the numerous studies the actual role of CCK_A or CCK_B receptors in opioid analgesia remains to be established (Dourish *et al.*, 1990; Benedetti *et al.*, 1997; Benedetti, 1997). The ability of CCK_B antagonists to prevent the development of opioid tolerance and dependence is shown (Idanpaan-Heikkila *et al.*, 1997; Kayser *et al.*, 1998). There is also evidence that CCK_A receptors are responsible for the rewarding properties of morphine, whereas CCK_B receptors modulate the analgesic activity of morphine (Singh *et al.*, 1996). Interestingly, the increased number of CCK receptors in the supraoptic nucleus after the chronic morphine administration is described, suggesting a role of increased sensitivity to the endogenous CCK in the development of tolerance to the analgesic effect of morphine (Munro *et al.*, 1998). The anatomical substrate for CCK-opioid interplay is not definite as yet. A clear anti-opioid action of CCK is described on the neuronal level in the spinal cord (Suh and Tseng, 1990). However, the supraspinal structures, particularly periaqueductal grey, are also involved (Hendrie *et al.*, 1989a; Dourish, 1992). The cellular mechanisms for that interaction are unclear. The electrophysiological studies demonstrated that CCK diminished morphine-induced inhibition of dorsal horn neuronal firing in response to the painful stimuli, whereas CCK antibodies and antagonists enhanced it (Suberg *et al.*, 1985; Suberg and Watkins, 1987). Otherwise, CCK has been described to increase the level of intracellular calcium in the presynaptic terminal by the mobilisation from the intracellular stores, thus antagonising the suppression of the increase of internal calcium by opioids (Wang *et al.*, 1992).

However, only few studies have been conducted to explore the role of opioid peptides in CCK-induced behavioural effects. The importance of opioid peptides has been established in the regulation of exploratory behaviour. Motta and Brandao (1993) have shown that the systemic administration of morphine at low doses or its injection into the dorsal periaqueductal grey induced anxiolytic-like action in the elevated plus-maze. Moreover, the environmental stimuli seem to be important in the regulation of tone of the endogenous opioid system. Stressful stimuli can increase the release of opioid peptides (Rodgers and Deacon, 1979). The CCK antagonists devazepide and L-365,260 potentiate morphine antinociception only in a novel but not in a familiar environment (Lavigne *et al.*, 1992). According to a recent study, μ-opioid receptors play a crucial role in the discriminative properties of CCK (Riley and Melton, 1997).

2.4. CCK-serotonin (5-HT) interactions

Several preclinical and clinical studies have shown the involvement of interplay between 5-HT and CCK in the development of anxiety. The anatomical substrate for this interaction seems to be the pathway arising from the raphe nucleus (median raphe nucleus) and terminating in the limbic and prefrontal cortical structures (Andrews *et al.*, 1997; Coplan and Lydiard, 1998). CCK-8 excites 5-HT neurones in the dorsal raphe nucleus (Boden *et al.*, 1991; Boden and Woodruff, 1994) and facilitates 5-HT release in the rat hypothalamus (Voigt *et al.*, 1998). The exposure of animals to a novel aversive environment (e.g. the elevated plus-maze) induces the cortical and hippocampal release of 5-HT, and this effect can be potentiated by CCK-4 (File *et al.*, 1993; Rex *et al.*, 1994; Rex and Fink, 1998). The administration of 5-HT_{1A} receptor agonist 8-OHDPAT or CCK_B receptor antagonist L-365, 260 can reverse this effect of CCK-4 (Rex *et al.*, 1994; Rex and Fink, 1998). Selective 5-HT re-uptake inhibitors (SSRIs) have been found to reduce the anxiolytic-like action induced by CCK receptor antagonists (Bickerdike *et al.*, 1994).

The changes in 5-HT neurotransmission seem to be crucial in mediating of behavioural reactions to the unpleasant events. However, the neuroanatomical substrate is not established as yet. Several studies support the importance of hippocampal 5-HT release as the trigger of anxiogenic-like behaviour (Andrews and File, 1993). Other studies have been paid main attention to the cortical changes and found 5-HT release in that site also plays a role (Rex *et al.*, 1993). Also, it is established that anxiogenic stimuli influence differently 5-HT metabolism in the different brain regions. The exposure of rats to the cat odour and diazepam withdrawal increased the cortical 5-HT and 5-HIAA levels. However, in the hippocampus only diazepam withdrawal increased content of

5-HT, whereas the cat odour exposure decreased the levels of 5-HT and 5-HIAA (Andrews *et al.*, 1993).

However, most experiments have studied the role of 5-HT in the action of drugs influencing CCK-ergic transmission, whereas the opposite interaction has remained out of scope. Nevertheless, few studies have been conducted in this field and it has been shown that 5-HT can increase the release of CCK in the cerebral cortex and nucleus accumbens (Raiteri *et al.*, 1993). The acute administration of alaproclate, the selective 5-HT reuptake inhibitor (SSRI), significantly elevates the levels of CCK in the cingulate cortex and periaqueductal grey (Rosen *et al.*, 1995). Fluoxetine, the 5-HT reuptake inhibitor, dose-dependently reduces the exploratory behaviour of rats in the elevated plus-maze (Handley and McBlane, 1993). The anxiogenic-like effect of drugs, increasing the content of 5-HT in the brain, is also supported by the human data. Den Boer and Westenberg (1996) have found that acute administration of 5-HT reuptake inhibitors can increase anxiety in patients suffering from anxiety disorders. On the other hand, the chronic treatment (for 8 weeks) with SSRI-s results in the decreased sensitivity to the anxiogenic properties of CCK-4 in panic disorder patients (Boyer, 1993; van Megen *et al.*, 1997). Therefore, one aim of our study was to verify the CCK-5-HT interaction in the action of SSRIs (paroxetine) and in the development of exploratory behaviour.

3. OBJECTIVES

According to the recent investigations the role of CCK in animal models of anxiety remains to be controversial. There are studies supporting the role of CCK, whereas the others do not confirm that. The background of these inconsistencies is not clear and therefore will need further clarification. In the present study a stress was put on the environmental factors influencing the level of anxiety in rats and affecting the response of animals to the anxiogenic action of CCK. According to these studies the conditions were selected where the anxiogenic action of CCK agonists was the strongest. In these particular conditions the interaction of CCK with endopioid mechanisms and 5-HT was explored.

The more specific purposes of our study were as follows:

1. **To explore the influence of pre-experimental stress on the action of CCK agonist and the seasonal variations of exploratory activity.** For these aims two pre-experimental procedures — handling and isolation were used. We studied the action of caerulein, an unspecific CCK agonist, in the elevated plus-maze after different pre-test manipulations. As the occurrence of panic attacks has been shown to be higher in summer besides to winter, we compared also the exploratory activity of rats and CCK neurotransmission in summer and in winter.
2. **To reveal the biochemical mechanisms those underlie the individual differences of exploratory behaviour of rats in the elevated plus-maze.** The rats were selected according to exploratory behaviour in the elevated plus-maze. The radioligand binding and hormonal studies were performed to establish a role of CCK in the individual differences of exploratory behaviour.
3. **To establish the role and nature of the interplay between CCK and endopioid mechanisms in the regulation of anxiety.** For this goal agonists and antagonists for CCK and opioid receptors were combined, and their action in the elevated plus-maze was studied.
4. **To study the involvement of 5-HT-CCK interaction in the regulation of anxiety.** For that purposes we studied the influence of paroxetine, a selective serotonin reuptake inhibitor (SSRI), on the exploratory behaviour and CCK neurotransmission.

4. MATERIALS AND METHODS

4.1. Animals

Male Wistar rats (Han/Kuo: WIST) were obtained from the National Animal Centre, Kuopio, Finland. The animals weighing 200–360 g were used for experiments. The animals were housed four per cage under artificial conditions with free access to water and food pellets. The animal house had controlled temperature at 20°C±2°C and 12 h light/dark illumination cycle (lights on at 7:00). At least one hour before an experiment the animals were moved with their home cages to the experimental room.

4.2. Drugs

Caerulein (CCK-like decapeptide, Sigma Co), BOC-CCK-4 (N-tert-Butoxycarbonyl-CCK-4, Sigma Co), morphine sulphate (Boehringer-Ingelheim), naloxone HCl (Sigma Co), paroxetine (Smith Kline and Beecham, U.K.) were dissolved in physiological saline (0.9% sodium chloride solution). L-365,260 [(3R(+)-N-2,3-dihydo-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine)-3-yl)-N^1-(3-methylphenyl)urea] Merck Sharp & Dohme], L-364,718 or MK-329 or devazepide (3S(-)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide, Merck Sharp & Dohme), LY 288,513 [trans-N-(4-bromophenyl)-3-oxo-4,5-diphenyl-1-pyrazolidinecarboxamide, Ely Lilly & Co, U.S.A.] and diazepam (Sigma, U.S.A.) were suspended in 1% Tween-80 (Sigma Co) solution in saline.

Caerulein, BOC-CCK-4 and morphine were administered subcutaneously, whereas naloxone, paroxetine, L-365,260 and LY 288,513 were given intraperitoneally.

For radioligand binding experiments raclopride (S-3,5-dichloro-N[(1-ethyl-2-pyrrolidinyl)methyl]-6-methoxysalicylamide tartrate, Astra AB, Sweden), and ketanserin (Janssen) were dissolved in saline. Clonazepam (Hoffman La Roche, Switzerland) was dissolved in ethanol and therefore diluted in the incubation buffer, where the final concentration of ethanol was about 0.05%.

The radioligands [Propionyl-^{3}H]-propionylated-CCK-8-sulphated ([^{3}H]p CCK-8), [^{3}H]-spiperone and [^{3}H]-flunitrazepam were obtained from Amersham Radiochemicals (U.K.). The other chemicals for radioligand binding studies (caerulein, HEPES (N-[2-hydroxyethyl]piperazine-N'-[-2-ethane-sulfonic acid]), NaCl, $MgCl_2$, KCl and EDTA [ethylenediamine-tetraacetic acid]) were purchased from Sigma (U.S.A.).

4.3. Behavioural studies

4.3.1. Pre-experimental manipulations

The animals were brought into the experimental room 1 hour before the experiment. The rats were handling-naive and were not adapted to the experimental situation, if not specified otherwise. Each rat was used only once. All experiments were carried out between 12:00 to 19:00.

To study the effects of pre-experimental manipulations on the exploratory behaviour and anxiogenic-like effect of CCK in the elevated plus-maze, an experiment was performed in November and December. Before the experiment handling and brief isolation were combined as two different stressors. The rats were divided into four different groups. Two groups of rats were handled in the experimental room on three consecutive days (twice daily) before the experiment. The other two groups of animals were brought to the experimental room immediately before the beginning of the experiment. The handled and non-handled rats were divided into two groups after the injection of caerulein (5 μg/kg s.c., Sigma), an agonist of CCK receptors. Caerulein or saline was injected 15 min before the beginning of the plus-maze study. One half of animals was isolated after the injection, whereas the other half was placed back into the home-cage. The action of the CCK_B antagonist L-365,260 (1–100 μg/kg i.p., Merck Sharp & Dohme) and CCK_A antagonist devazepide (1–100 μg/kg i.p., Merck Sharp & Dohme) on the anxiogenic-like action of caerulein was also studied. CCK antagonists or vehicle (2% Tween-85 in physiological saline) were injected 30 min before the plus-maze exposure.

In the second half of the experiment, the possible seasonal differences in the exploratory activity of rats were studied. Two studies were performed — one study was conducted at the beginning of July 1993 (summer) and the other one in late November of 1994 (winter). This study was performed in 40 handling-naive rats (in both experiments). The animals were decapitated immediately after the plus-maze exposure, and the blood and brain samples were taken for the neurohormonal studies.

4.3.2. Elevated plus-maze

The method initially suggested by Handley and Mithani (1984) for the measurement of exploratory activity was employed with some modifications (Pellow *et al.*, 1985). The apparatus consisted of two opposite open arms (50×10 cm) without side walls and two enclosed arms (50×10×40 cm) with side walls and an end wall, extending from a central square (10×10 cm). The maze was elevated to the height of 75 cm, and placed in a lit room. During a 5-min observation session an observer took the following measures:

1) latency of the first open part entry;
2) time spent in exploring the open part and open arms of plus-maze;
3) number of closed and open arm entries;
4) number of lines crossed in the open part;
5) ratio between open and total arm entries (also % open entries).

At the beginning of the experiment an animal was placed into the centre of the plus-maze, facing towards the closed arm. An arm entry was counted only when all four limbs of the rat were within the given arm. Time spent in open arms, number of open arm entries and the ratio between open and total arm entries are the "classical" measures of anxiety in the elevated plus-maze (Rodgers and Johnson, 1995). By contrast, the number of closed arm entries and the number of line crossings are the measures reflecting the locomotor activity of rats (Rodgers and Johnson, 1995; Rodgers *et al.*, 1997).

4.3.3. Motility test

Exploratory activity of rats was measured by means of photoelectric motility boxes (448×448×450 mm) connected to a computer (TSE, Technical & Scientific Equipment GmbH, Germany). Animals, naive to the test situation were placed singly into the box. Time in exploration (sec), distance of exploration (in metres), number and duration of rearing were registered at 5-min intervals during the 15-min observation period.

4.4. Radioligand binding studies

4.4.1. Preparation of brain samples

After decapitation the brains were quickly dissected on ice according to the method of Glowinski and Iversen (1966). The binding studies for CCK receptors were performed in the frontal cortex (also containing the anterior cingulate and frontoparietal cortex) and hippocampus. Benzodiazepine and 5-HT receptors were analysed in the frontal cortex (contains the anterior cingulate and frontoparietal cortex). For the study of dopamine receptors the striatum was used. These brain structures were selected according to the previous studies since the most prominent changes due to the exploratory behaviour have occurred in these brain regions (File *et al.*, 1993; Rex *et al.*, 1994; Pratt and Brett, 1995). Brain tissues were homogenised in 20 volumes of ice-cold 50 mM Tris-HCl (pH 7.4 at 4°C) using a Potter-S glass-teflon homogeniser (1000 rpm, 12 passes). The membranes were washed twice in the same buffer by centrifugation (48 000×g for 20 min) and resuspension.

Thereafter brain samples were resuspended in the appropriate incubation buffer at a concentration of 10 mg wet weight/ml.

4.4.2. [Propionyl-^{3}H]-propionylated-CCK-8-sulphated binding for CCK receptors

After the last centrifugation, the crude brain membranes were homogenised in HEPES buffer (10 mM HEPES; 130 mM NaCl; 5 mM KCl; 1 mM $MgCl_2$; 1 mM EDTA; pH 6.5 adjusted with 1 N NaOH) containing bovine serum albumin (0.5 mg/ml). The parameters of CCK receptors were determined in the presence of 0.05–2.4 nM [^{3}H]pCCK-8 (specific activity 79 Ci/mmol) at 23°C in a total incubation volume of 0.5 ml. Caerulein (100 nM) was added to determine the non-specific binding. The incubation was terminated after 120 min by the rapid filtration over Whatman GF/B filters pre-soaked with the bovine serum albumin (0.5 mg/ml). The filters were washed with 3×3 ml of ice-cold HEPES buffer. In a separate study paroxetine (0.01–1 mM) was added to the incubation medium to reveal the possible direct interaction of paroxetine with CCK binding sites in the frontal cortex.

4.4.3. [^{3}H]-spiperone binding for serotonin (5-HT_{2A}) receptors

After the final washing the crude brain membranes were homogenised in the incubation buffer consisting of 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ (pH 7.4). [^{3}H]-spiperone (specific activity 105 Ci/mmol) at final concentrations 0.06–2 nM was incubated 30 min at 37°C with the membrane preparation in 0.5 ml of the reaction volume. Non-specific binding was determined in the presence of 1 μM ketanserin. Incubation was stopped by the rapid filtration through Whatman GF/B glass fibre filters (pre-soaked with 0.05% polyethylenimine).

4.4.4. [^{3}H]-spiperone binding for dopamine (D_2) receptors

After the washing the crude brain membranes were homogenised in the incubation buffer consisting of 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ (pH 7.4). [^{3}H]-spiperone (specific activity 105 Ci/mmol) at final concentrations 0.06–2 nM was added to the tissue preparation. Nonspecific binding was determined in the presence of 1 μM raclopride. The incubation was carried out in a total volume of 0.5 ml for 30 min at 37°C and was terminated by the rapid filtration over pre-soaked

Whatman GF/B filters and the filters were washed 3×3 ml with ice-cold incubation buffer.

4.4.5. [^{3}H]-flunitrazepam binding for benzodiazepine receptors

The pellets were homogenised in Tris-HCl buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4). The membranes were incubated in the presence of 0.25–16 nM N-methyl-[^{3}H]-flunitrazepam (specific activity 90 Ci/mmol) at 4°C in a total volume of 0.5 ml in the presence or absence of unlabelled clonazepam (1 μM) to determine non-specific binding. Incubation was terminated after 60 minutes by rapid filtration over Whatman GF/B filters, thereafter washed with 9 ml ice-cold buffer.

The filters were dried and left overnight in Wallac High Safe III scintillation cocktail. Radioactivity was assayed by liquid scintillation spectrometry (Wallac β-scintillation counter) at 50 per cent efficiency level. The protein content was measured according to a dye-binding assay (Bradford, 1976). Saturation curves were analysed using non-linear least squares regression (Leatherbarrow, 1987).

4.5. Hormonal studies

In all cases truncal blood was collected, serum was separated by centrifugation and samples were stored at –20°C until prolactin (PRL), thyrotropin (TSH) and growth hormone (GH) concentrations were determined from duplicate samples (0.1 ml) by specific radioimmunoassays. The rat PRL, TSH and GH kits were gifts from NIH. PRL results are expressed in ng/ml of NIDDK-rPRL-RP-2 standard. TSH data are expressed in ng/ml of NIDDK-TSH-RP-2 standard. GH results are expressed in ng/ml of NIDDK-GH-RP-2 standard. The intra-assay coefficient of variation was less than 15%.

4.6. Statistical analysis

The analysis of data was performed by means of the Statistica for Windows (Statsoft Inc., USA) software. The results are expressed as mean values ± S.E.M. Behavioural and hormonal data were analysed by one-way analysis of variance (ANOVA). *Post hoc* comparisons were performed by means of Newman-Keuls or Tukey HSD tests. The data of radioligand-binding experiments were assayed by means of Student's *t*-test.

5. RESULTS AND DISCUSSION

5.1. Pre-experimental stress and seasonal variations in the action of CCK (paper V)

Nonspecific CCK agonist caerulein (5 μg/kg) caused the strongest action in animals brought to the experimental room immediately before the experiment (not subjected to pre-experimental handling) and kept in isolation after the administration of caerulein (Figure 1). The effect of CCK agonist was antagonised by L-365,260 (1–100 μg/kg), an antagonist of CCK_B receptors, in a dose-dependent manner with a statistically significant effect at a dose of 100 μg/kg (Figure 2). One hundred μg/kg of devazepide, an antagonist of CCK_A receptors, was also effective in reversing the anxiogenic-like action of caerulein in stressed animals, but this effect was not as clear as in the case of L-365,260. In the experiments where CCK antagonists were given as a single treatment, only 10 μg/kg of L-365,260 induced anxiolytic-like action, whereas devazepide was without any effect. One and 100 μg/kg of L-365,260 were without any significant influence (Figure 2).

This study revealed a clear relation between the anti-exploratory effect of caerulein and pre-experimental stress. This finding is in good accordance with the previous studies showing the presence of an anxiogenic-like action of CCK only in a novel environment (Dauge *et al.*, 1989). Neurochemically, CCK-4 potentiated an increase in the 5-HT levels in the lateral prefrontal cortex only during the exposure of guinea pigs to the elevated plus-maze, but it had no effect in the animals that remained in their home-cage (Rex and Fink, 1998). Moreover, CCK antagonised morphine-induced analgesia in rats in novel, but not in familiar, experimental conditions (Wiertelak *et al.*, 1992). Accordingly, caerulein seems to potentiate neophobia in rats. However, novelty is not the only factor determining the action of caerulein in the plus-maze. The keeping of rats in social isolation after the injection of CCK also contributes to the action of caerulein. It is likely that the social isolation of rats may sensitise the animals to the anxiogenic-like effect of caerulein. The effective dose of agonist and behavioural patterns after CCK challenge depend on baseline anxiety of the animal. It has been shown that 10–50 times lower doses of caerulein and pentagastrin are anxiogenic in rats and mice housed under stressful over-crowded conditions (Harro *et al.*, 1995b). In our previous studies we have found that the social isolation of rats for 7 days induced anxiety in animals, but it also increased the density of CCK but not benzodiazepine receptors in the frontal cortex (Vasar *et al.*, 1993). According to our recent study CCK agonists produced the anxiogenic-like action only in rats not acclimatised to the experimental situation (Vasar *et al.*, 1997). It has been shown that pre-

experimental stress also increases the effectiveness of anxiolytic drugs in the plus-maze showing that the endogenous tone is an important factor in studying anxiety in rodents (Rodgers *et al.*, 1997). It is worthy to point out that patients suffering from anxiety disorders are also more sensitive to CCK-4- and pentagastrin-induced panic attacks than healthy volunteers (Bradwejn *et al.*, 1994; Shlik *et al.*, 1997). The characteristic feature of anxiety disorders is the increased serum level of stress hormones (ACTH, cortisol) (Abelson *et al.*, 1994; Arborelius *et al.*, 1999). Collectively, the level of pre-experimental stress is a factor that determines the potential response both in animals and in man to the anxiogenic-like action of CCK agonists.

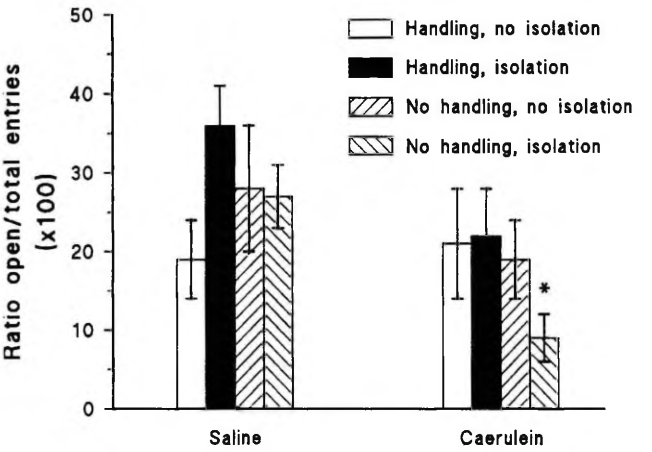

Figure 1. The effect of pre-experimental handling and isolation on the anti-exploratory action of caerulein measured in the elevated plus-maze. * — $p<0.05$ (compared to the respective saline group, the Newman-Keuls test after significant one-way ANOVA).

The potentiation of neophobia induced by caerulein was dose-dependently antagonised by the CCK antagonists L-365,260 and devazepide. The nearly equal potency of L-365,260 and devazepide against caerulein makes it unlikely that this effect of CCK antagonists may be mediated primarily via CCK_A receptors. This statement is based on the knowledge that devazepide has very high affinity for CCK_A, but it is relatively non-selective and has much better penetration, compared to L-365,260, into the brain (Hargreaves and Lin, 1992). CCK_B receptors are likely targets for the anxiogenic-like action of caerulein as 100 μg/kg of devazepide interacts also with this subtype (Woodruff and Hughes, 1991). This is in an agreement with the previous studies showing the key role of CCK_B receptors in CCK-induced anxiety both in the human and animal studies (Harro *et al.*, 1995b). The single treatment with L-365,260, but not with devazepide, induced the anxiolytic-like action in rats. However, the action of L-365,260 was not dose-dependent since only one dose of the CCK_B

receptor antagonist (10 μg/kg) was effective, and the lower and higher doses did not change the exploratory behaviour of rats. The U-shape action of L-365,260 has been also established in our previous studies (Harro and Vasar, 1991), but the background of this peculiar action remains to be established.

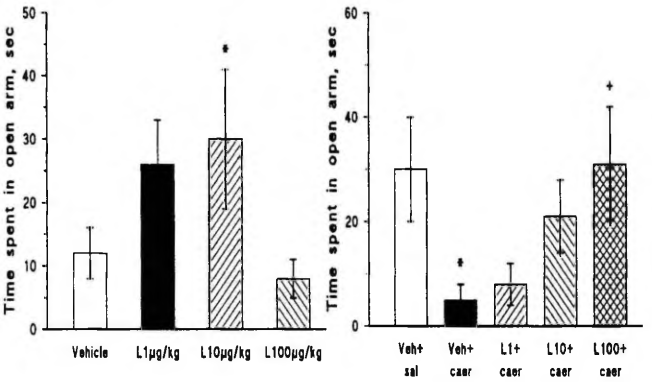

Figure 2. The effect of L-365,260 on the exploratory behaviour of rats in the plus-maze and on the anxiogenic-like action of caerulein and upon the exploratory behaviour in the elevated plus-maze. * — $p<0.05$ (compared to vehicle or vehicle + saline treated, Newman-Keuls after significant ANOVA); + — $p<0.05$ (compared to vehicle + caerulein treated, Newman-Keuls after significant ANOVA).

The second part of this study established seasonal fluctuations in the exploratory behaviour. Indeed, the exploratory activity of rats was much lower in July compared to the study performed in November (Table 2). The animals with a low exploratory activity displayed an increased number of CCK receptors in the frontal cortex and hippocampus and elevated density of 5-HT receptors in the frontal cortex (Figure 3). Also, the serum levels of the growth hormone were higher in July compared to November (Figure 3).

Table 2. Comparison of the results of two distinct experiments, performed in July and November — plus-maze exploration

Plus-maze parameters	July	November
Time in open part (sec)	46 ± 6	91 ± 6*
Number of open arm enrtries	0.2 ± 0.1	1.3 ± 0.3*
Time in open arm (sec)	4 ± 2	14 ± 3*
Number of total arm entries	3.1 ± 0.3	7.8 ± 0.6*
% open entries	4 ± 2	19 ± 4*

* — $p<0.05$ (Student's *t*-test).

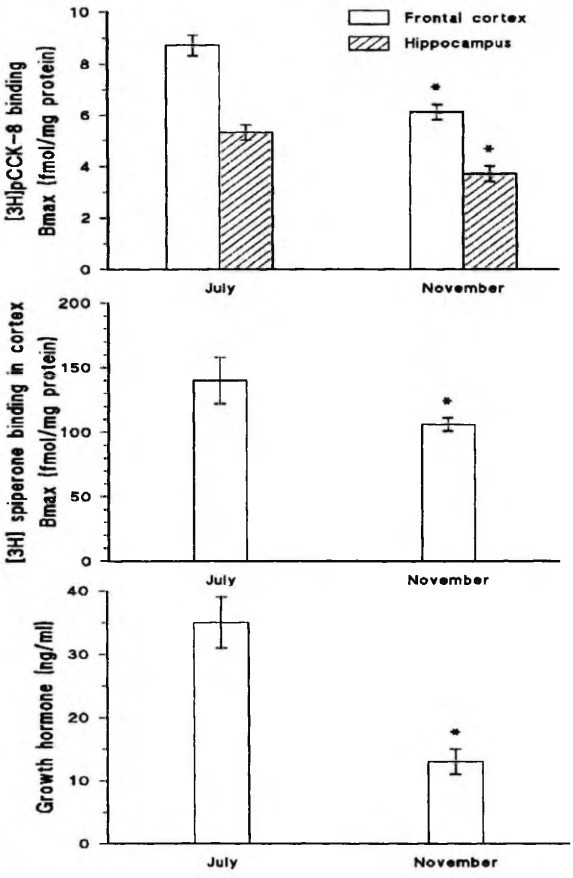

Figure 3. Comparison of the results of two experiments performed in July and in November: CCK and 5-HT receptors in the brain and serum levels of growth hormone. * — $p<0.05$ Student's *t*-test.

The present study provides a strong evidence of seasonal variations in the exploratory activity of rats. The comparison of exploratory activity of rats in two experiments conducted in July (summer) and in November (winter) revealed a clear difference. The exploratory activity of rats was much lower in summer compared to winter. Harro and others (1997) obtained similar results showing significant variation in the exploratory activity of rats in two studies, in November and in June. Moreover, it is important to stress that the anxiogenic-like action of caerulein is much weaker in summer compared to the studies performed in winter (our unpublished data). This was the reason why the influence of pre-experimental stress to the anxiogenic-like action of CCK agonist was studied in winter but not in summer. Also, these data are interesting in the light of recent reports describing significantly higher summer incidence in the occurrence of the first panic attack compared to winter (Cameron, 1989;

Lepine *et al.*, 1991; Marriott *et al.*, 1994). The hypothetical reason for such seasonality is the increased activity of people that leads to the overcrowding of public places (Lelliott *et al.*, 1989). In rats the increased level of anxiety in summer could be explained by the higher pressure from the surrounding nature since in summer the number of potential predators is much higher than in winter when life in nature apparently slows down. This statement is supported by the recent findings showing the seasonal fluctuations in the serum levels of ACTH in the sand rat with the maximum concentration during the late spring and summer (Amirat and Brudieux, 1993).

In summer the rats also had significantly increased levels of the growth hormone, whereas the levels of prolactin and thyrotropin remained unchanged. A recent study has established that healthy subjects respond to CCK-4 challenge with an increased level of the growth hormone, which was higher in those who panicked after CCK-4 than in non-panickers (Koszycki *et al.*, 1998). This finding is also in line with our study that neither the exposure of rats to the plus-maze nor distinctive exploratory activity correlated with the serum levels of the anterior pituitary hormone prolactin or thyrotropin (see part 5.2). Only the concentrations of the growth hormone were significantly different in the rats selected according to the exploratory behaviour. Namely, the animals with a higher exploratory activity had markedly lower levels of the growth hormone compared to the animals with a low activity. 5-HT stimulates the release of the growth hormone in response to stress (Charney *et al.*, 1987). Moreover, the activation of CCK_B receptors has been shown to increase the baseline release on the growth hormone (Männistö *et al.*, 1994; Peuranen *et al.*, 1994). Therefore, the enhanced levels of the growth hormone in animals in summer might be caused by the increased activity of 5-HT and CCK in the brain.

The reduced exploratory activity of rats in summer appears to be related to the increased number of CCK receptors in the frontal cortex and hippocampus. Harro and others (1997) have reached the same results that in June, when the exploratory activity was lower, the density of CCK receptors was higher in the frontal cortex, hippocampus, and striatum but not in the hypothalamus. Moreover, in summer the content of different CCK fragments (CCK-8s, CCK-8us, CCK-4, CCK-5) in the frontal cortex and in the hippocampus was much higher compared to the experiments conducted in November. In the light of these data, it is likely that the tone of CCK-ergic transmission may be higher in summer compared to winter. This could be a possible explanation why the anxiogenic-like action of caerulein is much weaker in summer since CCK receptors seem to be occupied by the endogenous ligand. In addition, we have noted an increased density of serotonin $5\text{-}HT_2$ receptors in the frontal cortex of rats having a decreased exploratory activity. By contrast, the number of dopamine D_2 receptors in the striatum remained unchanged. Accordingly, increased susceptibility to the unpleasant events in rats seems to be related to the increased density of CCK and $5\text{-}HT_2$ receptors in the forebrain.

Taken together, different pre-experimental manipulations and seasonal variations could be the reason for inconsistent findings in studies with CCK-ergic compounds. Behavioural validation and unifying of the test conditions before the ethological studies are needed to reduce the variations.

In conclusion, the anxiogenic-like action of caerulein was dependent on pre-experimental stress in animals. The CCK agonist caused the strongest action in rats not adapted to the experimental conditions. In addition, seasonal variations of exploratory activity were revealed in the present study. The rats were more active in winter than in summer. The reduced exploratory activity of rats was apparently related to the increased density of CCK and 5-HT_2 receptors in the brain. Also, the levels of the growth hormone were markedly higher in animals displaying reduced exploratory activity. Therefore, the lower exploratory activity in summer could be caused by the activation of CCK-ergic and 5-HT-ergic neurotransmission.

5.2. Relation of exploratory behaviour to neurochemical changes (paper I)

Forty-five male Wistar rats were selected according to their exploratory behaviour in the elevated plus-maze. They were separated as follows: "anxious" rats (7 animals) with low exploratory activity, "intermediate" rats (30 animals) with intermediate exploratory activity, and "non-anxious" rats (8 animals) having high exploratory activity (Table 3). In "anxious" rats the affinity of 5-HT_{2A} receptors was lower compared to home-cage controls and "non-anxious" rats. Also, the number of CCK receptors in the hippocampus was elevated in the "anxious" rats compared to home-cage controls (Figure 4). Additionally, the blood levels of the growth hormone (GH) were significantly lower in "non-anxious" rats compared to "anxious" counterparts (Figure 4).

Table 3. Selection of rats according to exploratory behavior in the elevated plus-maze (mean values ± S.E.M.)

Behavioural parameters	"Anxious" N=8	"Intermediate" N=30	"Non-anxious" N=7
Time spent in central square (sec)	0.5±0.3	69±4[a]	98±10[a,b]
Time spent in open arm (sec)	0	8±3	38±8[a,b]
Number of open arm entries	0	0.6±0.2	4.0±0.7[a,b]
Number of closed arm entries	1.0±0	5.5±0.5[a]	10.4±0.8[a,b]
Ratio open arm entries/closed arm entries	0	0.10±0.03	0.38±0.05[a,b]

a — $p<0.05$ (compared to "anxious" animals, the Newman-Keuls test after significant one-way ANOVA); b — $p<0.05$ (compared to the "intermediate" group).

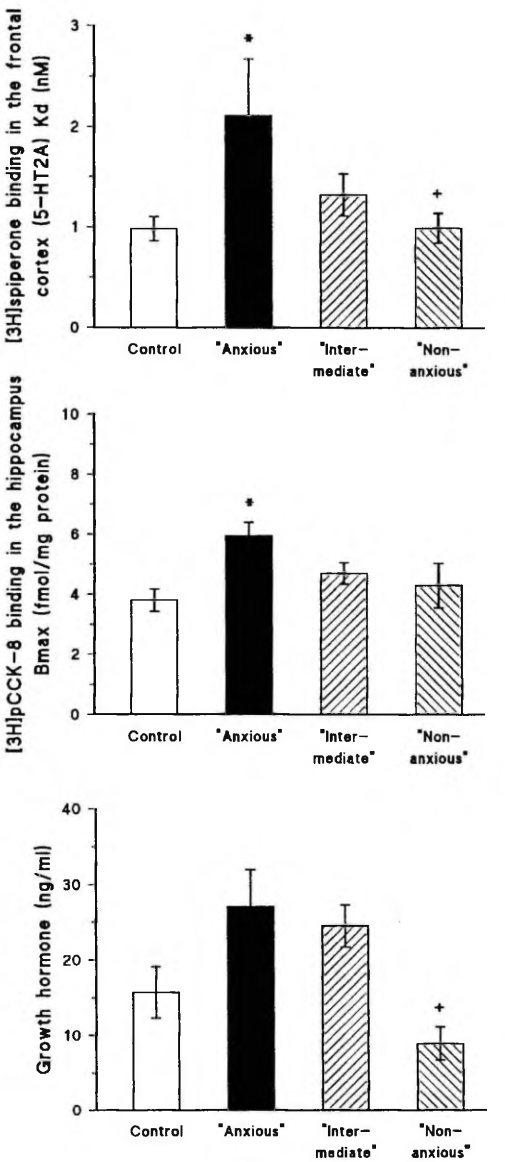

Figure 4. Parameters of serotonin 5-HT_{2A} and CCK receptors and the serum levels of the growth hormone in selected rats. * — $p<0.05$ (compared to home-cage controls, the Newman-Keuls test after significant one-way ANOVA); + — $p<0.05$ (compared to "anxious").

These behavioural findings are in agreement with the previous experiments confirming the significant variability of exploratory behaviour of rats in the elevated plus-maze (Harro *et al.*, 1990; Rägo *et al.*, 1991). Among the 45 selected rats we established a subgroup of animals eagerly exploring the open

arms. The open arms were considered to be the most aversive parts of the plus-maze (Pellow *et al.*, 1985). Moreover, a recent study of factor analysis behaviour (Rodgers and Johnson, 1995). These "non-anxious" rats performed revealed a positive correlation between open arm entries and anxiolytic-like three or more open arm entries per session. On the other hand, the other subgroup of rats ("anxious") did not leave the enclosed arms at all. It is worthy to note that these "anxious" animals made several unsuccessful attempts to enter from the enclosed arm into the central square reflecting the increased risk assessment behaviour. Accordingly, the central square located between the opened and enclosed arms is too aversive for "anxious" rats to explore. The exploratory behaviour of the "intermediate" group represents the mean exploratory activity of the whole population. Their behavioural activity was mainly restricted to the central square.

The radioligand binding data are somewhat different from the previously published results. Harro et al. (1990) have found that "anxious" animals have a significantly increased density of CCK receptors in the frontal cortex and reduced amount of these receptors in the hippocampus. In the present study, a similar tendency was established in the frontal cortex. The "anxious" animals had more CCK binding sites compared to the "non-anxious" counterparts, but this difference was not statistically significant. In the hippocampus the situation was completely different. Namely, we found an increased number of [^{3}H]pCCK-8 binding sites in the "anxious" animals compared to home-cage controls. Some support for the involvement of hippocampal CCK in the anxiogenic-like behaviour has been published. The recent studies indicate that CCK receptors in the hippocampus belong to the CCK_B (brain) receptor subtype (Honda *et al.*, 1993). Therefore, it is likely that the variations of exploratory behaviour in rats may be related to some extent to the differences in the density of CCK_B receptors. There is evidence that unavoidable electric foot-shocks increase the levels of CCK-like immunoreactivity in limbic regions (Siegel *et al.*, 1995). Moreover, the cessation of chronic 14-day diazepam treatment increased the number of CCK receptors in the hippocampus (Harro *et al.*, 1990). The elevated levels of preproCCK mRNA in the hippocampus has been found after withdrawal from the 3-week diazepam treatment (Rattray *et al.*, 1993). However, Pratt and Brett (1995) did not detect any changes in the content of preproCCK mRNA in the frontal cortex and hippocampus after the plus-maze exposure. Nevertheless, the administration of inverse benzodiazepine agonist FG 7142 at anxiogenic doses markedly increased the levels of preproCCK mRNA in both brain regions (Pratt and Brett, 1995). Despite the significantly distinctive behaviour, the plus-maze exposure may not be strong enough to increase CCK binding sites in the brain of all rats. Therefore, only in the most susceptible animals exposure to the elevated plus-maze caused the marked elevation of CCK binding sites in the hippocampus.

The "anxious" rats displayed the reduced affinity of 5-HT_{2A} receptors in the frontal cortex compared to home-cage controls and "non-anxious" rats. This change can be explained in the light of recent findings. Namely, Rex *et al.* (1993; 1994) and Marsden *et al.* (1993) established that the exposure of rodents to the elevated plus-maze increased the release of 5-HT in the frontal cortex. Similar alterations have also been described in the hippocampus (File *et al.*, 1993; Crawley and Corwin, 1994). Therefore, we are trying to speculate that the reduced affinity of 5-HT_{2A} receptors in the frontal cortex is related to the increased release of 5-HT in the "anxious" rats. Moreover, two recent neurochemical studies have revealed a link between CCK and 5-HT in the regulation of exploratory behaviour. More specifically, the anxiogenic-like action of CCK agonists in the plus-maze is related to the increased release of 5-HT in the prefrontal cortex (Rex *et al.*, 1997; Rex and Fink, 1998).

Besides CCK and 5-HT, also dopamine is shown to be involved in the regulation of anxiety (Glavin, 1993; Ladurelle *et al.*, 1995). However, we did not find any significant modifications of dopamine D_2 receptors in the striatum of selected rats. Accordingly, we were unable to establish a link between striatal dopamine receptors and exploratory behaviour.

We investigated also the release of anterior pituitary hormones in relation to the exploratory behaviour of rats. In the present study neither the exposure of rats to the plus-maze nor distinctive exploratory activity correlated with the serum levels of anterior pituitary hormones prolactin and thyrotropin. This is in line with results from the human studies where no difference between panic patients and healthy volunteers in the serum levels of thyrotropin-releasing hormone was found (Fossey *et al.*, 1993). Only the concentrations of the growth hormone (GH) were significantly different in the selected rats. Namely, the "non-anxious" rats had markedly lower levels of GH compared to the "anxious" and intermediate groups. Anterior pituitary hormones are regulated by a multitude of classic neurotransmitters such as 5-HT and dopamine but also by neuropeptide transmitters (Tuomisto and Männistö, 1985). It has been shown that 5-HT stimulates the baseline release of GH and may be involved in the stress-induced GH elevation in man (Charney *et al.*, 1987). CCK also participates in the regulation of GH. The activation of CCK_A receptors inhibits GH secretion while the activation of CCK_B receptors caused the opposite effect (Männistö *et al.*, 1994; Peuranen *et al.*, 1994). Therefore, the increased levels of GH in the "anxious" animals might be caused by the increased activity of 5-HT and CCK in the brain.

In conclusion, the present study confirms the significant individual differences in the exploratory behaviour of rats. Therefore, for performing of studies on animals involving exploratory behaviour, the animal groups should be big enough, consist of 10–12 animals at least to reduce the effect of individual variability. We were able to confirm the relation of individual behavioural differences to the activity of CCK-ergic neural circuits However,

the variations in hormonal levels and receptor parameters between the selected groups are not as marked as in the behavioural studies. Nevertheless, the increased density of CCK receptors in the hippocampus and reduced affinity of 5-HT_{2A} receptors in the frontal cortex seem to be linked to the decreased exploratory activity of rats in the elevated plus-maze. The background of changes in the content of GH remains to be elucidated, but it may be also related to the altered activity of 5-HT and CCK neurotransmission in the brain.

5.3. Interaction between CCK and endopioid mechanisms in anxiety (papers II, IV)

The opioid agonist morphine (1.0 mg/kg) significantly increased the exploratory behaviour of rats in the plus-maze (Figure 5). The locomotor activity in the motility boxes remained unchanged at 1.0 mg/kg of morphine. Lower (0.5 mg/kg) and higher doses of morphine (2.5 mg/kg) were without any significant effect in the plus-maze, whereas a higher dose tended to reduce the locomotor activity of animals in the motility boxes. That could be a reason for the lack of anxiolytic-like activity of a higher dose of morphine as the reduced motor activity masks the changes in exploratory behaviour. Reduced locomotor activity accompanying the administration of a higher dose (2.5 mg/kg) of morphine is possibly due to the inhibition of dopaminergic neurotransmission in the mesolimbic structures (Lang *et al.*, 1995). Opioid antagonist naloxone (0.5 mg/kg and 10 mg/kg) itself was unable to modify exploratory behaviour. Nevertheless, 10 mg/kg of naloxone tended to reduce the activity of rats in the plus-maze (Figure 5). The administration of non-selective CCK agonist caerulein (1 and 5 μg/kg) and a selective CCK_B receptor agonist BOC-CCK-4 (1, 10 and 50 μg/kg) dose-dependently reduced the exploratory activity of rats in the elevated plus-maze (Figure 5). One μg/kg of caerulein was unable to decrease the exploratory behaviour, whereas 5 μg/kg induced a significant reduction in all the studied parameters. Also, treatment with BOC-CCK-4 (10 and 50 μg/kg) caused the anxiogenic-like behaviour of rats. The CCK_B receptor antagonist L-365,260 (10 μg/kg) displayed anxiolytic-like behaviour increasing the ratio between open and total arm entries (Figure 5).

Naloxone (0.5 mg/kg) potently antagonised the anxiolytic-like effect of morphine. CCK_B receptor agonist BOC-CCK-4 (10 μg/kg), also blocked the action of morphine (Figure 6). The combination of naloxone (0.5 mg/kg) with the sub-effective doses of caerulein (1 μg/kg) and BOC-CCK-4 (1 μg/kg) induced a robust decrease in the exploratory behaviour of rats (Figure 7). The combination of L-365,260 (100 μg/kg) with the sub-effective dose of morphine (0.5 mg/kg) caused the anxiolytic-like action in the plus-maze not seen if the drugs were given alone (Figure 8).

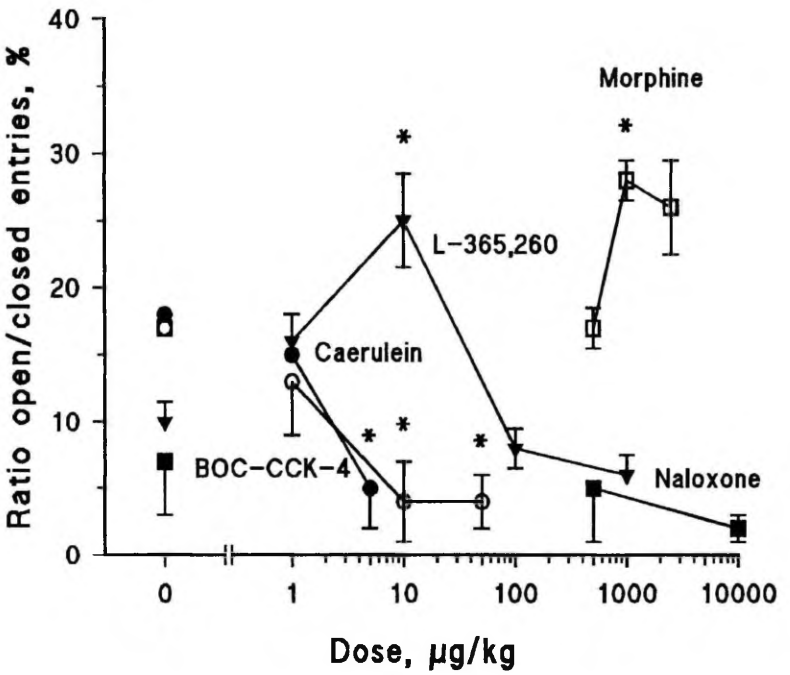

Figure 5. The effects of caerulein, BOC-CCK-4, morphine, L-365,260 and naloxone upon the elevated behaviour of rats in the plus-maze. * — $p<0.05$, compared to vehicle-treated rats.

In the single-treatment experiments we established clear antagonistic influence of CCK agonists and opioid agonist on the exploratory behaviour of rats. Thus, CCK agonists have anxiogenic-like, while morphine has anxiolytic-like effect. It has been shown that microinjections of morphine into the central nucleus of amygdala counteracted the reduction in social interaction caused by an unfamiliar test arena but not by the increased lighting conditions (File and Rodgers, 1979). Moreover, it has been suggested that opioid peptides may be released under non-painful stress (exposure to the open-field test) (Rodgers and Deacon, 1979). BOC-CCK-4 antagonised the anxiolytic-like effect of morphine, but CCK agonist possessed U-shaped action against morphine in the plus-maze (Figure 6). Namely, 10 µg/kg of BOC-CCK-4 reversed the anxiolytic-like effect of morphine, whereas the other doses of CCK agonists were ineffective. Antagonistic interaction between CCK and endogenous opioids has described in the regulation of pain processing (Dourish *et al.*, 1988). In our opinion such interplay is valuable also in the regulation of exploratory behaviour.

In addition to the anxiogenic-like effect of CCK-agonists we found the anxiogenic-like action enhancing effect of naloxone (Figure 5). BOC-CCK-4 and caerulein induce the anxiogenic-like action via the CCK_B receptors as we have shown above. The coadministration of naloxone (0.5 mg/kg) with the sub-effective doses of caerulein and BOC-CCK-4 induced a potent anxiogenic-like action in the elevated plus-maze (Figure 7). This finding is apparently in favour of an antagonistic interplay between CCK and endogenous opioid peptides in the regulation of anxiety and the importance of μ-opioid receptors in that. Neurochemically, a biphasic effect of morphine upon the release of CCK-LI has been described. Lower doses of morphine decreased the release of CCK, and this effect was preventable with μ-antagonist naloxone, whereas 100 times higher doses increased the release of CCK-LI by stimulating δ-opioid receptors (Benoliel *et al.*, 1994).

As morphine is an agonist for μ-opioid receptors and a low dose of naloxone is able to reverse the action of morphine, it is very likely that μ-receptors participate in the regulation of exploratory behaviour. Indeed, Motta and Brandao (1993) have shown that the systemic administration of morphine at low doses or its injection into the dorsal periaqueductal grey induced anxiolytic-like action in the elevated plus-maze. Microdialysis studies on freely moving rats confirmed the inhibition of CCK release in the frontal cortex by the stimulation of μ-opioid receptors (Benoliel *et al.*, 1998). Some studies have found the ability of CCK to antagonise the effects of morphine on cellular levels and influence the properties of μ-opioid receptors (Wang *et al.*, 1992; Liu *et al.*, 1995). In relation to our study, these findings confirmed the μ-opioid receptors as site for the described interaction.

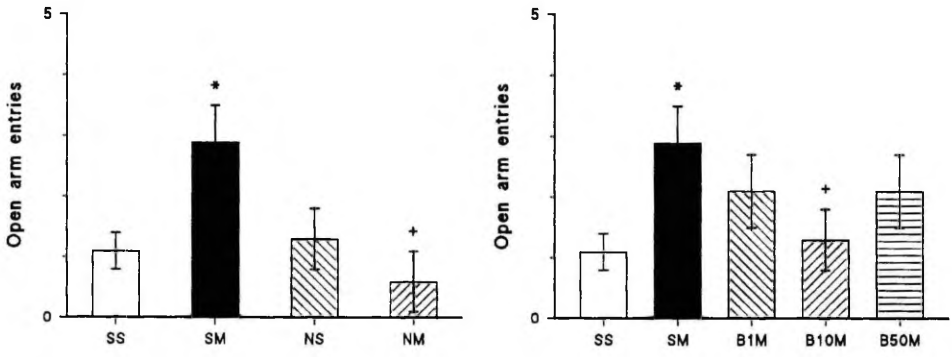

Figure 6. The effect of naloxone (0.5 mg/kg) and BOC-CCK-4 (1–50 μg/kg) on the anxiolytic-like action of morphine (1 mg/kg) in the elevated plus-maze. S — saline, M — morphine, N — naloxone, B1 — BOC-CCK-4 1 μg/kg, B10 — BOC-CCK-4 10 μg/kg and B50 — BOC-CCK-4 50 μg/kg. * — $p<0.05$ compared to saline-treated animals; + — $p<0.05$ compared to morphine-treated animals.

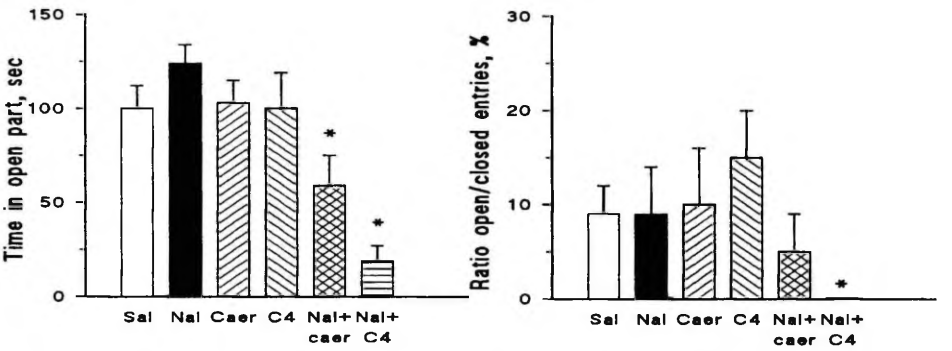

Figure 7. The action of sub-effective doses of caerulein (1 μg/kg) and BOC-CCK-4 (1 μg/kg) alone and in combination with naloxone (0.5 mg/kg) in the elevated plus-maze. Sal — saline, Nal — naloxone, Caer — caerulein, C4 — BOC-CCK-4. * — $p<0.05$ compared to saline-treated animals; + — $p<0.05$ compared to BOC-CCK-4-treated animals.

CCK_B receptor antagonist L-365,260 alone expressed the anxiolytic-like activity only at one dose (10 μg/kg), having the characteristic bell-shaped dose-response curve described by several authors earlier (Hughes *et al.*, 1990; Ravard *et al.*, 1990; Harro and Vasar, 1991). Also, the combination of L-365,260 with the sub-effective dose of morphine (0.5 mg/kg) induced an anxiolytic-like action. Again L-365,260 was effective only at one dose 100 μg/kg (Figure 8). It is worthy to note that this dose of L-365,260 did not change the exploratory activity of rats *per se* showing that the interaction between L-365,260 and morphine was specific. The potentiation of the action of morphine by the CCK_B receptor antagonist could also be explained in the light of the recent finding that stimulation of μ-receptors decreases the release of CCK in the frontal cortex of rat (Benoliel *et al.*, 1998). Moreover, this dose of L-365,260 (100 μg/kg), but not the lower doses, antagonised the anxiogenic-like action of caerulein, an agonist of CCK receptors (Figure 2) (Vasar *et al.*, 1997). Altogether these data are in favour of the antagonistic interaction between morphine and CCK in the regulation of exploratory behaviour.

Several studies indicate the antagonistic interaction between CCK and endopioid peptides in the regulation of pain sensitivity. However, as in the present work, the action of CCK was dependent on the pre-experimental stress of rats. Wiertelak *et al.* (1992) have shown that CCK antagonises morphine-induced antinociception only in the novel but not in the familiar environment. This finding was confirmed by Lavigne *et al.* (1992) demonstrating that CCK antagonists devazepide and L-365,260 enhanced morphine-induced analgesia

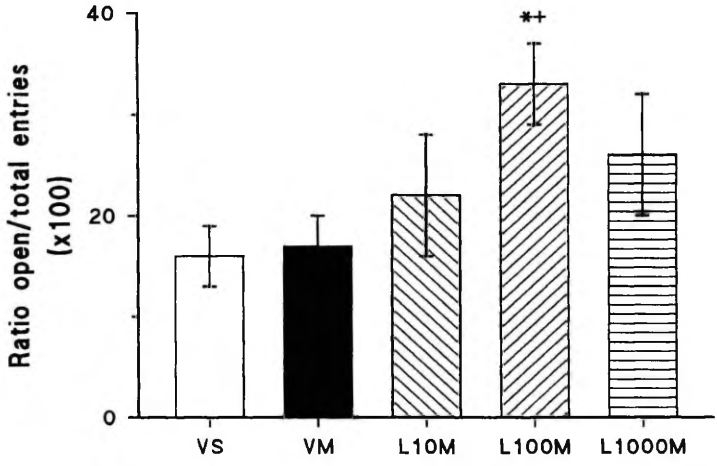

Figure 8. Interaction of L-365,260 (10–1000 μg/kg) with the sub-effective dose (0.5 mg/kg) of morphine. V — vehicle, S — saline, M — morphine, L10 — L-365,260 10 μg/kg, L100 — L-365,260 100 μg/kg, L1000 — L-365,260 1000 μg/kg. * — $p<0.05$ compared to saline-treated animals; + — $p<0.05$ compared to morphine-treated animals.

only in non-acclimatised rats exposed to the novel environment. Therefore, it is likely that even in the case of pain regulation the interplay between CCK and endogenous opioid peptides may be actually dependent on the level of anxiety. This suggestion is supported by the recent study of Benedetti *et al.* (1997) who found that nocebo-induced hyperalgesia was reversed dose-dependently by CCK antagonist proglumide but not by naloxone. Since the nocebo procedure represents an anxiogenic stimulus, it is likely that nocebo hyperalgesia may be due to a CCK-dependent increase of anxiety.

In conclusion, CCK and endopioid mechanisms have an opposite role in the behavioural response of animals towards the novel environment. The simultaneous stimulation of CCK_B receptors and the blockade of μ-opioid receptors apparently increase anxiety in rats. On the other hand, morphine, an agonist for μ-opioid receptors, potently increased the exploratory behaviour of rats in the elevated plus-maze, and that effect was completely antagonised by stimulation of CCK_B receptors with BOC-CCK-4. Moreover, the combination of the sub-effective dose of morphine with CCK_B receptor antagonist L-365,260 potentiated the anxiolytic-like action of μ-receptor agonist. According to these series of experiments, the antagonistic interplay between CCK_B and μ-opioid receptors in the regulation of exploratory behaviour is evident.

5.4. Serotonin release induces anti-exploratory action and increases the activity of CCK-ergic transmission (paper III)

The administration of SSRI paroxetine (0.5, 2, 4, 8 mg/kg) induced a dose-dependent reduction of exploratory activity of rats in the motility test (Figure 9). Also, the same doses of paroxetine (4 and 8 mg/kg) elevated the density of CCK binding sites in the frontal cortex but not in the hippocampus (Figure 10). Paroxetine itself was unable to modify the binding of CCK (data not shown). In the elevated plus-maze only 8 mg/kg of paroxetine had a clear anxiogenic-like action. Moreover, CCK_B antagonist LY 288,513 dose-dependently reversed the anti-exploratory activity of 2 mg/kg paroxetine (Figure 11) but not the effect of higher dose of paroxetine (8 mg/kg). Diazepam at a high dose (2.5 mg/kg) suppressed the exploratory behaviour of rats in the motility test. The combination of diazepam (0.5, 1, 2.5 mg/kg) with paroxetine (2 mg/kg) did not modify the anxiogenic-like effect of SSRI.

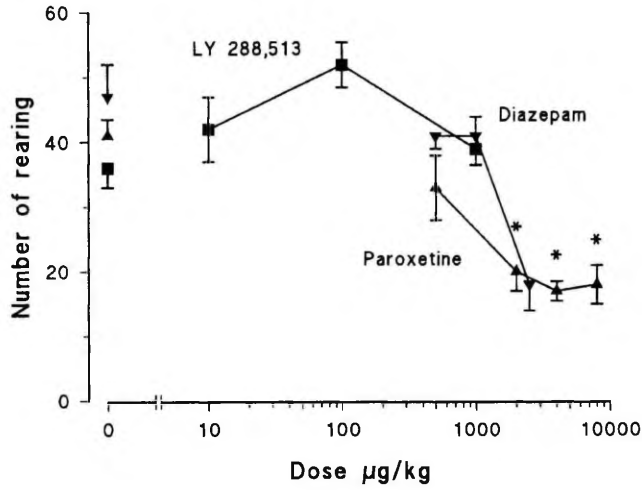

Figure 9. The effect of paroxetine (0.5–8 mg/kg), diazepam (0.5–2.5 mg/kg), and LY288, 513 (0.01–1 mg/kg) on the exploratory behaviour of rats in the motility box. * — $p<0.05$ compared to saline (paroxetine) or vehicle-treated (diazepam, LY288,513) rats.

The administration of paroxetine, a selective inhibitor of 5-HT reuptake, induced a significant suppression of the exploratory activity of rats in the motility test. Already 2 mg/kg of paroxetine inhibited the frequency of rearing and time spent in rearing. The frequency of rearing and time spent in rearing are the most sensitive parameters in revealing the anti-exploratory action of paroxetine in the motility test. Moreover, paroxetine was apparently less potent in reducing the exploratory activity of rats in the elevated plus-maze. Only

8 mg/kg of paroxetine decreased the exploratory behaviour of animals. The decreased number of closed arm entries reflecting the suppression of locomotor activity accompanied the anti-exploratory action of paroxetine in the plus-maze. Therefore, it is very likely that the decreased locomotor activity may mask the "pure" anti-exploratory effect of paroxetine in the elevated plus-maze. Similar results are described in a recent paper, where only 0.0027 μmol/kg (1 mg/kg) of paroxetine decreased exploratory behaviour and higher doses tended to suppress the locomotor activity of animals (Sanchez and Meier, 1997). The existing experimental data support the role of 5-HT in the regulation of anxiety. Handley and McBlane (1993) have shown that the administration of fluoxetine, the 5-HT reuptake inhibitor, induces the anxiogenic-like action in the rat elevated plus-maze. The administration of paroxetine produced an anxiogenic-like profile in the two-compartment exploration test of rat (Sanchez and Meier, 1997). The exposure of rats to the aversive environment clearly increases the release of 5-HT in the frontal cortex and hippocampus (File *et al.*, 1993; Rex *et al.*, 1994). However, as diazepam was not able to reverse the effect of paroxetine, the present behavioural study suggests that the anti-exploratory action of paroxetine is related rather to the decrease of exploratory drive than to the increase in anxiety.

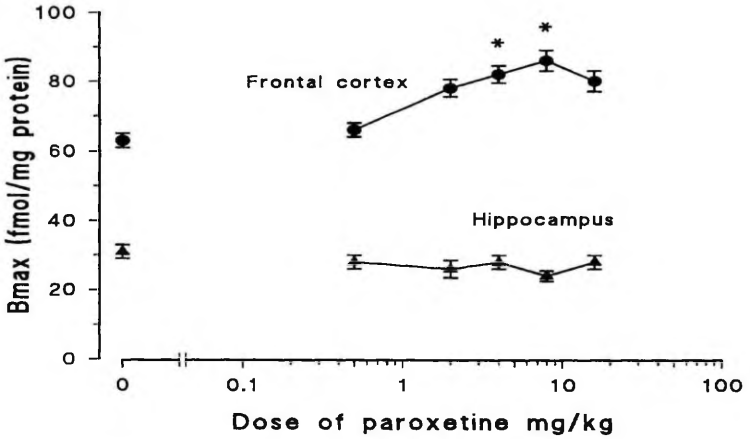

Figure 10. The effect of paroxetine (0.5–16 mg/kg) on the parameters of CCK binding in the frontal cortex and hippocampus. * — $p<0.05$ compared to saline-treated rats.

Rosén *et al.* (1995) have shown that 5-HT reuptake inhibitor alaproclate increases the levels of CCK in the cingulate cortex and periaqueductal grey. The direct application of 5-HT to the neurones of the cerebral cortex and nucleus accumbens evoked the release of CCK (Raiteri *et al.*, 1993). In the present study paroxetine, the compound with the highest 5-HT uptake inhibitory potency *in vivo*, elevated the number of CCK binding sites in the frontal

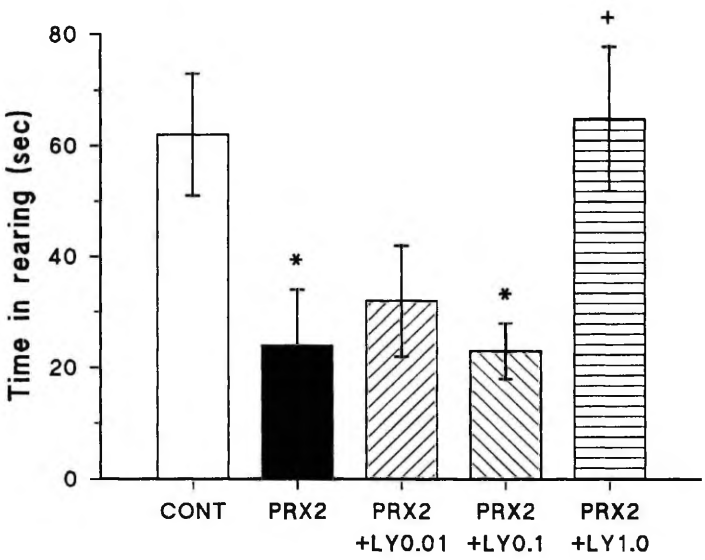

Figure 11. The effect of LY288,513 (0.01–1 mg/kg) on the anti-exploratory action of paroxetine (2 mg/kg). CONT — saline + vehicle, PRX2 — paroxetine 2 mg/kg + vehicle, LY0.01 — LY288,513 0.01 mg/kg, LY0.1 — LY288,513 0.1 mg/kg, LY1.0 — LY288,513 1.0 mg/kg. * — $p<0.05$ compared to saline + vehicle-treated rats, + — $p<0.05$ compared to paroxetine-treated rats.

cortex but not in the hippocampus. The stressful manipulations in rats are shown to increase the density of CCK receptors and mRNA levels of preproCCK in the frontal cortex (Pratt and Brett, 1995; Harro *et al.*, 1995a). The social isolation of rats for seven days induced an anxiogenic-like action and elevated the number of CCK receptors in the frontal cortex but not in the hippocampus (Vasar *et al.*, 1993). Therefore, it is not surprising that the pre-treatment of rats with the CCK_B receptor antagonist LY 288,513 dose-dependently reversed the anti-exploratory action of paroxetine (2 mg/kg). This is in line with the study of Matto et al. (1996) showing the ability of the CCK_B receptor antagonist L-365,260 to antagonise the anti-exploratory effect of the 5-HT reuptake inhibitor citalopram in the elevated plus-maze. These data are in favour of the hypothesis that the administration of 5-HT reuptake inhibitors increases CCK-mediated neurotransmission in the brain. The antagonism of LY 288,513 against paroxetine seems to be specific since the CCK_B receptor antagonist does not increase the exploratory activity *per se*. Moreover, it has been shown that SSRIs are able to antagonise the anxiolytic-like effect of CCK_B antagonist, CI-988 (Bickerdike *et al.*, 1994). Therefore, synergistic interaction between 5-HT and CCK in the regulation of exploratory behaviour is evident. Nevertheless, LY 288,513 did not block the effect of the higher dose of paroxetine (8 mg/kg) showing a difference in the action of two doses of 5-HT reuptake inhibitor. This discrepancy could explain that paroxet-

ine at higher doses induces a more pronounced increase in the concentration of 5-HT in the synaptic cleft masking a possible interaction with CCK. Unlike the present study, Harro et al. (1997) did not find any changes in the density of CCK receptors and in the content of CCK-related peptides after a long-term treatment with various antidepressant drugs, including 5-HT reuptake inhibitors. However, they analysed the action of long-term administration of antidepressant drugs, whereas the effect of acute treatment was not examined.

In conclusion, the selective 5-HT reuptake inhibitor paroxetine induced an anti-exploratory effect in the motility boxes. However, the above-described behavioural studies do not reflect the increase in anxiety after the acute treatment with paroxetine. This statement is supported by the findings that paroxetine did not cause the anxiogenic-like action in the elevated plus-maze and diazepam, a potent anxiolytic drug, did not antagonise the anti-exploratory action of paroxetine in the motility test. Therefore, it is likely that paroxetine may reduce the exploratory drive in rats and makes animals more susceptible to the novel environment. This effect of paroxetine seems to be mediated via the increase in CCK-ergic neurotransmission.

6. GENERAL DISCUSSION

The present study provides further evidence that the exploratory behaviour of rats is influenced by the different environmental stimuli. Moreover, we confirmed the synergistic interaction between CCK and 5-HT and the antagonistic interplay between CCK-endopioid mechanisms in the regulation of anxiety. It has been shown that CCK-ergic neurotransmission is activated in the cortical and hippocampal areas during anxiogenic events (Rattray *et al.*, 1993). Moreover, an increased release of 5-HT in the frontal cortex is a clear neurochemical change during the exposure to the aversive environment (Rex and Fink, 1998). The animals who are more susceptible to the aversive environmental stimuli and who respond with anxiogenic-like behaviour to novel situations have activated CCK- ergic and 5-HT-ergic neural circuits. This feature seems to decrease their ability for adaptation to environmental changes. Interestingly, in panic patients several biochemical changes reflecting the alterations in the CCK neurotransmission have been described (Koszycki, 1995). Additionally, patients with panic attacks have been shown to be more sensitive to panicogenic CCK-4 (Bradwejn *et al.*, 1991). Moreover, in humans the vulnerability to unpleasant situations or anxiety sensitivity has been described as a prominent characteristic of panic patients (Koszycki *et al.*, 1993). On the other hand, no straightforward correlation between anxiety sensitivity scale and response to the CCK-4 has been established as yet (Koszycki *et al.*, 1996). However, results from studies with animals are supportive to the hypothesis that vulnerability to the anxiety disorders is a consequence of the activation of CCK neurotransmission.

According to the hypothesis of Wiertelak *et al.* (1992), the environmental stimuli that signal the occurrence of aversive or dangerous events activate endogenous opioid analgesia systems. The signals for safety (the non-occurrence of aversive events) produce the opposite and inhibit environmentally produced analgesia. Wiertelak *et al.* (1992) believe that CCK is a mediator of that kind of safety signals. However, the present study is not in favour of that statement since CCK apparently increases anxiety in the novel environment and the anxiogenic-like action of CCK was potentiated by opioid antagonist naloxone. Moreover, CCK agonist reversed the anxiolytic-like activity of morphine. Therefore, CCK-ergic pathways would rather signal the unsafe and aversive aspects of environment.

Seasonal fluctuations in the activity of 5-HT circuits are well known and have been described by several authors (Nagayama and Lu, 1998). Such variation is believed to be the reason for the seasonality of affective disorders (Maes *et al.*, 1993). This kind of seasonality exists also in the patients suffering from the panic disorder, but the biochemical correlates causally linked to the

circannual variations are not known as yet. We were able to confirm the annual rhythm in the responsiveness to the aversive environment in the animals kept under constant temperature and the light-dark period. Accordingly, decreased exploratory behaviour of animals in summer correlates with increased activity in the CCK-ergic and 5-HT- ergic neurotransmission in the cortical regions. This finding strongly suggests that circannual rhythm is endogenous based on the changes in the "internal clock". As concentration of ACTH has been described to follow a similar fluctuation (Amirat and Brudieux, 1993), we are tempting to speculate the connection between CCK and HPA axis in the circannual variations. In our opinion, the natural pressure activates neural "fear" circuits, which is an important adaptive event in wildlife where fear is a life-saving response to the threatening dangers. Therefore, in summer laboratory animals become more susceptible to the unpleasant environmental stimuli, and their exploratory activity or "curiosity" decreases. This kind of defence reaction is highly conserved in evolution and is indispensable for survival in the continuously changing world. Over-reactivity in that system leads to various psychopathological diseases, named anxiety disorders.

7. CONCLUSIONS

1. Pre-experimental stress and isolation of animals after injection makes them sensitive to the anxiogenic-like effect of caerulein, an agonist of CCK receptors. Decreased exploratory behaviour of rats in summer is related to the increased CCK-ergic and 5-HT-ergic activity in the brain areas that are most involved in the generation of response to the unpleasant stimulus.

2. Differences in the exploratory behaviour are probably caused by the individual differences in the CCK-ergic and 5-HT-ergic neurotransmissions. Increased activity in the CCK-ergic neural circuits in the hippocampus seems to make animals more susceptible to the exposure to the unpleasant environment. This finding supports the opinion that the "endogenous tone" in the interaction of CCK-ergic and 5-HT-ergic neural circuits modifies the "basal" exploratory activity of animals. Animals having higher "endogenous tone" in those circuits express marked avoidance behaviour in the elevated plus-maze.

3. Taken together, in planning of the ethological experiments to study the exploratory behaviour, the design and conditions of test should be cautiously analysed to reduce the variability of the results. Most probably, according to our present work, inconsistencies in the studies with the CCK-ergic compounds are caused by significant seasonal and individual fluctuations in the exploratory behaviour.

4. CCK and endopioid mechanisms have an opposite influence in the regulation of exploratory behaviour. Activation of CCK_B receptors decreases, whereas the stimulation of μ-opioid receptors increases the exploratory behaviour of animals. Most likely, activation of endogenous opioid mechanisms signals that the surrounding environment is safe and not life-threatening.

5. Interaction between 5-HT and CCK is synergistic in the generation of anxiogenic-like behaviour. Activation of 5-HT neurotransmission decreases the exploratory drive and increases CCK-ergic transmission. An increased release of 5-HT induced by paroxetine forces animals to avoid the unfamiliar and potentially dangerous environment. Thus, CCK and 5-HT are signals that environment is unsafe and dangerous and should be avoided.

8. REFERENCES

Abelson JL, Nesse RM. Pentagastrin infusions in patients with panic disorder. I. Symptoms and cardiovascular responses . Biol Psychiatry 1994; 36: 73–83.

Abelson JL, Nesse RM, Vinik AI. Pentagastrin infusions in patients with panic disorder. II. Neuroendocrinology. Biol Psychiatry 1994; 36: 84–96.

Amirat Z, Brudieux R. Seasonal changes in in vivo cortisol release to ACTH and plasma and pituitary concentrations of ACTH in a desert rodent, the sand rat (Psammomys obesus). Comp Biochem Physiol A Physiol 1993; 104: 29–34.

Andrews N, Barnes NM, Steward LJ, West KE, Cunningham J, Wu PY, Zangrossi HJ, File SE. A comparison of rat brain amino acid and monoamine content in diazepam withdrawal and after exposure to a phobic stimulus. Br J Pharmacol 1993; 109: 171–174.

Andrews N, File SE. Increased 5-HT release mediates the anxiogenic response during benzodiazepine withdrawal: a review of supporting neurochemical and behavioural evidence. Psychopharmacology (Berl) 1993; 112: 21–25.

Andrews N, File SE, Fernandes C, Gonzalez LE, Barnes NM. Evidence that the median raphe nucleus-dorsal hippocampal pathway mediates diazepam withdrawal-induced anxiety. Psychopharmacology (Berl) 1997; 130: 228–234.

Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 1999; 160: 1–12.

Baber NS, Dourish CT, Hill DR. The role of CCK caerulein, and CCK antagonists in nociception. Pain 1989; 39: 307–328.

Benedetti F. Cholecystokinin type A and type B receptors and their modulation of opioid analgesia. News Physiol Sci 1997; 12: 263–268.

Benedetti F, Amanzio M, Casadio C, Oliaro A, Maggi G. Blockade of nocebo hyperalgesia by the cholecystokinin antagonist proglumide. Pain 1997; 71: 135–140.

Benoliel JJ, Becker C, Mauborgne A, Bourgoin S, Hamon M, Cesselin F. Interactions between central opioidergic and cholecystokininergic systems in rats: possible significance for the development of of opioid tolerance. Bull Acad Natl Med 1998; 182: 311–324.

Benoliel JJ, Collin E, Mauborgne A, Bourgoin S, Legrand JC, Hamon M, Cesselin F. Mu and delta opioid receptors mediate opposite modulations by morphine of the spinal release of cholecystokinin-like material. Brain Res 1994; 653: 81–91.

Bickerdike MJ, Marsden CA, Dourish CT, Fletcher A. The influence of 5-hydroxytryptamine re-uptake blockade on CCK receptor antagonist effects in the rat elevated zero-maze. Eur J Pharmacol 1994; 271: 403–411.

Biro E, Sarnyai Z, Penke B, Szabo G, Telegdy G. Role of endogenous corticotropin-releasing factor in mediation of neuroendocrine and behavioral responses to cholecystokinin octapeptide sulfate ester in rats. Neuroendocrinology 1993; 57: 340–345.

Boden P, Hill RG. Effects of cholecystokinin and related peptides on neuronal activity in the ventromedial nucleus of the rat hypothalamus. Br J Pharmacol 1988; 94: 246–252.

Boden P, Woodruff GN. Ionic mechanisms underlying cholecystokinin action in rat brain. Ann NY Acad Sci 1994; 713: 129–137.

Boden PR, Woodruff GN, Pinnock RD. Pharmacology of a cholecystokinin receptor on 5-hydroxytryptamine neurones in the dorsal raphe of the rat brain. Br J Pharmacol 1991; 102: 635–638.

Boyer W. Serotonin uptake inhibitors are superior to imipramine and alprazolam in alleviaviating panic attecks: a meta-analysis. Int Clin Psychopharmacol 1993; 10: 45–49.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248–254.

Bradwejn J, de Montigny C. Benzodiazepines antagonize cholecystokinin-induced activation of rat hippocampal neurones. Nature 1984; 312: 363–364.

Bradwejn J, de Montigny C. Effects of PK 8165, a partial benzodiazepine receptor agonist, on cholecystokinin-induced activation of hippocampal pyramidal neurons: a microiontophoretic study in the rat. Eur J Pharmacol 1985; 112: 415–418.

Bradwejn J, Koszycki D, Couetoux du Tertre A, Paradis M, Bourin M. Effects of flumazenil on cholecystokinin-tetrapeptide-induced panic symptoms in healthy volunteers. Psychopharmacology (Berl) 1994; 114: 257–261.

Bradwejn J, Koszycki D, Couetoux du Tertre A, van M, den Boer J, Westenberg H. The panicogenic effects of cholecystokinin-tetrapeptide are antagonized by L-365,260, a central cholecystokinin receptor antagonist, in patients with panic disorder. Arch Gen Psychiatry 1994; 51: 486–493.

Bradwejn J, Koszycki D, Meterissian G. Cholecystokinin-tetrapeptide induces panic attacks in patients with panic disorder. Can J Psychiatry 1990; 35: 83–85.

Bradwejn J, Koszycki D, Shriqui C. Enhanced sensitivity to cholecystokinin tetrapeptide in panic disorder. Clinical and behavioral findings. Arch Gen Psychiatry 1991; 48: 603–610.

Cameron OG. Frequency of panic disorder in summer. Am J Psychiatry 1989; 146: 123.

Chang RS, Lotti VJ. Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. Proc Natl Acad Sci USA 1986; 83: 4923–4926.

Chang RS, Lotti VJ, Chen TB, Kunkel KA. Characterization of the binding of [^{3}H]-(+/-)-L-364,718: a new potent, nonpeptide cholecystokinin antagonist radioligand selective for peripheral receptors. Mol Pharmacol 1986; 30: 212–217.

Charney DS, Woods SW, Goodman WK, Heninger GR. Serotonin function in anxiety: II. Effects of the serotonin agonist mCPP in panic disorder patients and healthy subjects. Psychopharmacology (Berl) 1987; 92: 14–24.

Chopin P, Briley M. The benzodiazepine antagonist flumazenil blocks the effects of CCK receptor agonists and antagonists in the elevated plus-maze. Psychopharmacology (Berl) 1993; 110: 409–414.

Coplan JD, Lydiard RB. Brain circuits in panic disorder. Biol Psychiatry 1998; 44: 1264–1276.

Costall B, Domeney AM, Hughes J, Kelly ME, Naylor RJ, Woodruff GN. Anxiolytic effects of CCK-B antagonists. Neuropeptides 1991; 19 Suppl: 65–73.

Crawley JN, Corwin RL. Biological actions of cholecystokinin. Peptides 1994; 15: 731–755.

Dauge V, Dor A, Feger J, Roques BP. The behavioral effects of CCK8 injected into the medial nucleus accumbens are dependent on the motivational state of the rat. Eur J Pharmacol 1989; 163: 25–32.

Dawson GR, Rupniak NM, Iversen SD, Curnow R, Tye S, Stanhope KJ, Tricklebank MD. Lack of effect of CCK_B receptor antagonists in ethological and conditioned animal screens for anxiolytic drugs. Psychopharmacology (Berl) 1995; 121: 109–117.

Dawson GR, Rupniak NMJ, Iversen SD, Tricklebank MD. Lack of effect of CCK_B receptor antagonists in animal screens for anxiolytic drugs. Journal of Psychopharmacology 1994; 8: A47

de Montigny C. Cholecystokinin tetrapeptide induces panic-like attacks in healthy-volunteers — preliminary findings. Arch Gen Psychiatry 1989; 46: 511–517.

de Weerth A, Jonas L, Schade R, Schöneberg T, Wolf G, Pace A, Kirchhoff F, Schulz M, Heinig T, Greten H, von Schrenk T. Gastrin/cholecystokinin type B receptors in the kidney: molecular, pharmacological, functional characterization, and localization. Eur J Clin Invest 1998; 28: 592–601.

Della-Fera MA, Baile CA. Cholecystokinin octapeptide: continuous picomole injections into the cerebral ventricles of sheep suppress feeding. Science 1979; 206: 471–473.

den Boer JA, Westenberg HGM. Involvement of serotonin receptor subtypes in panic disorder. In Westenberg HGM, den Boer JA, Murphy DL, (eds). Advances in the Neurobiology of Anxiety Disorders. Chichester, New York, John Wiley & Sons Ltd, 1996, pp 139–172.

Dockray GJ, Taylor IL. Heptadecapeptide gastrin: measurement in blood by specific radioimmunoassay. Gastroenterology 1976; 71: 971–977.

Dourish CT. The role of CCK_A and CCK_B receptors in mediating the inhibitory effect of CCK on opioid analgesia. In Dourish CT, Cooper SJ, Iversen SD, Iversen LL, (eds). Multiple Cholecystokinin Receptors in the CNS. New York, Oxford University Press, 1992, pp 456–479.

Dourish CT, Hawley D, Iversen SD. Enhancement of morphine analgesia and prevention of morphine tolerance in the rat by the cholecystokinin antagonist L-364,718. Eur J Pharmacol 1988; 147: 469–472.

Dourish CT, O'Neill MF, Coughlan J, Kitchener SJ, Hawley D, Iversen SD. The selective CCK-B receptor antagonist L-365,260 enhances morphine analgesia and prevents morphine tolerance in the rat. Eur J Pharmacol 1990; 176: 35–44.

Durieux C, Pelaprat D, Charpentier B, Morgat JL, Roques BP. Characterization of [^{3}H] CCK4 binding sites in mouse and rat brain. Neuropeptides 1988; 12: 141–148.

Egrise D, Rubinstein M, Schoutens A, Cantraine F, Mendlewicz J. Seasonal variation of platelet serotonin uptake and [^{3}H]-imipramine binding in normal and depressed subjects. Biol Psychiatry 1986; 21: 283–292.

Emson PC, Rehfeld JF, Rossor MN. Distribution of cholecystokinin-like peptides in the human-brain. J Neurochem 1982; 38: 1177–1179.

Faris PL. Opiate antagonistic function of cholecystokinin in analgesia and energy balance systems. Ann N Y Acad Sci 1985b; 448:437–47: 437–447.

Faris PL. Role of cholecystokinin in the control of nociception and food intake. Prog Clin Biol Res 1985a; 192: 159–166.

Faris PL, Komisaruk BR, Watkins LR, Mayer DJ. Evidence for the neuropeptide cholecystokinin as an antagonist of opiate analgesia. Science 1983; 219: 310–312.

File SE, Rodgers RJ. Partial anxiolytic action of morphine sulphate following micro-injection into the central nucleus of the amygdala in rats. Pharmacol Biochem Behav 1979; 11: 313–318.

File SE, Zangrossi HJ, Andrews N. Novel environment and cat odor change GABA and 5-HT release and uptake in the rat. Pharmacol Biochem Behav 1993; 45: 931–934.

File SE, Zangrossi HJ, Andrews N. Social interaction and elevated plus-maze tests: changes in release and uptake of 5-HT and GABA. Neuropharmacology 1993; 32: 217–221.

File SE, Zangrossi HJ, Sanders FL, Mabbutt PS. Dissociation between behavioral and corticosterone responses on repeated exposures to cat odor. Physiol Behav 1993; 54: 1109–1111.

File SE, Zangrossi HJ, Sanders FL, Mabbutt PS. Raised corticosterone in the rat after exposure to the elevated plus-maze. Psychopharmacology (Berl) 1994; 113: 543–546.

Fossey MD, Lydiard RB, Ballenger JC, Laraia MT, Bissette G, Nemeroff CB. Cerebrospinal fluid thyrotropin-releasing hormone concentrations in patients with anxiety disorders. J Neuropsychiatry Clin Neurosci 1993; 5: 335–337.

Gall C, Lauterborn J, Burks D, Seroogy K. Co-localization of enkephalin and cholecystokinin in discrete areas of rat brain. Brain Res 1987; 403: 403–408.

Gaudreau P, Quirion R, St-Pierre S, Pert CB. Characterization and visualization of cholecystokinin receptors in rat brain using [^{3}H]pentagastrin. Peptides 1983; 4: 755–762.

Glavin GB. Vulnerability to stress ulcerogenesis in rats differing in anxiety: a dopamine correlate. J Physiol Paris 1993; 87: 239–243.

Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain-I. the disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain. J Neurochem 1966; 13: 655–669.

Handley SL, McBlane JW. 5-HT drugs in animal models of anxiety. Psychopharmacology (Berl) 1993; 112: 13–20.

Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'- motivated behaviour. Naunyn-Schmiedebergs Arch Pharmacol 1984; 327: 1–5.

Hargreaves RJ, Lin JH. Blood-brain transfer of the CCK antagonists L-365,260 and devazepide. In Dourish CT, Cooper SJ, Iversen SD, Iversen LL, (eds). Multiple Cholecystokinin Receptors in the CNS. New York, Oxford University Press, 1992, pp 107–114.

Harro J, Kiivet RA, Lang A, Vasar E. Rats with anxious or non-anxious type of exploratory behaviour differ in their brain CCK-8 and benzodiazepine receptor characteristics. Behav Brain Res 1990; 39: 63–71.

Harro J, Lang A, Vasar E. Long-term diazepam treatment produces changes in cholecystokinin receptor binding in rat brain. Eur J Pharmacol 1990; 180: 77–83.

Harro J, Löfberg C, Pähkla R, Matto V, Rägo L, Oreland L, Allikmets L. Different molecular forms of cholecystokinin and CCKB receptor binding in the rat brain after chronic antidepressant treatment. Naunyn-Schmiedebergs Arch Pharmacol 1997; 355: 57–63.

Harro J, Põld M, Vasar E. Anxiogenic-like action of caerulein, a CCK-8 receptor agonist, in the mouse: influence of acute and subchronic diazepam treatment. Naunyn-Schmiedebergs Arch Pharmacol 1990; 341: 62–67.

Harro J, Vasar E. Cholecystokinin-induced anxiety: how is it reflected in studies on exploratory behaviour? Neurosci Biobehav Rev 1991; 15: 473–477.

Harro J, Vasar E. Evidence that CCK_B receptors mediate the regulation of exploratory behaviour in the rat. Eur J Pharmacol 1991; 193: 379–381.

Harro J, Vasar E, Koszycki D, Bradwejn J. Cholecystokinin in panic and anxiety disorders. In Panksepp J, (ed). Advances in Biological Psychiatry. Greenwich, CT, JAI Press Inc, 1995a, pp 235–262.

Harro J, Vasar E, Oreland L, Männistö PT. Animal studies on CCK and anxiety. In Bradwejn J, Vasar E, (eds). Cholecystokinin and Anxiety: from Neuron to Behavior. Austin, R.G. Landes Company, 1995b, pp 57–72.

Hendrie CA, Neill JC, Shepherd JK, Dourish CT. The effects of CCK_A and CCK_B antagonists on activity in the black/white exploration model of anxiety in mice. Physiol Behav 1993b; 54: 689–693.

Hendrie CA, Shepherd JK, Rodgers RJ. Differential effects of the CCK antagonist, MK-329, on analgesia induced by morphine, social conflict (opioid) and defeat experience (non-opioid) in male mice. Neuropharmacology 1989a; 28: 1025–1032.

Hendry SHC, Jones EG, Defelipe J, Schmechel D, Brandon C, Emson PC. Neuropeptide-containing neurons of the cerebral cortex are also GABAergic. Proc Natl Acad Sci USA 1984; 81: 6526

Hill DR, Singh L, Boden P, Pinnock RD, Woodruff GN, Hughes J. Detection of CCK receptor subtypes in mammalian brain using highly selective non-peptide antagonists. In Dourish CT, Cooper SJ, Iversen SD, Iversen LL, (eds). Multiple Cholecystokinin Receptors in the CNS. New York, Oxford University Press, 1992, pp 57–76.

Hill DR, Woodruff GN. Differentiation of central cholecystokinin receptor binding sites using the non-peptide antagonists MK-329 and L-365,260. Brain Res 1990; 526: 276–283.

Honda T, Wada E, Battey JF, Wank SA. Differential gene expression of CCK_A and CCK_B receptors in the rat brain. Mol Cell Neurosci 1993; 4: 143–154.

Hughes J, Boden P, Costall B, Domeney A, Kelly E, Horwell DC, Hunter JC, Pinnock RD, Woodruff GN. Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. Proc Natl Acad Sci USA 1990; 87: 6728–6732.

Hökfelt T, Skirboll L, Rehfeld JF, Goldstein M, Markey K, Dann O. A sub-population of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide — evidence from immunohistochemistry combined with retrograde tracing. Neuroscience 1980; 5: 2093

Idanpaan-Heikkila JJ, Guilbaud G, Kayser V. Prevention of tolerance to the antinociceptive effects of systemic morphine by a selective cholecystokinin-B receptor antagonist in a rat model of peripheral neuropathy. J Pharmacol Exp Ther 1997; 282: 1366–1372.

Innis RB, Snyder SH. Cholecystokinin receptor binding in brain and pancreas: regulation of pancreatic binding by cyclic and acyclic guanine nucleotides. Eur J Pharmacol 1980; 65: 123

Ishibashi S, Oomura Y, Okajima T, Shibata S. Cholecystokinin, motilin and secretin effects on the central nervous system. Physiol Behav 1979; 23: 401–403.

Itoh S, Katsuura G, Yoshikawa K, Rehfeld JF. Potentiation of beta-endorphin effects by cholecystokinin antiserum in rats. Can J Physiol Pharmacol 1985; 63: 81–83.

Jagerschmidt A, Popovici T, O'Donohue M, Roques BP. Identification and characterization of various cholecystokinin B receptor mRNA forms in rat brain tissue and partial determination of the cholecystokinin B receptor gene structure. J Neurochem 1994; 63: 1199–1206.

Jensen RT, Lemp GF, Gardner JD. Interactions of COOH-terminal fragments of cholecystokinin with receptors on dispersed acini from guinea pig pancreas. J Biol Chem 1982; 257: 5554–5559.

Johnson NJ, Rodgers RJ. Ethological analysis of cholecystokinin (CCK_A and CCK_B) receptor ligands in the elevated plus-maze test of anxiety in mice. Psychopharmacology (Berl) 1996; 124: 355–364.

Kamilaris TC, Johnson EO, Calogero AE, Kalogeras KT, Bernardini R, Chrousos GP, Gold PW. Cholecystokinin-octapeptide stimulates hypothalamic-pituitary-adrenal function in rats: role of corticotropin-releasing hormone. Endocrinology 1992; 130: 1764–1774.

Kayser V, Idanpaan-Hekkila JJ, Christensen D, Guilbaud G. The selective cholecystokinin-B receptor antagonist L-365,260 diminishes the expression of naloxone-induced morphine withdrawal symptoms in normal and neuropathic rats. Life Sci 1998; 62: 947–952.

Knight M, Tamminga CA, Steardo L, Beck ME, Barone P, Chase TN. Cholecystokinin-octapeptide fragments: binding to brain cholecystokinin receptors. Eur J Pharmacol 1984; 105: 49–55.

Koszycki D. Psychological factors and response to cholecystokinin. In Bradwejn J, Vasar E, (eds). Cholecystokinin and Anxiety: from Neuron to Behavior. Austin, R.G. Landes Company, 1995, pp 87–99.

Koszycki D, Cox BJ, Bradwejn J. Anxiety sensitivity and response to cholecystokinin tetrapeptide in healthy volunteers. Am J Psychiatry 1993; 150: 1881–1883.

Koszycki D, Zacharko RM, Bradwejn J. Influence of personality on behavioral response to cholecystokinin-tetrapeptide in patients with panic disorder. Psychiatry Res 1996; 62: 131–138.

Koszycki D, Zacharko RM, Le Melledo JM, Bradwejn J. Behavioral, cardiovascular, and neuroendocrine profiles following CCK-4 challenge in healthy volunteers: a comparison of panickers and nonpanickers. Depress Anxiety 1998; 8: 1–7.

Kramer MS, Cutler NR, Ballenger JC, Patterson WM, Mendels J, Chenault A, Shrivastava R, Matzura-Wolfe D, Lines C, Reines S. A placebo-controlled trial of L-365,260, a CCK_B antagonist, in panic disorder. Biol Psychiatry 1995; 37: 462–466.

Ladurelle N, Roques BP, Dauge V. The transfer of rats from a familiar to a novel environment prolongs the increase of extracellular dopamine efflux induced by CCK8 in the posterior nucleus accumbens. J Neurosci 1995; 15: 3118–3127.

Lang A, Harro J, Soosaar A, Kõks S, Volke V, Oreland L, Bourin M, Vasar E, Bradwejn J, Männistö PT. Role of N-methyl-D-aspartic acid and cholecystokinin receptors in apomorphine-induced aggressive behaviour in rats. Naunyn-Schmiedebergs Arch Pharmacol 1995; 351: 363–370.

Larsson LI, Rehfeld JF. Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous system. Brain Res 1979; 165: 201–218.

Lavigne GJ, Millington WR, Mueller GP. The CCK-A and CCK-B receptor antagonists, devazepide and L-365,260, enhance morphine antinociception only in non-acclimated rats exposed to a novel environment. Neuropeptides 1992; 21: 119–129.

Leatherbarrow RJ. Enzfitter, a non-linear regression data analysis program for the IBM PC 1987: Amsterdam, Elsevier Science Publishers.

Lee YM, Beinborn M, McBride EW, Lu M, Kolakowski LF, Kopin AS. The human brain cholecystokinin-B/gastrin receptor. Cloning and characterization. J Biol Chem 1993; 268: 8164–8169.

Lelliott P, Marks I, McNamee G, Tobena A. Onset of panic disorder with agoraphobia. Toward an integrated model [see comments]. Arch Gen Psychiatry 1989; 46: 1000–1004.

Lepine JP, Chignon JM, Teherani M. Suicidal behavior and onset of panic disorder [letter; comment]. Arch Gen Psychiatry 1991; 48: 668–669.

Lindefors N, Brené S, Kopp J, Lindén A, Brodin E, Sedvall G, Persson H. Distribution of cholecystokinin mRNA and peptides in the human brain. Neuroscience 1991; 42: 813–821.

Lindefors N, Lindén A, Brené S, Sedvall G, Persson H. CCK peptides and mRNA in the human brain. Prog Neurobiol 1993; 40: 671–690.

Lines C, Challenor J, Traub M. Cholecystokinin and anxiety in normal volunteers: an investigation of the anxiogenic properties of pentagastrin and reversal by the cholecystokinin receptor subtype B antagonist L-365,260. Br J Clin Pharmacol 1995; 39: 235–242.

Liu H, Chandler S, Beitz AJ, Shipley MT, Behbehani MM. Characterization of the effect of cholecystokinin (CCK) on neurons in the periaqueductal gray of the rat: immunocytochemical and in vivo and in vitro electrophysiological studies. Brain Res 1994; 642: 83–94.

Liu NJ, Xu T, Xu C, Li CQ, Yu YX, Kang HG, Han JS. Cholecystokinin octapeptide reverses mu-opioid-receptor-mediated inhibition of calcium current in rat dorsal root ganglion neurons. J Pharmacol Exp Ther 1995; 275: 1293–1299.

Lotti VJ, Chang RS. A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260. Eur J Pharmacol 1989; 162: 273–280.

Maes M, Meltzer HY, Suy E, De Meyer F. Seasonality in severity of depression: relationships to suicide and honicide occurence. Acta Psychiatr Scand 1993; 88: 156–161.

Marriott PF, Greenwood KM, Armstrong SM. Seasonality in panic disorder. J Affect Disord 1994; 31: 75–80.

Marsden CA, Bickerdike MJ, Cadogan AK, Wright IK, Rex A, Fink H. Serotoninergic mechanisms and animal models of anxiety. In Hamon M, Ollat H, Thiebot MH, (eds). Anxiety: Neurobiology, Clinic and Therapeutic Perspectives. Montrouge, John Libbey Eurotext Ltd., 1993, pp 75–82.

Matto V, Harro J, Allikmets L. The effects of cholecystokinin A and B receptor antagonists, devazepide and L 365260, on citalopram-induced decrease of exploratory behaviour in rat. J Physiol Pharmacol 1996; 47: 661–669.

Mezey E, Reisine TD, Skirboll L, Beinfeld M, Kiss JZ. Cholecystokinin in the medial parvocellular subdivision of the paraventricular nucleus — co-existence with corticotropin-releasing hormone. Ann NY Acad Sci 1985; 448: 152

Moran TH, Robinson PH, Goldrich MS, McHugh PR. Two brain cholecystokinin receptors: implications for behavioural actions. Brain Res 1986; 362: 175–179.

Motta V, Brandao ML. Aversive and antiaversive effects of morphine in the dorsal periaquaductal gray of rats submitted to the elevated plus-maze test. Pharmacol Biochem Behav 1993; 44: 119–125.

Munro G, Pumford KM, Russell JA. Altered cholecystokinin binding site density in the supraoptic nucleus of morphine-tolerant and -dependent rats. Brain Res 1998; 780: 190–198.

Mutt V. Chemistry, isolation and purification of gastrointestinal hormones. Biochem Soc Trans 1980; 8: 11–14.

Männistö PT, Lang A, Harro J, Peuranen E, Bradwejn J, Vasar E. Opposite effects mediated by CCK_A and CCK_B receptors in behavioural and hormonal studies in rats. Naunyn-Schmiedebergs Arch Pharmacol 1994; 349: 478–484.

Nagayama H, Lu J-Q. Circadian and circannual rhythms in the function of central $5\text{-}HT_{1A}$ receptors in laboratory rats. Psychopharmacology (Berl) 1998; 135: 279–283.

Pavlasevic S, Bednar I, Qureshi GA, Sodersten P. Brain cholecystokinin tetrapeptide levels are increased in a rat model of anxiety. NeuroReport 1993; 5: 225–228.

Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985; 14: 149–167.

Peuranen E, Lang A, Harro J, Vasar E, Männistö PT. Effect of CCK-agonists and antagonists on hypophyseal hormone secretion in single-housed and group-housed male rats. In Seredenin S, Longo VG, Gavirachi G, (eds). Biological Basis of Individual Sensitivity to Psychotropic Drugs (Proc. 2nd Intern. Golden Ring Confer.). Edinburgh, Graffham Press, 1994, pp 63–77.

Pisegna JR, de Weerth A, Huppi K, Wank SA. Molecular cloning of the human brain and gastric cholecystokinin receptor: structure, functional expression and chromosomal localization. Biochem Biophys Res Commun 1992; 189: 296–303.

Pratt JA, Brett RR. The benzodiazepine receptor inverse agonist FG 7142 induces cholecystokinin gene expression in rat brain. Neurosci Lett 1995; 184: 197–200.

Raiteri M, Paudice P, Vallebuona F. Release of cholecystokinin in the central nervous system. Neurochem Int 1993; 22: 519–527.

Rattray M, Singhvi S, Wu PY, Andrews N, File SE. Benzodiazepines increase preprocholecystokinin messenger RNA levels in rat brain. Eur J Pharmacol 1993; 245: 193–196.

Ravard S, Dourish CT, Iversen SD. Evidence that the anxiolytic-like effects of the CCK antagonists devazepide and L-365,260 in the elevated plus-maze paradigm in rats are mediated by CCK receptors. Br J Pharmacol 1990; 101: P576

Rehfeld JF. Immunochemical studies on cholecystokinin. II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog. J Biol Chem 1978; 253: 4022–4030.

Rehfeld JF. CCK and anxiety: introduction. In Dourish CT, Cooper SJ, Iversen SD, Iversen LL, (eds). Multiple Cholecystokinin Receptors in the CNS. New York, Oxford University Press, 1992, pp 117–120.

Rehfeld JF, Mogensen NW, Bardram L, Hilsted L, Monstein, HJ. Expression, but failing maturation of procholecystokinin in cerebellum. Brain Res 1992; 576: 111–119.

Rehfeld JF, Nielsen FC. Molecular forms and regional distribution of cholecystokinin in the central nervous system. In Bradwejn J, Vasar E, (eds). Cholecystokinin and Anxiety: from Neuron to Behavior. Austin, R.G. Landes Company, 1995, pp 33–56.

Reisine TD, Jensen RT. Cholecystokinin-8 stimulates adrenocorticotropin release from anterior pituitary cells. J Pharmacol Exp Ther 1986; 236: 621–626.

Rex A, Barth T, Voigt JP, Domeney AM, Fink H. Effects of cholecystokinin tetrapeptide and sulfated cholecystokinin octapeptide in rat models of anxiety. Neurosci Lett 1994; 172: 139–142.

Rex A, Fink H. Effects of cholecystokinin-receptor agonists on cortical 5-HT release in guinea pigs on the X-maze. Peptides 1998; 19: 519–526.

Rex A, Fink H, Marsden CA. Effects of BOC-CCK-4 and L-365,260 on cortical 5-HT release in guinea-pigs on exposure to the elevated plus maze. Neuropharmacology 1994; 33: 559–565.

Rex A, Marsden CA, Fink H. Cortical 5-HT-CCK interactions and anxiety-related behaviour of guinea-pigs: a microdialysis study. Neurosci Lett 1997; 228: 79–82.

Rex A, Marsden CA, Fink M. Effect of diazepam on cortical 5-HT release and behavior in the guinea-pig exposure to the elevated plus-maze. Psychopharmacology (Berl) 1993; 110: 233–236.

Riley AL, Melton PM. Effects of mu- and delta-opioid-receptor antagonists on the stimulus properties of cholecystokinin. Pharmacol Biochem Behav 1997; 57: 57–62.

Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: an ethological perspective. Braz J Med Biol Res 1997; 30: 289–304.

Rodgers RJ, Deacon RM. Effect of naloxone on the behaviour of rats exposed to a novel environment. Psychopharmacology (Berl) 1979; 65: 103–105.

Rodgers RJ, Johnson NJ. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol Biochem Behav 1995; 52: 297–303.

Rosen A, Franck J, Brodin E. Effects of acute systemic treatment with the 5 HT-uptake blocker alaproclate on tissue levels and release of substance P in rat periaqueductal grey. Neuropeptides 1995; 28: 317–324.

Rägo L, Adojaan A, Harro J, Kiivet RA. Correlation between exploratory activity in an elevated plus-maze and number of central and peripheral benzodiazepine binding sites. Naunyn-Schmiedebergs Arch Pharmacol 1991; 343: 301–306.

Saito A, Sankaran H, Goldfine ID, Williams JA. Cholecystokinin receptors in the brain: characterization and distribution. Science 1980; 208: 1155–1156.

Saito A, Sankaran H, Goldfine ID, Williams JA. Characterization of receptors for cholecystokinin and related peptides in mouse cerebral cortex. J Neurochem 1981; 37: 483–490.

Saito T, Ukai K, Masuda T, Nakagawa T, Kimura K, Fujii M, Wakatsuki K, Saeki M, Kasai H. General pharmacological profile of the novel cholecystokinin-A antagonist loxiglumide. Arzneimittelforschung 1997; 47: 1375–1382.

Sakamoto N, Takatsuji K, Shiosaka S, Tateishi K, Hasimurs E, Miura S, Hamaoka T. Cholecystokinin-8-like immunoreactivity in the pre- and post-central gyri of the human cerebral cortex. Brain Res 1984; 307: 77–83.

Sanchez C, Meier E. Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? Psychopharmacology (Berl) 1997; 129: 197–205.

Shlik J, Aluoja A, Vasar V, Vasar E, Podar T, Bradwejn J. Effects of citalopram treatment on behavioural, cardiovascular and neuroendocrine response to cholecystokinin tetrapeptide challenge in patients with panic disorder. J Psychiatry Neurosci 1997; 22: 332–340.

Shlik J, Vasar E, Bradwejn J. Cholecystokinin and Psychiatric Disorders. Role in Aetiology and Potential of Receptor Antagonists in Therapy. CNS Drugs 1997; 8: 134–152.

Siegel RA, Dueker E-M, Fuchs E. Responsiveness of mesolimbic, mesocortical, septal and hippocampal cholecystokinin and substance P neuronal systems to stress, in the male rat. Neurochem Int 1995; 6: 783–789.

Singh L, Field MJ, Hughes J, Menzies R, Oles RJ, Vass CA, Woodruff GN. The behavioural properties of CI-988, a selective cholecystokininB receptor antagonist. Br J Pharmacol 1991; 104: 239–245.

Singh L, Oles RJ, Field MJ, Atwal P, Woodruff GN, Hunter JC. Effect of CCK receptor antagonists on the antinociceptive, reinforcing and gut motility properties of morphine. Br J Pharmacol 1996; 118: 1317–1325.

Skinner K, Basbaum AI, Fields HL. Cholecystokinin and enkephalin in brain stem pain modulating circuits. NeuroReport 1997; 8: 2995–2998.

Skirboll L, Hokfelt T, Rehfeld JF, CuellO AC, Dockray GJ. Coexistence of substance P-like and cholecystokinin-like immunoreactivity in neurons of the mesencephalic periaqueductal central grey. Neurosci Lett 1982; 28: 35

Spath-Schwalbe E, Piroth L, Pietrowsky R. Stimulation of the pituitary adrenocortical system in man by cerulein, a cholecystokinin-8-like peptide. Clin Physiol Biochem 1988; 6: 316–320.

Stengaard-Pedersen K, Larsson LI. Localization and opiate receptor binding of enkephalin, CCK, and ACTH/β-endorphin in the rat central nervous system. Peptides 1981; 2 (suppl): 3–19.

Suberg SN, Culhane ES, Carstens E, Watkins LR. Behavioral and electrophysiological investigations of opiate/cholecystokinin interactions. Adv Pain Res Ther 1985; 9: 541–553.

Suberg SN, Watkins LR. Interaction of cholecystokinin and opioids in pain modulation. In AnonymousPain and Headache. Basel, Karger, 1987, pp 247–265.

Suh HH, Tseng LF. Differential effects of sulfated cholecystokinin octapeptide and proglumide injected intrathecally on antinociception induced by beta-endorphin and morphine administered intracerebroventricularly in mice. Eur J Pharmacol 1990; 179: 329–338.

Taquet H, Javoy-Agid F, Mauborgne A, Benoliel JJ, Agid Y, Legrand JC, Tramu G, Cesselin F, Hamon M. Biochemical mapping of cholecystokinin-, substance P-, [Met]enkephalin- , [Leu]enkephalin- and dynorphin A (1-8)-like immunoreactivities in the human cerebral cortex. Neuroscience 1988; 27: 871–883.

Tuomisto J, Männistö PT. Neurotransmitter regulation of anterior pituitary hormones. Pharmacol Rev 1985; 37: 249–332.

van Megen HJ, Westenberg HG, den Boer JA, Haigh JR, Traub M. Pentagastrin induced panic attacks: enhanced sensitivity in panic disorder patients. Psychopharmacology (Berl) 1994; 114: 449–455.

van Megen HJ, Westenberg HG, den Boer JA, Slaap B, Scheepmakers A. Effect of the selective serotonin reuptake inhibitor fluvoxamine on CCK-4 induced panic attacks. Psychopharmacology (Berl) 1997; 129: 357–364.

Vanderhaeghen JJ, Crawley JN. Neuronal cholecystokinin. Ann NY Acad Sci 1985; 448: 1

Vanderhaeghen JJ, Signeau JC, Gepts W. New peptide in the vertebrate CNS reacting with antigastrin antibodies. Nature 1975; 257: 604–605.

Vasar E, Kõks S, Volke V, Võikar V. Cholecystokinin in animal models of anxiety. Biol Psychiatry 1997; 42, 1S: 196–197.

Vasar E, Peuranen E, Harro J, Lang A, Oreland L, Männistö PT. Social isolation of rats increases the density of cholecystokinin receptors in the frontal cortex and abolishes the anti-exploratory effect of caerulein. Naunyn-Schmiedebergs Arch Pharmacol 1993; 348: 96–101.

Voigt JP, Sohr R, Fink H. CCK-8S facilitates 5-HT release in the rat hypothalamus. Pharmacol Biochem Behav 1998; 59: 179–182.

Wang J, Ren M, Han J. Mobilization of calcium from intracellular stores as one of the mechanisms underlying the antiopioid effect of cholecystokinin octapeptide. Peptides 1992; 13: 947–951.

Wank SA. Cholecystokinin receptors. Am J Physiol 1995; 269: G628–G646

Wank SA, Harkins R, Jensen RT, Shapira H, de Weerth A, Slattery T. Purification, molecular cloning, and functional expression of the cholecystokinin receptor from rat pancreas. Proc Natl Acad Sci USA 1992; 89: 3125–3129.

Wank SA, Jensen RT, Gardner JD. Kinetics of binding of cholecystokinin to pancreatic acini. Am J Physiol 1988; 255: G106–G112

Wank SA, Jensen RT, Gardner JD. Stimulation of secretion by secretagogues. Methods Enzymol 1990; 192: 247–255.

Wank SA, Pisegna JR, de Weerth A. Brain and gastrointestinal cholecystokinin receptor family: structure and functional expression. Proc Natl Acad Sci USA 1992; 89: 8691–8695.

Wank SA, Pisegna JR, de Weerth A. Cholecystokinin receptor family. Molecular cloning, structure, and functional expression in rat, guinea pig, and human. Ann NY Acad Sci 1994; 713: 49–66.

Wiertelak EP, Maier SF, Watkins LR. Cholecystokinin antianalgesia: safety cues abolish morphine analgesia. Science 1992; 256: 830–833.

Woodruff GN, Hughes J. Cholecystokinin antagonists. Annu Rev Pharmacol Toxicol 1991; 31: 469–501.

ACKNOWLEDGEMENTS

This study was mostly carried out at the Department of Physiology, University of Tartu. Some experiments were performed at the Department of Pharmacology and Toxicology, University of Helsinki. I am very grateful to every person at labs in both sides of the Gulf of Finland who has contributed to my thesis.

Especially I would like to stress my gratefulness to Professor Eero Vasar for supervising my experimental work and this thesis, for his continuous encouragement and supportive guidance, for his friendship and support. Of course he is the person who opened the gate to the neuroscience research for me.

I thank Professor Pekka T. Männistö for his helpful advises, for the possibilities to visit his department.

I am thankful to Professor Mart Ustav for his supervision in the molecular biology and for his proverbs.

I am grateful to Associate Professor Andres Soosaar for reviewing my manuscript(s), for his suggestions, and of course for his appetence for endless discussions.

I would like to thank Associate Professor Enn Veldi for the linguistic correction made in the manuscript.

I am grateful to all my co-workers at the Department of Physiology and at the Institute of Molecular and Cell Biology.

I would like to express my gratitude to my parents for giving me possibilities to educate myself and for creating mental atmosphere at my childhood's home.

Last but not least I am very grateful to my friend and wife Gea for her love and understanding. I thank Gea for continuous help and for her impressive attitude of mind making life more colourful.

The Estonian Science Foundation, the Sigrid Juselius Foundation and the Academy of Finland supported financially this work.

SUMMARY IN ESTONIAN

KOLETSÜSTOKINIINIST PÕHJUSTATUD ÄREVUS — KESKKONNASTIIMULITE MÕJU NING SEOSED ENDOPIOIDSETE MEHHANISMIDEGA JA SEROTONIINIGA

Koletsüstokiniin (CCK) on peptiid, mis avastati esmakordselt seedetraktis. Järgnevad uuringud näitasid, et tegemist on ainega, mida esineb laialdaselt ka ajus. Tänapäeva seisukohtade järgi ongi CCK ajus enim levinud peptiidse struktuuriga neuromediaator. Kesknärvisüsteemis (KNS) leiduv CCK on seotud toitumiskäitumise kujunemisega, valuaistingu modulatsiooniga, motivatsioonide ja emotsioonide regulatsiooniga. Bioloogilises psühhiaatrias on CCK pälvinud tähelepanu kui virgatsaine, mis osaleb ärevus- ja paanikahäirete patogeneesis. CCK-4 manustamine kutsub esile paanikahooge nii katseloomadel kui ka inimestel. Täheldatud on CCK_B alatüübi retseptorite antagonistide anksiolüütilist toimet. Vaatamata esialgsetele paljulubavatele katsetulemustele, on edasised uuringud olnud üsna vasturääkivad ning tulemuste varieeruvus eri katsetes suur. Kirjanduse andmed viitavad ka teiste neuromediaatorite, eeskätt serotoniini (5-HT) ja endopioidide rollile CCK poolt indutseeritud ärevuse tekkes.

Uurimuse eesmärgid

1. Uurida katse-eelse stressi mõju CCK agonisti tseruleiini anksiogeensele toimele ning selgitada eksploratiivse aktiivsuse sesoonsete erinevuste tagamaid.
2. Selgitada neurokeemilisi protsesse, mis tingivad katseloomade uudistamisaktiivsuse varieeruvuse tõstetud plusspuuris.
3. Uurida ja selgitada CCK ning endopioidsete mehhanismide vahelist interaktsiooni eksploratiivse käitumise regulatsioonis.
4. Selgitada 5-HT ja CCK vahelist interaktsiooni ärevuskäitumise tekkes.

Uurimismeetodid

Käitumiskatsetes kasutati isaseid Wistari liini rotte (Han/Kuo: WIST). Käitumiskatseseadmeteks olid tõstetud plusspuur ja automatiseeritud motoorika jälgimise süsteem. Osale loomadele rakendati katse-eelse manipulatsioonina kas

loomade "käsitsemist" (ingl. k. *handling*) või lühiajalist isolatsiooni pärast tseruleiini manustamist. Leidmaks eksploratiivse aktiivsuse individuaalsete erinevuste neurokeemilisi põhjuseid, jagati rotid vastavalt uudistamisaktiivsusele gruppidesse. Käitumiskatsetega paralleelsetelt tehti ka neurokeemilisi uuringuid, milles hinnati aju retseptoorsete süsteemide seisundit ja adenohüpofüsaarsete hormoonide sisaldust veres. Selgitamaks mediaatorsüsteemidevahelisi interaktsioone, kasutati vastavatesse süsteemidesse toimivaid agoniste või antagoniste.

Tulemused

Uuringud katse-eelsete stressoritega näitasid, et tseruleiini anksiogeenne toime sõltub katseloomadega tehtavatest katse-eelsetest manipulatsioonidest. Nimelt avaldas tseruleiin (5 μg/kg) kõige tugevamat anksiogeenset toimet neisse rottidesse, keda eelnevalt ei "käsitsetud" ega asetatud pärast süsti üksikult ridapuuri. Loomadele, keda oli "käsitsetud" ja kes asetati pärast süsti tagasi kodupuuri, ei avaldanud tseruleiin mingit toimet.

Uuringu teises osas võrreldi katseloomade uudistamisaktiivsust kahel aastaajal — suvel (juuni) ja talvel (november). Leiti katseloomade väga suur uudistamisaktiivsuse sesoonne erinevus. Rottide eksploratiivne aktiivsus oli suvel oluliselt väiksem kui talvel. Peale selle oli suvel rottidel oluliselt rohkem CCK-retseptoreid ajukoores ja hipokampuses ning 5-HT_{2A}-retseptoreid ajukoores. Samuti oli kasvuhormooni kontsentratsioon seerumis suvistel rottidel suurem kui talvistel.

Järgnevates uuringutes leiti, et katseloomade uudistamisaktiivsus erines indiviiditi väga oluliselt. Selline jaotumine võimaldas jagada rotid kolme gruppi: ärevad, vahepealsed ja mitteärevad. Mitteärevad rotid viibisid pluss-puuri avatud õlgadel keskmiselt 38±8 sekundit (s.o 13% katseajast), samas kui ärevad ei väljunud üldse avatud õlgadele. Radioligandsidumiskatsetega leiti, et vähese uudistamisaktiivsusega (ärevatel) rottidel oli hipokampuses CCK- retseptorite arv suurenenud, ajukoores aga 5-HT_{2A}-retseptorite afiinsus vähenenud. Adenohüpofüüsi hormoonidest ilmnesid muutused ainult kasvuhormooni sisalduses, mitteärevatel ja plusspuuril mittekäinud rottidel oli võrreldes ärevate loomadega veres oluliselt vähem kasvuhormooni.

CCK ja endopioidide vahelist interaktsiooni uurides sedastati, et morfiinil oli selge anksiolüütiline toime (1,0 mg/kg), naloksoon ei mõjutanud aga uudistamiskäitumist üldse. CCK agonistid tseruleiin ja BOC-CCK-4 avaldasid doosist sõltuvat anksiogeenset toimet. CCK_B-retseptorite antagonistil L-365,360-l oli anksiolüütiline toime, kuid annuse toime kõver oli iseloomuliku tagurpidise U kujuga, ainsaks efektiivseks doosiks oli 10 μg/kg. Kombineerides alaläviseid tseruleiini või BOC-CCK-4 annuseid naloksooni väikese annusega (0,5 mg/kg), saadi tugev anksiogeenne toime. Selgus ka, et morfiini anksio-

lüütiline toime oli kõrvaldatav naloksooni 0,5 mg/kg annusega ja 10 μg/kg BOC-CCK-4-ga. Teisalt, morfiini alalävise annuse (0,5 mg/kg) kombinatsioon L-365,360-ga doosis 100 μg/kg andis anksiolüütilise toime.

Paroksetiin on tugevaima toimega selektiivne serotoniini tagasihaarde inhibiitor (SSRI) nii *in vitro* kui ka *in vivo*. Paroksetiin langetas doosist sõltuvalt katseloomade uudistamisaktiivsust, efektiivsete doosidega 2, 4 ja 8 mg/kg. Doosid 4 ja 8 mg/kg suurendasid ka CCK_B-retseptorite arvu roti ajukoores, kuid mitte hipokampuses. Diasepaam ei suutnud kõrvaldada paroksetiini anksiogeenset toimet, kui CCK_B antagonist LY 288,513 annuses 1,0 mg/kg kõrvaldas täielikult paroksetiini toime annuses 2 mg/kg. Suuremate paroksetiini annuste antieksploratiivset toimet CCK_B antagonist ei kõrvaldanud.

Järeldused

1. CCK-ergiliste ainete toime on tugevam pärast katse-eelseid stressoorseid (ilma "käsitsemiseta" ja isolatsioon) manipulatsioone. Seega sõltub CCK-ergiliste ainete toime endogeensest CCK-neuronite toonusest. Katseloomade uudistamiskäitumise vähenemine suvekuudel on seotud CCK- ja 5-HT-ergiliste närviringide aktivatsiooniga.
2. Loomade eksploratiivne aktiivsus sõltub CCK- ja 5-HT-ergiliste närviringide aktivatsioonist. Suurenenud aktiivsus nimetatud ringides viib organismi kohanemisvõime vähenemisele, mis katseloomadel avaldub uudistamisaktiivsuse vähenemises.
3. Loomade eksploratiivse käitumise kirjeldatud individuaalsed ja sesoonsed erinevused on kindlasti üheks eri uurimisgruppide ebapüsivate tulemuste põhjuseks. Seetõttu tuleb käitumiskatsete planeerimisel arvestada kirjeldatud variatsioonidega ja võimalusel katsetingimusi ühtlustada.
4. CCK-l ja endopioidsetel mehhanismidel on uudistamiskäitumise regulatsioonis teineteise suhtes antagonistlik mõju. CCK_B-retseptorite aktivatsioon vähendab, samas kui μ-opioidiretseptorite aktivatsioon suurendab katseloomadel uudistamisaktiivsust.
5. CCK ja 5-HT vaheline interaktsioon anksiogeense käitumise kujunemisel on sünergistlik. 5-HT neurotransmissiooni aktivatsioon vähendab uudistamisaktiivsust ja see toime korreleerub CCK-ergiliste neuronite aktivatsiooniga. 5-HT suurem vabanemine põhjustab katseloomadel uudset situatsiooni vältiva käitumise.

Kokkuvõtvalt võib öelda, et katseloomade uudistamiskäitumise kujunemises osalevad närviringid on olulised ka loomade reaktsioonis ebameeldivale ja uudsele keskkonnale. CCK- ja 5-HT-ergiliste ringide aktivatsioon halvendab adaptatsiooni uudse situatsiooniga ning tingib sellest hoidumise. Endopioidsete mehhanismide aktivatsioon teisalt aga parandab isendi kohanemist uudse kesk-

konnaga. CCK poolt kontrollitava vältiva käitumise näol on tegemist evolutsiooniliselt vana ja konserveerunud käitumisega, mis tagab isendi püsimajäämise muutuvas maailmas. Et kirjeldatud skeem on tähtis lüli ärevushäirete patogeneesis, on oluline nende närviringide põhjalikum tundmaõppimine ja defineerimine.

PUBLICATIONS

I

Kõks S., Vasar E., Soosaar A., Lang A., Volke V., Võikar V.,
Bourin M., Männistö P. T.
Relation of exploratory behavior of rats in elevated plus-maze to brain
receptor properties and serum growth hormone levels.
Eur Neuropsychopharmacol 1997; 7: 289–294.

ELSEVIER

European Neuropsychopharmacology 7 (1997) 289–294

EUROPEAN NEURO-PSYCHOPHARMACOLOGY

Relation of exploratory behavior of rats in elevated plus-maze to brain receptor binding properties and serum growth hormone levels

Sulev Kõks[a,*], Eero Vasar[a], Andres Soosaar[a], Aavo Lang[a], Vallo Volke[a], Vootele Võikar[a], Michel Bourin[b], Pekka T. Männistö[c]

[a]*Department of Physiology, University of Tartu, 2 Näituse Street, EE-2400 Tartu, Estonia*
[b]*Laboratory of Pharmacology, University of Nantes, Nantes Cedex 44035, France*
[c]*Department of Pharmacology and Toxicology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland*

Received 17 October 1996; accepted 26 March 1997

Abstract

Forty-five male Wistar rats were selected according to their behavior in the elevated plus-maze. They were separated as follows: animals with low exploratory activity ('anxious'), an 'intermediate' group and animals having high exploratory activity ('non-anxious'). Various receptor binding studies and hormonal assays were also performed in these selected rats. The affinity of 5-hydroxytryptamine 5-HT_{2A} receptors in the frontal cortex was lower in the 'anxious' rats compared to home-cage controls and 'non-anxious' animals. Moreover, the number of cholecystokinin (CCK) receptors in the hippocampus was significantly elevated in the 'anxious' group compared to home-cage control animals. The blood levels of growth hormone (GH) were significantly lower in the 'non-anxious' rats compared to 'anxious' counterparts. In conclusion, it seems likely that the decreased exploratory activity of rats is related to the increased 5-hydroxytryptamine (5-HT) and CCK mediated neurotransmission in the brain. The different serum levels of GH in the selected rats probably reflect alterations in the activity of 5-HT and CCK. © 1997 Elsevier Science B.V.

Keywords: Elevated plus-maze; Exploratory activity; Selection of rats; Neurotransmitter receptors; Blood hormone levels

1. Introduction

Rägo et al. (1988, 1991) and Harro et al. (1990) showed that it was possible to categorise rodents according to their exploratory behavior in the elevated plus-maze. The exploratory behavior of rodents having the lowest activity resembled to that of animals treated with the anxiogenic β-carbolines (DMCM, FG 7142), whereas the behavior of high activity animals was similar to the administration of diazepam. In addition, Rägo et al. (1988) have established the reduced number of benzodiazepine and $GABA_A$ receptors in the cerebral cortex of 'anxious' mice compared to their 'non-anxious' counterparts. Harro et al. (1990) also reported a reduced density of benzodiazepine receptors in the 'anxious' rats simultaneously with an increased number of cholecystokinin (CCK) receptors in the frontal cortex. The administration of the benzodiazepine inverse agonist FG 7142 in anxiogenic doses (10–20 mg/kg) increased the density of CCK receptors in rat frontal cortex (Harro et al., 1990). These findings support the antagonistic interaction between CCK and benzodiazepine/$GABA_A$ receptors first established in the electrophysiological study of Bradwejn and de Montigny (1984).

However, not only are CCK (Crawley and Corwin, 1994; Harro et al., 1993) and benzodiazepine/$GABA_A$ (Handley, 1994) receptors are involved in the regulation of anxiety, but neuronal 5-hydroxytryptamine (5-HT) (Iversen, 1984; Andrews and File, 1993; Handley and McBlane, 1993; Marsden et al., 1993) and dopamine (Pratt, 1992; Glavin, 1993; Vaccarino, 1994; Ladurelle et al., 1995) also have a role in the control of negative emotions. The administration of 5-HT receptor agonist as mCPP has been shown to cause anxiety in rodents as well

*Corresponding author. Tel.: +372 7 465334; fax: +372 7 422044; e-mail: sulev.koks@ut.ee

PII S0924-977X(97)00034-5

in man (Handley, 1994). On the other hand, the drugs blocking 5-HT_2 receptors have been proven to have an anxiolytic action (Barrett and Vanover, 1993). CCK and 5-HT seem to interact in the regulation of anxiety since the administration of CCK-4 potentiates the release of 5-HT induced by the exposure of rodents to the plus-maze (Rex et al., 1994). L-365,260, an antagonist of CCK_B (brain subtype) receptors, potently reversed this action of CCK-4 (Rex et al., 1994). There is also some evidence that 5-HT may be a mediator of the anxiogenic-like effect of CCK in the plus-maze test (Vasar et al., 1993). Dopamine is the other neurotransmitter closely interacting with CCK in the regulation of behavior (Ladurelle et al., 1993; Crawley and Corwin, 1994). Evidence from the study of Glavin (1993) suggests that dopamine is also involved in the modulation of anxiety in rodents.

On the other hand, CCK, 5-HT and dopamine have also been shown to regulate the blood levels of anterior pituitary hormones (thyrotropin, growth hormone, prolactin) (Tuomisto and Männistö, 1985; Charney et al., 1987; Peuranen et al., 1995). We may therefore propose that anxiogenic-like behavior is related to neurochemical changes in the brain and these changes can also influence serum anterior pituitary hormone content. This stimulated the study of the relationship between the exploratory behavior and levels of anterior pituitary hormones. Accordingly, the aim of present study was to confirm and extend the data of experiments performed by Rägo et al. (1988, 1991) and Harro et al. (1990), and to find out whether the exploratory behavior in the elevated plus-maze is correlated with some radioligand binding and hormonal parameters.

The male Wistar rats were selected according to their exploratory behavior in the elevated plus-maze. The above mentioned studies suggest the implication of four different neurotransmitter systems (CCK, GABA, dopamine and 5-HT) in the regulation of anxiety (Glavin, 1993; Handley, 1994). Therefore, the various brain structures of selected rats were used for radioligand binding studies to reveal the parameters of dopamine D_2 (in the striatum), 5-hydroxytryptamine 5-HT_{2A} (in the frontal cortex), benzodiazepine (in the frontal cortex) and CCK receptors (in the frontal cortex and hippocampus). The levels of anterior pituitary hormones in serum were also measured.

2. Experimental procedures

2.1. Animals

The experiments were performed on male Wistar rats (Han/Kuo; Finnish National Laboratory Animal Center, Kuopio, Finland) weighing 260–360 g. The rats were kept four to five per cage at 21±2°C in a silent, dark room illuminated artificially from 7 a.m. to 7 p.m. They had laboratory pellets and water ad libitum. The experiments were performed 10 days after the arrival of rats to the animal house from the breeding company. For the selection procedure 80 handling-naive animals were used. All the behavioral experiments were done between 12:00 and 17:00. The animals were killed by decapitation immediately after the plus-maze exposure. Their brain structures were used for radioligand binding studies and the blood samples were taken to perform the hormonal experiments. The rats randomly taken from the home-cage (altogether 16 rats) were employed as the control animals for the hormonal and receptor binding studies.

2.2. Exploratory activity in an elevated plus-maze

The method suggested initially by Handley and Mithani (1984) for measuring exploratory activity was employed to study rats, with modifications as given by Pellow et al. (1985). The apparatus consisted of two opposite open arms (50×10 cm) without side walls and two enclosed arms (50×10×40 cm) with side walls and end wall, extending from a central square (10×10 cm). The maze was elevated to the height of 50 cm, and placed in a lit room (110 radiometric lux). During a 5 min observation session the following measures were taken by an observer: 1) number of attempts to enter from closed arm into the central square; 2) time spent in exploring central square and open arms of plus-maze; 3) number of closed and open arm entries. Subsequently, the ratio between open and closed arm entries was calculated. At the beginning of the experiment an animal was placed at the center of the plus-maze, facing the closed arm. An arm entry was counted only when all four limbs of the rat were within a given arm.

2.3. Radioligand binding studies

After decapitation the brains were quickly dissected on ice. The binding studies were performed on the various brain structures of the rats. The striatum was used to determine the density and affinity of dopamine D_2 receptors, the frontal cortex for 5-hydroxytryptamine 5-HT_{2A}, benzodiazepine and CCK receptors, and the hippocampus for CCK receptors (Table 1). These brain structures were selected according to results from previous studies since in these brain regions the most significant neurochemical changes had been established due to the elevated plus-maze exposure (Rägo et al., 1988, 1991; Harro et al., 1990; Rex et al., 1993; File et al., 1993). The brain tissue was homogenized in 20 volumes of ice-cold 50 mM Tris–HCl (pH 7.4 at 4°C) using a Potter-S glass-teflon homogenizer (1000 rpm, 12 passes). The membranes were washed twice in the same buffer by centrifugation (48 000×g for 20 min) and resuspension. After the last centrifugation the crude brain membranes were suspended in the incubation buffer for the appropriate binding assay.

The protein content was measured according to a dye-

Table 1
Basic data about the methods of receptor binding studies.

Receptor	Ligand	Specific activity	Brain area	Nonspecific binding		Reference
Dopamine D_2	[^{3}H]-spiperone	105 Ci/mmole	Striatum	Raclopride	1 μM	Lang et al., 1992
5-hydroxytryptamine 5-HT_{2A}	[^{3}H]-spiperone	105 Ci/mmole	Frontal cortex	Ketanserin	1 μM	Lang et al., 1992
CCK	[^{3}H]-pCCK-8	75 Ci/mmole	Frontal cortex and hippocampus	Caerulein	100 nM	Lang et al., 1995
Benzodiazepine	[^{3}H]-flunitrazepam	90 Ci/mmole	Frontal cortex	Clonazepam	1 μM	Braestrup and Squires, 1977

binding assay (Bradford, 1976). Saturation curves were analyzed using non-linear least squares regression (Leatherbarrow, 1987).

2.4. Hormonal studies

In all cases truncal blood was collected, serum was separated by centrifugation and samples were stored at −20°C until prolactin (PRL), thyrotropin (TSH) and growth hormone (GH) concentrations were determined from duplicate samples (0.1 ml) by specific radioimmunoassays. The rat PRL, TSH and GH kits were gifts from NIH. PRL results are expressed in ng/ml of NIDKK-rPRL-RP-2 standard. TSH data are expressed in ng/ml of NIDKK-TSH-RP-2 standard. GH results are expressed in ng/ml of NIDKK-GH-RP-2 standard. The intra-assay coefficient of variation was less than 15%.

2.5. Statistics

Results are expressed in the tables as mean values ± S.E.M. Behavioral, hormonal and radioligand binding data were analyzed by means of one-way analysis of variance (ANOVA). Post hoc comparisons were performed using Newman-Keuls test. Pearson r correlation coefficient (simple linear correlation test) was employed for correlation analysis.

3. Results

3.1. The selection of rats in the elevated plus-maze

Eighty handling-naive rats were used for the elevated plus-maze experiment. Forty five animals were selected for the further studies. The remaining 35 were discarded since their behavior did not differ from the mean activity of all population. The selected 45 rats were divided into three subgroups according to their exploratory behavior (Table 2):

1) rats not leaving the closed arms; they were called 'anxious' (8 animals);

2) rats with moderate exploratory activity, exploring mainly the central square located between open and enclosed arms and making less than two open arm entries per session; this group was named as 'intermediate' (30 animals);

3) animals with high exploratory activity, making at least three open arm entries; they were called 'non-anxious' (7 animals).

There were the significant correlations between several parameters of plus-maze exploration. The ratio between open and closed arm entries, a classical measure of anxiety in rodents (Pellow et al., 1985), was correlated with the open arm exploration time ($r=0.89$, $P<0.01$) and number of open arm entries ($r=0.91$, $P<0.01$). A good correlation was also established between the number of closed arm entries and time spent in the central square ($r=0.76$, $P<0.01$).

3.2. The radioligand binding studies in the selected rats

The 'anxious' rats had significantly lower affinity of 5-HT_{2A} receptors in the frontal cortex (Table 3) compared to home-cage controls and 'non-anxious' rats ($F_{3,58}=2.30$, $P<0.05$). The density of 5-HT_{2A} receptors in the frontal cortex did not differ among the selected rats. The affinity and density of dopamine D_2 receptors in the striatum was not affected by the exposure of rats to the plus-maze.

The 'non-anxious' rats had less [^{3}H]pCCK-8 binding sites in the frontal cortex compared to the 'anxious'

Table 2
The selection of rats according to their exploratory behavior in the elevated plus-maze (mean values ± S.E.M.).

Behavioral parameters	'Anxious'	'Intermediate'	'Non-anxious'
Number of attempts to enter into central square	2.8±0.5	6.0±0.7	5.1±0.7
Time spent in central square (sec)	0.5±0.3	69±4	98±10
Time spent in open arm (sec)	0	8±3	38±8
Number of open arm entries	0	0.6±0.2	4.0±0.7
Number of closed arm entries	1.0±0	5.5±0.5	10.4±0.8
Ratio open arm entries/closed arm entries	0	0.10±0.03	0.38±0.05

Table 3
Parameters of dopamine D_2, 5-hydroxytryptamine 5-HT_{1A}, CCK and benzodiazepine receptors in selected rats (mean values ± S.E.M.).

	Home-cage controls	'Anxious'	'Intermediate'	'Non-anxious'
[^{3}H]-spiperone binding in the frontal cortex (5-HT_{1A})				
K_d (nM)	0.98±0.12	2.11±0.56[a]	1.32±0.21	0.99±0.15[b]
B_{max} (fmol/mg protein)	156±21	180±43	181±17	191±14
[^{3}H]-spiperone binding in the striatum (D_2)				
K_d (nM)	0.90±0.18	0.57±0.09	0.80±0.09	0.86±0.21
B_{max} (fmol/mg protein)	221±8	197±10	199±9	220±21
[^{3}H]pCCK-8 binding in the frontal cortex				
K_d (nM)	0.85±0.07	0.73±0.07	0.81±0.07	0.97±0.16
B_{max} (fmol/mg protein)	7.99±0.75	8.89±0.95	7.16±0.37	6.31±0.46
[^{3}H]pCCK-8 binding in the hippocampus				
K_d (nM)	0.52±0.07	0.70±0.07	0.77±0.08	0.64±0.16
B_{max} (fmol/mg protein)	3.80±0.37	5.96±0.44[a]	4.70±0.36	4.30±0.74
[^{3}H]-flunitrazepam binding in the frontal cortex				
K_d (nM)	2.58±0.36	1.96±0.29	2.39±0.36	1.97±0.36
B_{max} (fmol/mg protein)	536±89	581±62	621±28	556±113

[a] $P<0.05$ (compared to home-cage controls, Newman–Keuls test after significant one-way ANOVA); [b] $P<0.05$ (compared to 'anxious')

animals. However, this difference was not statistically significant. In the hippocampus, the 'anxious' animals had apparently higher density of CCK receptors than home-cage controls ($F_{3,58}=2.64$, $P<0.05$). The 'non-anxious' animals also tended to have less CCK receptors in the hippocampus compared to the 'anxious' counterparts. By contrast, the binding parameters of benzodiazepine receptors did not differ among the selected rats.

The correlation analysis revealed few weak, but significant correlations between the plus-maze activity and radioligand binding parameters. The affinity of 5-HT_2 receptors and the number of CCK_B binding sites in hippocampus negatively correlated with the exploratory behavior ($r=-0.38$, $P<0.05$; $r=-0.31$, $P<0.05$, respectively).

3.3. The hormonal studies in the selected rats

The only significant change (Table 4) was established in the case of GH ($F_{3,58}=2.92$, $P<0.05$). The 'non-anxious' animals had markedly lower levels of GH compared to 'anxious' and intermediate rats.

4. Discussion

The present study is in good agreement with previous experiments confirming the significant variability of exploratory behavior of rats in the elevated plus-maze (Harro et al., 1990; Rägo et al., 1991). Among the 45 rats selected, we established a subgroup of animals eagerly exploring the open arms. The open arms are considered to be the most aversive parts of plus-maze (Pellow et al., 1985). These 'non-anxious' rats performed three or more open arm entries per session. On the other hand, the other population of rats did not leave the enclosed arms at all. It is worth noting that these 'anxious' animals made several unsuccessful attempts to enter from the enclosed arm into the central square. Accordingly, the central square located between the opened and enclosed arms is too aversive a place for the 'anxious' rats to explore. The exploratory behavior of the 'intermediate' group represents the mean exploratory activity of whole population. Their behavioral activity was mainly restricted to the central square.

The results obtained from the radioligand binding studies are somewhat different from the previously published data. Harro et al. (1990) found that the 'anxious' animals have a significantly increased density of CCK receptors in the frontal cortex and reduced amount of these receptors in the hippocampus. In the present study, the similar tendency was established in the frontal cortex. The 'anxious' animals had more CCK binding sites compared to the 'non-anxious' counterparts, but this difference was not statistically significant. However, the situation was different in the hippocampus. Namely, we found increased number of [^{3}H]pCCK-8 binding sites in the 'anxious'

Table 4
Serum concentrations of anterior pituitary hormones in selected rats (mean values ± S.E.M.).

	Home-cage controls	'Anxious'	'Intermediate'	'Non-anxious'
PRL (ng/ml)	17.1±4.4	12.7±3.0	13.1±2.2	5.5±1.0
GH (ng/ml)	15.7±3.4	27.1±4.9[a]	24.5±2.8[a]	8.9±2.2
TSH (ng/ml)	1.3±0.4	2.2±0.6	1.3±0.2	1.3±0.4

[a] $P<0.05$ (compared to 'non-anxious' animals, Newman–Keuls test after significant one-way ANOVA)

animals compared to home-cage controls. A similar, but much smaller elevation of CCK receptors was also observable in the other selected groups, reflecting a possible link between the exploratory activity and density of CCK receptors in the hippocampus. The recent studies indicate that CCK receptors in the hippocampus belong to the CCK_B (brain) receptor subtype (Honda et al., 1993). It is likely that the variations of exploratory behavior in rats are related in some extent to the differences in the density of CCK_B receptors. However, Pratt and Brett (1995) did not detect any changes in the content of preproCCK mRNA in the frontal cortex and hippocampus after the plus-maze exposure. Nevertheless, the administration of inverse benzodiazepine agonist FG 7142 at the anxiogenic doses markedly increased the levels of preproCCK mRNA in both brain regions (Pratt and Brett, 1995). Despite the significantly distinctive behavior it could be that the plus-maze exposure is not enough strong stress to increase CCK binding sites in the brain of all rats. Therefore, only in the most susceptible animals exposure to the elevated plus-maze caused the marked elevation of CCK binding sites in the hippocampus.

Contrary to previous studies (Harro et al., 1990; Rägo et al., 1991), we did not find a link between the exploratory activity and density of benzodiazepine receptors in the frontal cortex. Two suggestions should be considered to explain this discrepancy. Firstly, the animals used in the earlier studies had a much higher level of basal anxiety (Harro et al., 1990; Rägo et al., 1991). Secondly, the animals in the previous experiments were kept in over-crowded conditions (20–25 rats per cage) which is very stressful for experimental animals. Therefore, the high pre-experimental anxiety is a likely reason to explain the differences between the present and previous studies.

The 'anxious' rats displayed the reduced affinity of $5\text{-}HT_{2A}$ receptors in the frontal cortex compared to home-cage controls and 'non-anxious' rats. The background of this decrease remains to be established. However, this change can be explained in the light of recent findings. Namely, Rex et al. (1993, 1994) and Marsden et al. (1993) established that the exposure of rodents to the elevated plus-maze increased the release of 5-HT in the frontal cortex. Similar alterations are also described in the hippocampus (Crawley and Corwin, 1994; File et al., 1993). Therefore, we are tempted to speculate that the reduced affinity of $5\text{-}HT_{2A}$ receptors in the frontal cortex is related to the increased release of 5-HT in the 'anxious' rats.

Besides CCK and 5-HT, dopamine has also been shown to be involved in the regulation of anxiety (Glavin, 1993; Ladurelle et al., 1995). However, we did not find any significant modifications of dopamine D_2 receptors in the striatum of selected rats. Accordingly, we were not able to establish a link between striatal dopamine receptors and exploratory behavior.

We investigated also the release of anterior pituitary hormones in relation to the exploratory behavior of rats. In the present study neither the exposure of rats to the plus-maze nor distinctive exploratory activity correlated with the serum levels of anterior pituitary hormones prolactin and thyrotropin. Only the concentrations of growth hormone (GH) were significantly different in the selected rats. Namely, the 'non-anxious' rats had markedly lower levels of GH compared to the 'anxious' and intermediate groups. Anterior pituitary hormones are regulated by a multitude of classic neurotransmitters like 5-HT and dopamine but also by neuropeptide transmitters (Tuomisto and Männistö, 1985). It has been shown that 5-HT stimulates the baseline release of GH and may be involved in the stress-induced GH elevation in man (Charney et al., 1987). CCK also participates in the regulation of GH. The activation of cholecystokinin CCK_A (alimentary) receptors inhibits GH secretion while the activation of CCK_B receptors caused an opposite effect (Männistö et al., 1994; Peuranen et al., 1994). Therefore, the augmented levels of GH in the 'anxious' animals might be caused by the increased activity of 5-HT and CCK in the brain.

In conclusion, the present study confirms significant individual differences in the exploratory behavior of rats. However, the variations in hormonal and receptoral levels between the selected groups are not as marked as in the behavioral studies. Nevertheless, the increased density of CCK receptors in the hippocampus and the reduced affinity of $5\text{-}HT_{2A}$ receptors in the frontal cortex seem to be linked to the decreased exploratory activity of rats in the elevated plus-maze. The elevated plus-maze is a forced exploratory model (Belzung et al., 1994). Therefore, we suggest that the described neurochemical changes eare induced by the exposure of rats to the test situation and that they reflect the 'state' but not 'trait' anxiety. The background of changes in the content of GH remains to be elucidated, but it may be also related to the altered activity of 5-HT and CCK neurotransmission in the brain.

Acknowledgements

The support to this study from the Sigrid Juselius Foundation to E.V. and P.T.M. and Academy of Finland to P.T.M. is gratefully acknowledged. This study was also supported by grant No. 1409 from the Estonian Science Foundation.

References

Andrews, N., File, S.E., 1993. Increased 5-HT release mediates the anxiogenic response during benzodiazepine withdrawal: a review of supporting neurochemical and behavioral evidence. Psychopharmacology 112, 21–25.

Barrett, J.E., Vanover, K.E., 1993. 5-HT receptors as targets for the development of novel anxiolytic drugs: models, mechanisms and future directions. Psychopharmacology 112, 1–12.

Belzung, C., Pineau, N., Beuzen, A., Misslin, R., 1994. PD135158, a CCK-B antagonist, reduces 'state', but not 'trait' anxiety in mice. Pharmacol. Biochem. Behav. 49, 433–436.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Bradwejn, J., de Montigny, C., 1984. Benzodiazepines antagonize cholecystokinin-induced activation of rat hippocampal neurons. Nature 312, 363–364.

Braestrup, C., Squires, R.F., 1977. Specific benzodiazepine receptors in rat brain characterized by high affinity [^{3}H]-diazepam binding. Proc. Natl. Acad. Sci. USA 74, 3805–3809.

Charney, D.S., Woods, S.W., Goodman, W.K., Heninger, G.R., 1987. Serotonin function in anxiety. II. Effects of serotonin agonist mCPP in panic disorder patients and healthy subjects. Psychopharmacology 92, 14–24.

Crawley, J.N., Corwin, R.L., 1994. Biological actions of cholecystokinin. Peptides 15, 731–755.

File, S.E., Zangrossi, J.R., Andrews, N., 1993. Social interaction and elevated plus-maze tests: changes in release and uptake of 5-HT and GABA. Neuropharmacology 32, 217–221.

Glavin, G.B., 1993. Vulnerability to stress ulcerogenesis in rats differing in anxiety: a dopaminergic correlate. J. Physiol. Paris 87, 239–243.

Handley, S.L., Mithani, S., 1984. Effects of alpha-adrenoreceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behavior. Naunyn-Schmiedeberg's Arch. Pharmacol. 327, 1–5.

Handley, S., McBlane, J.W., 1993. 5HT drugs in animal models of anxiety. Psychopharmacology 112, 13–20.

Handley, S., 1994. Future prospects for the pharmacological treatment of anxiety. CNS Drugs 2, 397–414.

Harro, J., Kiivet, R.A., Lang, A., Vasar, E., 1990. Rats with anxious or non-anxious type of exploratory behavior differ in their brain CCK-8 and benzodiazepine receptor characteristics. Behav. Brain Res. 39, 63–71.

Harro, J., Vasar, E., Bradwejn, J., 1993. Cholecystokinin in animal and human research of anxiety. Trends Pharmacol. Sci. 14, 244–249.

Honda, T., Wada, E., Battey, J.F., Wank, S.A., 1993. Differential gene expression of CCK_A and CCK_B receptors in the rat brain. Mol. Cell. Neurosci. 4, 143–154.

Iversen, S.D., 1984. 5-HT and anxiety. Neuropharmacology 23, 1553–1560.

Ladurelle, N., Keller, G., Roques, B.P., Dauge, V., 1993. Effects of CCK_A and of the CCK_B-selective agonist BC264 on extracellular dopamine content in the anterior and posterior nucleus accumbens: a microdialysis study in freely moving rats. Brain Res. 628, 254–262.

Ladurelle, N., Roques, B.P., Dauge, V., 1995. The transfer of rats from a familiar to a novel environment prolongs the increase of extracellular dopamine efflux induced by CCK8 in the posterior nucleus accumbens. J. Neurosci. 15, 3118–3127.

Lang, A., Vasar, E., Soosaar, A., Harro, J., 1992. The involvement of sigma and phencyclidine receptors in the action of antipsychotic drugs. Pharmacol. Toxicol. 71, 132–138.

Lang, A., Harro, J., Soosaar, A., Kõks, S., Volke, V., Oreland, L., Bourin, M., Vasar, E., Bradwejn, J., Männistö, P.T., 1995. Role of N-methyl-D-aspartic acid and cholecystokinin receptors in apomorphine-induced aggressive behavior in rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 351, 363–370.

Leatherbarrow, R.J., 1987. Enzfitter, a non-linear regression data analysis program for the IBM PC. Elsevier, Amsterdam.

Marsden, C.A., Bickerdike, M., Cadogan, A.K., Wright, I., Rex, A. and Fink, H., 1993. Serotoninergic mechanisms and animal models of anxiety. In: Hamon, M.; Ollat, H.; Thiebot, M. H., (Eds.), Anxiety: Neurobiology, Clinic and Therapeutic Perspectives. John Libbey Eurotext Ltd., Montrouge, pp. 75–82.

Männistö, P.T., Lang, A., Harro, J., Peuranen, E., Bradwejn, J., Vasar, E., 1994. Opposite effects mediated by CCK_A and CCK_B receptors in behavioral and hormonal studies in rats housed in social groups. Naunyn-Schmiedeberg's Arch. Pharmacol. 349, 478–484.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.

Peuranen, E., Lang, A., Harro, J., Vasar, E. and Männistö, P.T., 1994. Effect of CCK-agonists and antagonists on hypophyseal hormone secretion in single-housed and group-housed male rats. In: Seredenin, S.; Longo, V.G.; Gavirachi, G., (Eds.), Biological Basis of Individual Sensitivity to Psychotropic Drugs (Proc. 2nd Intern. Golden Ring Confer.) Graffham Press, Edinburgh, pp. 63–72.

Peuranen, E., Vasar, E., Kõks, S., Volke, V., Lang, A., Rauhala, P., Männistö, P.T., 1995. Further studies on the role of cholecystokinin-A and B receptors in secretion of anterior pituitary hormones in male rats. Neuropeptides 28, 1–11.

Pratt, J.A., 1992. The neuroanatomical basis of anxiety. Pharmacol. Ther. 55, 149–181.

Pratt, J.A., Brett, R.R., 1995. The benzodiazepine inverse agonist FG 7142 induces cholecystokinin gene expression in rat brain. Neurosci. Lett. 184, 197–200.

Rägo, L., Kiivet, R.A., Harro, J., Põld, M., 1988. Behavioral differences in an elevated plus-maze: correlation between anxiety and decreased number of GABA and benzodiazepine receptors in mouse cerebral cortex. Naunyn-Schmiedberg's Arch. Pharmacol. 337, 675–678.

Rägo, L., Adojaan, A., Harro, J., Kiivet, R.A., 1991. Correlation between exploratory activity in an elevated plus-maze and number of central and peripheral benzodiazepine binding sites. Naunyn-Schmiedberg's Arch. Pharmacol. 343, 301–306.

Rex, A., Marsden, C.A., Fink, H., 1993. Effect of diazepam on cortical 5-HT release and behavior in the guinea-pig exposure to the elevated plus-maze. Psychopharmacology 110, 233–236.

Rex, A., Fink, H., Marsden, C.A., 1994. Effects of BOC-CCK-4 and L-365,260 on cortical 5-HT release in guinea-pigs on exposure to the elevated plus-maze. Neuropharmacology 33, 559–565.

Tuomisto, J., Männistö, P.T., 1985. Neurotransmitter regulation of anterior pituitary hormones. Pharmacol. Rev. 37, 249–332.

Vaccarino, F., 1994. Nucleus accumbens dopamine-CCK interactions in psychostimulant reward and related behaviors. Neurosci. Biobehav. Rev. 18, 207–214.

Vasar, E., Peuranen, E., Ööpik, T., Harro, J., Männistö, P.T., 1993. Ondansetron, an antagonist of 5-HT3 receptors, antagonizes the anti-exploratory effect of caerulein, an agonist of CCK receptors, in the elevated plus-maze. Psychopharmacology 110, 213–218.

II

Kõks S., Soosaar A., Võikar V., Volke V., Ustav M., Männistö P. T.,
Bourin M., Vasar E.
Opioid antagonist naloxone potentiates anxiogenic-like action
of cholecystokinin agonists in elevated plus-maze.
Neuropeptides 1998; 32: 235–240.

Neuropeptides (1998) 32 (3), 235–240

Opioid antagonist naloxone potentiates anxiogenic-like action of cholecystokinin agonists in elevated plus-maze

S. Kõks,[1,2] A. Soosaar,[1] V. Võikar,[1] V. Volke,[1,2], M. Ustav,[2] P. T. Männistö,[3] M. Bourin,[4] E. Vasar[1]

[1]Department of Physiology, University of Tartu, Estonia
[2]Institute of Molecular and Cell Biology, University of Tartu, Estonia
[3]Department of Pharmacology and Toxicology, University of Kuopio, Finland
[4]Laboratory of Pharmacology, University of Nantes, France

Summary This study investigated the interplay of cholecystokinin (CCK) and endogenous opioid peptides in the regulation of anxiety. The acute administration of non-selective CCK agonist caerulein (1 and 5 μg/kg) and a selective CCK_B receptor agonist BOC-CCK-4 (1, 10 and 50 μg/kg) induced a dose-dependent anxiogenic-like action in the plus-maze model of anxiety. BOC-CCK-4 displayed a similar efficacy with caerulein, indicating that the described effect was mediated via CCK_B receptor subtype. The opioid antagonist naloxone itself (0.5 mg/kg) did not change the exploratory activity of rats in the plus-maze. However, the combination of naloxone with the sub-effective doses of caerulein (1 μg/kg) and BOC-CCK-4 (1 μg/kg) induced a significant inhibition of exploratory behaviour in rats. Accordingly, CCK and endogenous opioid peptides have an antagonistic role in the exploratory model of anxiety in rats.

INTRODUCTION

A growing body of evidence suggests the interaction of two neuropeptidergic systems – cholecystokinin (CCK) and opioid peptides – in the regulation of behaviour. The distribution of CCK in the brain parallels that of enkephalins and endorphins in the numerous brain regions.[1,2] Moreover, CCK and opioid peptides are colocalized in the same neurons in discrete brain areas.[3,4] Two different CCK receptor subtypes, namely CCK_A and CCK_B receptors, are described in the central nervous system.[5] These receptors have been pharmacologically characterized and cloned.[6–8] It is suggested that CCK might function via the interaction with CCK_B receptors as an opioid antagonist in various behavioural models.[9–11] CCK_B receptor antagonists, but not CCK_A antagonists, facilitate the antidepressant-like effect induced by opioid peptides in the conditioned suppression of motility test in mice.[9] Moreover, the selective CCK_B receptor antagonist PD-134,308 has been shown to potentiate the rewarding effect of morphine in the place preference paradigm[10] and to augment antinociception induced by opioid peptides.[12]

However, only a few studies[13] have been performed on the role of opioid peptides in CCK-induced behavioural effects. CCK is implicated in the neurobiology of anxiety[14,15] and therefore an attempt was made to study the role of endogenous opioid peptides in CCK-induced anxiety in rats. A non-selective antagonist of opioid receptors (naloxone) was combined with sub-effective doses of CCK agonists caerulein and BOC-CCK-4. The possible anxiogenic-like action of these combinations in rats was studied in the elevated plus-maze.

Received 20 October 1997
Accepted 12 January 1998

Correspondence to: Dr S. Kõks, Department of Physiology, University of Tartu, 2 Näituse Street, EE2400 Tartu, Estonia. Tel: +372–7–465334, Fax: +372–7–422044, e-mail: sulev.koks@ut.ee

MATERIALS AND METHODS

Animals

Male Wistar (Han/Kuo: WIST) rats (National Animal Centre, Kuopio, Finland) weighing 250–300 g were kept four per cage in the animal house at 20 ± 2°C with a 12 h light/dark cycle (light on at 7.00 a.m.). Tap water and food

pellets were available *ad libitum*. There were 12 animals in each group.

The elevated plus-maze

The method initially suggested by Handley and Mithani[16] for the measurement of exploratory activity was employed in rats with some modifications.[17] The apparatus consisted of two opposite open arms (50 × 10 cm) without side walls and two enclosed arms (50 × 10 × 40 cm) with side walls and end wall, extending from a central square (10 × 10 cm). The maze was elevated to a height of 50 cm, and placed in a lit room. During a 5 min observation session the following measures were taken by an observer: 1) time spent in exploration of open part and open arms of plus-maze; 2) number of closed and open arm entries; 3) number of line crossings in open part; and 4) ratio between open and total arm entries. At the beginning of the experiment an animal was placed into the centre of the plus-maze, facing toward a closed arm. An arm entry was counted only when all four limbs of the rat were within a given arm. The animals were brought into the experimental room 1 h before the experiment and were handling naive. After the injection of drugs the rats were placed in the single cages for 15 min before the plus-maze exposure. Each rat was tested only once.

Drugs

Caerulein (Sigma), BOC-CCK-4 (N-tert-butoxy-carbonyl-CCK-4; Sigma) and naloxone HCl (Sigma) were dissolved in physiological saline. Caerulein (1 and 5 μg/kg) and BOC-CCK-4 (1, 10 and 50 μg/kg) were injected subcutaneously, naloxone (0.5 and 10 mg/kg) intraperitoneally. These drugs or saline were given 15 min before the experiment.

Statistics

Results are expressed as mean values ± SEM. The data of behavioural studies were analysed by one-way analysis of variance (ANOVA). Post hoc comparisons between individual groups were performed by means of Newman-Keuls test with the Statistica for Windows software.

RESULTS

The subcutaneous administration of caerulein, a non-selective CCK_A/CCK_B receptor agonist, induced a dose-dependent reduction of exploratory activity of rats in the elevated plus-maze. The behaviour of rats treated with 1 μg/kg of caerulein did not differ from that of saline-treated animals, whereas 5 μg/kg of caerulein induced a statistically significant reduction in all studied parameters (Fig. 1, time in open part $F_{2,27} = 3.65$, $P < 0.05$; open

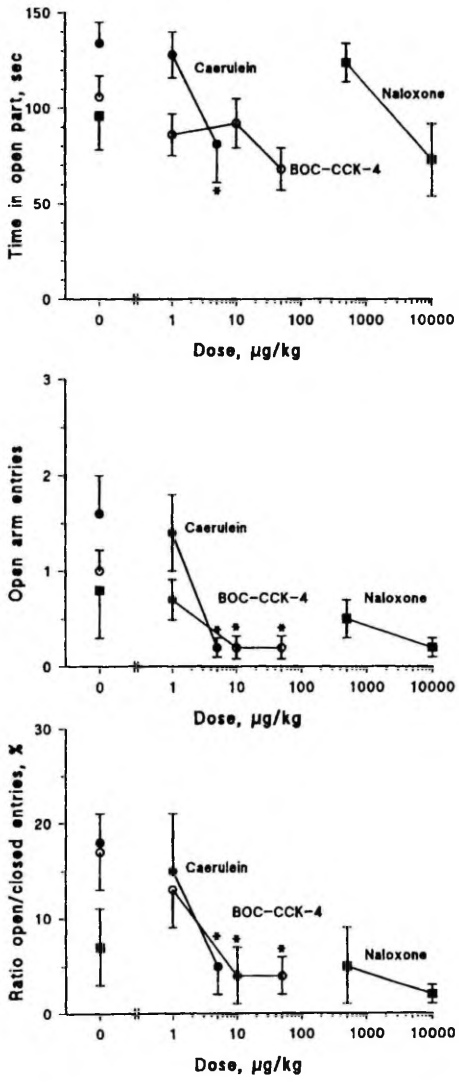

Fig. 1 The effects of caerulein, BOC-CCK-4 and naloxone upon the exploratory behaviour of rats in the plus-maze. All drugs were given 15 min before the experiment, naloxone was injected intraperitoneally, caerulein and BOC-CCK-4 subcutaneously. *$P < 0.05$, Newman-Keuls after significant ANOVA, compared with saline-treated animals.

arm entries $F_{2,27} = 3.89$, $P < 0.05$; ratio between open and closed arm entries $F_{2,27} = 3.62$, $P < 0.05$). The higher dose of caerulein also induced a significant reduction in line crossings (from 28 ± 3 to 10 ± 2, $F_{2,27} = 3.90$, $P < 0.05$) and closed arm entries (from 6.9 ± 0.7 to 3.2 ± 0.7, $F_{2,27} = 5.26$, $P < 0.05$). The treatment with BOC-CCK-4, a selective CCK_B receptor agonist, also induced a dose-dependent reduction of exploratory behaviour of rats in the plus-maze paradigm. One μg/kg of BOC-CCK-4 did not modify the behaviour of rats if compared with control rats. However, the higher doses of BOC-CCK-4 (10 and 50 μg/kg) induced a statistically significant reduction in the behavioural measures believed to reflect anxiety in rodents: the number of open arm entries ($F_{3,56} = 4,08$, $P < 0.01$) and ratio between open and total arm entries ($F_{3,56} = 3.14$, $P < 0.05$). The reduction of line crossings ($F_{3,56} = 2.51$, $P = 0.067$) and closed arm entries ($F_{3,56} = 2.50$, $P = 0.069$) did not reach the statistically significant level.

The administration of naloxone (0.5 and 10 mg/kg) did not modify the exploratory behaviour of rats. Nevertheless, 10 mg/kg of naloxone, unlike the lower dose (0.5 mg/kg), tended to reduce the activity of rats in the plus-maze without significant differences.

The combination of naloxone (0.5 mg/kg) with the sub-effective dose of caerulein (1 μg/kg) induced a statistically significant reduction of exploratory behaviour of rats (Fig. 2, line crossings $F_{3,46} = 2.85$, $P < 0.05$; time in open part $F_{3,46} = 3.62$, $P < 0.05$). The same was true with the combination of naloxone with the sub-effective dose of BOC-CCK-4 (1 μg/kg) (line crossings $F_{3,30} = 7.69$, $P < 0.01$; time in open part $F_{3,30} = 11.79$, $P < 0.01$; open arm entries $F_{3,30} = 3.45$, $P < 0.05$; time in open arm $F_{3,30} = 2.95$, $P < 0.05$; closed arm entries $F_{3,30} = 8.63$, $p < 0.01$; ratio between open and closed arm entries $F_{3,30} = 2.95$, $P < 0.05$). In fact, the combination of naloxone with the CCK_B receptor agonist induced much stronger inhibition of exploratory activity compared with the combination of opioid antagonist and caerulein.

DISCUSSION

A recent study established that the anxiogenic-like action of caerulein, a non-selective agonist of CCK_A/CCK_B receptors, in the elevated plus-maze is dependent on pre-experimental stress in rats.[15] The administration of caerulein induced the anti-exploratory effect only in rats not adapted to handling, i.e. CCK agonist increased neophobia. In the present study, two agonists of CCK receptors, caerulein and BOC-CCK-4, with different selectivity for CCK_B receptor subtype, caused a similar and dose-dependent reduction of exploratory behaviour in rats. Accordingly, this anxiogenic-like action of CCK agonists in the plus-maze was mediated via the CCK_B receptor subtype.

The administration of the opioid antagonist naloxone at a low dose (0.5 mg/kg) did not affect the exploratory behaviour of rats. However, the higher dose (10 mg/kg) of naloxone tended to reduce the activity of rats. This trend in the action of naloxone could be explained in the light of data showing that it blocks not only μ-opioid receptors, but also δ– and κ-receptors.[18] Indeed, Motta and Brandao[19] have shown that the systemic administration of morphine at low doses or its injection into the dorsal periaquaductal grey induced anxiolytic-like action in the elevated plus-maze. Naloxone at low doses reversed the anxiolytic-like effect of morphine. Privette and Terrian[20] found that the administration of κ-opioid agonists, U-50, 488H and U-69,593, induced a strong anxiolytic-like action in the elevated plus-maze. This action of κ-opioid agonists is reversed by naloxone, also suggesting an opioid receptor site of action.[20] Therefore, it is likely that naloxone administered at low doses interacts only with μ-opioid receptors, whereas at higher doses the interaction with κ-opioid receptors is also involved. The co-administration of naloxone (0.5 mg/kg) with the sub-effective doses of caerulein and BOC-CCK-4 induced a potent anxiogenic-like action in the elevated plus-maze. This finding is apparently in favour of an antagonistic interplay between CCK and endogenous opioid peptides in the regulation of anxiety.

There are several studies showing the negative interaction between CCK and endogenous opioid peptides in the regulation of pain sensitivity. However, as in the present work, the action of CCK was dependent on the pre-experimental stress of rats. Wiertelak et al[21] have shown that CCK antagonizes morphine-induced antinociception only in the novel, but not in the familiar environment. This finding was confirmed by Lavigne et al[22] demonstrating that CCK antagonists devazepide and L-365,260 enhanced morphine-induced analgesia only in non-acclimatized rats exposed to the novel environment. Therefore, it is likely that even in the case of pain regulation the interplay between CCK and endogenous opioid peptides is actually dependent on the level of anxiety. This suggestion is supported by a recent study by Benedetti et al[23] who found that nocebo-induced hyperalgesia was reversed dose dependently by the CCK antagonist proglumide, but not by naloxone. As the nocebo procedure represents an anxiogenic stimulus it is likely that nocebo hyperalgesia may be due to a CCK-dependent increase in anxiety.

According to the hypothesis of Wiertelak et al,[21] the environmental stimuli that signal the occurrence of aversive or dangerous events activate endogenous opioid analgesia systems. The signals for safety (the non-occurrence of aversive events) produce the opposite effect and inhibit environmentally produced analgesia. Wiertelak et al[21] believe that CCK is a mediator of that kind of safety

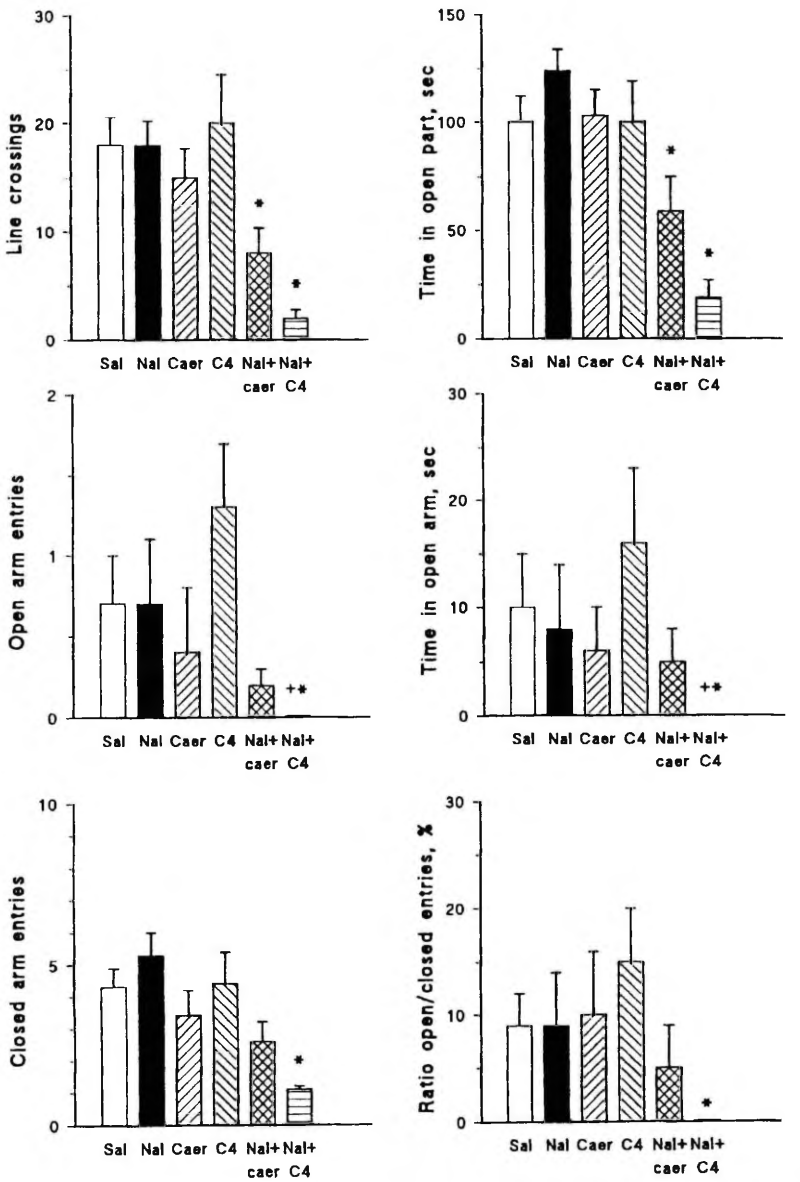

Fig. 2 The action of sub-effective doses of caerulein (1 μg/kg) and BOC-CCK-4 (1 μg/kg) alone and in combination with naloxone (0.5 mg/kg) in the elevated plus-maze. Drugs were administered 15 min before the experiment. Sal, saline; Nal, naloxone; Caer, caerulein; C4; BOC-CCK-4. *$P < 0.05$, Newman-Keuls after significant ANOVA, compared with saline-treated animals; +, $P < 0.05$, Newman-Keuls after significant ANOVA, compared with BOC-CCK-4-treated animals.

signal. However, the present study is not in favour of that statement, as CCK apparently increases anxiety in the novel environment and the anxiogenic-like action of CCK was potentiated by the opioid antagonist naloxone. Therefore, CCK is rather signalling that the novel environment is unsafe and aversive.

In fact, most studies have explored the action of CCK agonists and antagonists on the behavioural effects of morphine and endogenous opioid peptides, and few studies have been devoted to the investigation of endogenous opioid peptides and their receptors in the behavioural effects of CCK. However, a similar antagonistic interaction between CCK and opioid peptides was described by Riley and Melton.[13] Namely, the administration of naloxone strongly potentiated CCK-induced conditioned taste aversion. However, this action of CCK was mediated via CCK_A receptors. Moreover, the selective δ-opioid antagonist naltrindole, unlike naloxone, did not potentiate the aversive learning induced by CCK.[13] Accordingly, the antagonistic interaction between CCK and opioid peptides seems to occur at various levels: CCK_A receptors located in the periphery are likely targets for food aversion, whereas CCK_B receptors in the brain are responsible for the aversion toward environment.

There are some hints that the interplay between CCK and opioid peptides may also contribute to the mechanisms of human anxiety. Patients suffering from panic disorder have an increased sensitivity toward the panicogenic-like action of CCK-4,[14] but they also seem to have a reduced tone of endogenous opioid system reflected as reduced concentrations of β-endorphin in the cerebrospinal fluid.[24]

In conclusion, CCK and endogenous opioid peptides have an opposite role in the behavioural response of animals toward the novel environment. The simultaneous stimulation of CCK_B receptors and the blockade of μ-opioid receptors apparently increase anxiety in rats. Endogenous opiod peptides interacting with μ-opioid receptors seem to be the endogenous antagonists of CCK-induced anxiety.

ACKNOWLEDGEMENT

This study was supported by the Estonian Science Foundation (grant no. 1409).

REFERENCES

1. Stengaard-Pedersen K, Larsson L I. Localization and opiate receptor binding of enkephalin, CCK, and ACTH/β-endorphin in the rat central nervous system. Peptides (Suppl) 1981; 2: 3–19.
2. Skinner K, Busbaum A I, Fields H L. Cholecystokinin and enkephalin in brain stem pain modulating circuits. NeuroReport 1997; 8: 2995–2998.
3. Gall C, Lauterborn J, Burks D, Seroogy K. Co-localization of enkephalin and cholecystokinin in discrete areas of rat brain. Brain Res 1987; 403: 403–408.
4. Pohl M, Benoliel J J, Bourgoin S et al. Regional distribution of calcitonin gene-related peptide-, substance P-, cholecystokinin-, Met^5-enkephalin-, and dynorphin A (1–8)-like material in the spinal cord and dorsal root ganglia of adult rats. Effects of dorsal rhizotomy and neonatal capsaicin. J Neurochem 1990; 55: 1122–1130.
5. Moran T H, Robinson P H, Goldrich M S, McHugh P R. Two brain cholecystokinin receptors: implications for behavioural actions. Brain Res 1986; 362: 175–179.
6. Kopin A S, Lee Y-M, McBride E W et al. Expression, cloning and characterization of canine parietal cell gastrin receptor. Proc Natl Acad Sci USA 1992; 89: 3605–3609.
7. Wank S A, Pisenga J R, Weerth A. Brain and gastrointestinal cholecystokinin receptor family: structure and functional expression. Proc Natl Acad Sci USA 1992; 89: 8691–8695.
8. Wank S A. Cholecystokinin receptors. Am J Physiol 1995; 269: G628–646.
9. Smadja C, Maldonado R, Turcaud S, Fournie-Zaluski M C, Roques B P. Opposite role of CCK_A and CCK_B receptors in the modulation of endogenous enkephalin antidepressant-like effects. Psychopharmacology 1995; 120: 400–408.
10. Valverde O, Fournie-Zaluski M C, Roques B P, Maldonado R. The CCK_B antagonist PD-134,308 facilitates rewarding effects of endogenous enkephalins but does not induce place preference in rats. Psychopharmacology 1996; 123: 119–126.
11. Valverde O, Smadja C, Roques B P, Maldonado R. The attenuation of morphine place preference following chronic mild stress reversed by a CCK_B receptor antagonist. Psychopharmacology 1997; 131: 79–85.
12. Valverde O, Maldonado R, Fournie-Zaluski M C, Roques B P. Cholecystokinin B antagonists strongly potentiate antinociception mediated by endogenous enkephalins. J Pharmacol Exp Ther 1994; 270: 77–88.
13. Riley A, Melton P M. Effects of μ- and δ-opioid receptor agonists on the stimulus properties of cholecystokinin. Pharmacol Biochem Behav 1997; 57: 57–62.
14. Shlik J, Vasar E, Bradwejn J. Cholecystokinin and psychiatric disorders. Role in aetiology and potential of receptor antagonists in therapy. CNS Drugs 1997; 8: 134–152.
15. Vasar E, Kõks S, Volke V, Võikar V. Cholecystokinin in animal models of anxiety. Biol Psychiatry 1997; 42, 1S: 196–197.
16. Handley S L, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn- Schmiedebergs Arch Pharmacol 1984; 327: 1–5.
17. Pellow S, Chopin P, File S E, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985; 14: 149–167.
18. Tsuda M, Suzuki T, Misawa M, Nagase H. Involvement of the opioid system in the anxiolytic effect of diazepam in mice. Eur J Pharmacol 1996; 307: 7–14.
19. Motta V, Brandao M L. Aversive and antiaversive effects of morphine in the dorsal periaquaductal grey of rats submitted to the elevated plus-maze test. Pharmacol Biochem Behav 1993; 44: 119–125.
20. Privette T H, Terrian D M. Kappa opioid agonists produce anxiolytic-like behavior on the elevated plus-maze. Psychopharmacology 1995; 118: 444–450.
21. Wiertelak E P, Maier S F, Watkins L R. Cholecystokinin antianalgesia: safety cues abolish morphine analgesia. Science 1992; 256: 830–833.

22. Lavigne G J, Millington W R, Mueller G P. The CCK-A and CCK-B receptor antagonists, devazepide and L-365, 260, enhance morphine antinociception only in non-acclimated rats exposed to a novel environment. Neuropeptides 1992; 21: 119–129.
23. Benedetti F, Amanzio M, Casadio C, Oliaro A, Maggi G. Blockade of nocebo hyperalgesia by the cholecystokinin antagonist proglumide. Pain 1997; 71: 135–140.
24. Perez-Costillas L, Gurpegui M, Ortega E. Low serum beta-endorphin levels in panic disorder. American Psychiatric Association, 150th Annual Meeting, 17–22 May, 1997, San Diego, California, NR 491, 200–201.

III

Kõks S., Bourin M., Võikar V., Soosaar A., Vasar E.
Role of CCK in anti-exploratory action of paroxetine, 5-HT reuptake inhibitor.
Int J Neuropsychopharmacol 1999; 2 (in press).

International Journal of Neuropsychopharmacology (1999), 2, 000–000.

ARTICLE

Role of CCK in anti-exploratory action of paroxetine, 5-HT reuptake inhibitor

Sulev Kõks[1,2], Michel Bourin[3], Vootele Võikar[1], Andres Soosaar[1] and Eero Vasar[1]

[1] *Department of Physiology, University of Tartu, 2 Näituse Street, EE2400 Tartu, Estonia*

[2] *Institute of Molecular and Cell Biology, University of Tartu, 23 Riia Street, EE-2400 Tartu, Estonia*

[3] *Laboratory of Pharmacology, University of Nantes, 1 rue Gaston-Veil, Nantes Cedex 44035, France*

Abstract

The administration of paroxetine (0.5–8 mg/kg), a selective 5-HT reuptake inhibitor, induced a dose-dependent reduction of exploratory activity of rats in the motility test. In the elevated plus-maze paroxetine was less effective, only 8 mg/kg of paroxetine decreased the exploratory behaviour of rats. The doses of paroxetine (2–8 mg/kg) reducing the exploratory activity in the motility test increased the density of CCK receptors in the frontal cortex, but not in the hippocampus. The treatment of rats with the CCK_B receptor antagonist LY288,513 (0.01–1 mg/kg) did not change the exploratory activity. However, the reduction of exploratory activity induced by the low dose of paroxetine (2 mg/kg), but not by the higher dose (8 mg/kg), was dose-dependently reversed by the administration of LY288,513. Moreover, LY288,513 did not affect the anti-exploratory action of paroxetine (8 mg/kg) in the elevated plus-maze. Diazepam at doses (0.5–1.0 mg/kg) not suppressing the locomotor activity did not change the anti-exploratory action of paroxetine in the motility test. It is likely that the anti-exploratory action of a low dose of paroxetine (2 mg/kg) is not related to the increase in anxiety, but rather to the reduction of exploratory drive. Evidence exists that this effect of paroxetine is mediated via the activation of CCK-ergic transmission.

Received 7 September 1998; Reviewed 9 November 1998; Revised 1 December 1998; Accepted 6 December 1998

Key words: 5-hydroxytryptamine, cholecystokinin, motility test, elevated plus-maze, rat.

Introduction

There is a growing body of evidence that cholecystokinin (CCK) and 5-hydroxytryptamine (5-HT) interact in the regulation of behaviour. The administration of cholecystokinin tetrapeptide (CCK-4), an agonist of CCK_B (brain subtype, CCK_2) receptors, induces an anxiogenic-like action in the elevated plus-maze, and this behavioural action of CCK agonist is accompanied by the increased release of 5-HT in the cerebral cortex (Rex et al., 1994). On the other hand, CCK_B receptor antagonist L365,260 causes the opposite effect and antagonizes the behavioural and neurochemical action of CCK-4 (Rex et al., 1994). Peripherally administered CCK-8 reduces the food intake and elevates the levels of 5-HT in the hypothalamus (Voigt et al., 1998). The stimulation of somatodendritic 5-HT autoreceptors by 5-HT_{1A} receptor agonist 8-OH-DPAT reverses CCK-8 induced satiety and CCK-4 caused anxiety (Poeschla et al., 1992; Rex et al., 1997). However, it should be noted that the anxiogenic-like action of CCK is mediated via the CCK_B receptor subtype, whereas CCK_A (peripheral subtype, CCK_1) receptors are responsible for CCK-induced satiety (Shlik et al., 1997). The application of CCK-4 to the brain membranes increases the density of 5-hydroxytryptamine 5-HT_2 receptors in the frontal cortex (Agnati et al., 1983). Therefore, it is not surprising that the 5-HT_2 receptor antagonist deramciclane antagonizes the anxiogenic-like action of caerulein, an unselective CCK_A/CCK_B receptor agonist (Gacsalyi et al., 1997).

However most experiments have studied a role of 5-HT in the action of CCK receptor agonists and antagonists, whereas very little attention is paid to the opposite interaction. Nevertheless, it has been shown that 5-HT can increase the release of CCK in the cerebral cortex and nucleus accumbens (Raiteri et al., 1993). The acute administration of alaproclate, the 5-HT reuptake inhibitor, significantly elevates the levels of CCK in the cingulate cortex and periaquaductal grey (Rosén et al., 1995). Moreover, the exposure of rats to the novel aversive environment clearly increases the release of 5-HT in the frontal cortex and hippocampus (File et al., 1993; Rex et al., 1994). The administration of fluoxetine, the 5-HT reuptake inhibitor, dose-dependently reduces the exploratory behaviour of rats in the elevated plus-maze

Address for correspondence: Dr Sulev Kõks, Department of Physiology, University of Tartu, 2 Näituse Street, EE2400 Tartu, Estonia.
Tel: 3727 374330 *Fax*: 3727 374332
E-mail: sulev.koks@ut.ee

(Handley and McBlane, 1993). Handley and McBlane (1993) have considered the anti-exploratory action of fluoxetine as the anxiogenic-like action. Den Boer and Westenberg (1996) have shown in the clinical studies that the acute administration of 5-HT reuptake inhibitors may increase anxiety in patients suffering from anxiety disorders. Therefore, the aim of present experiments was to clarify a possible role of CCK in the anxiogenic-like action of drugs increasing 5-HT-ergic transmission. For that purpose a selective 5-HT reuptake inhibitor paroxetine was chosen. The behavioural effects of paroxetine were analysed in the motility box and elevated plus-maze. These two behavioural tests were selected to distinguish whether paroxetine-induced inhibition of exploratory behaviour is due to the increase in anxiety or the reduction of exploratory drive. Moreover, the action of paroxetine was studied on the parameters of CCK binding in the frontal cortex and hippocampus. Also the influence of CCK_B antagonist LY288,513 and diazepam, a benzodiazepine anxiolytic drug, on the behavioural effects of paroxetine was examined.

Materials and methods

Animals

Male Wistar (Han/Kuo) rats (National Animal Centre, Kuopio, Finland) weighing 200–220 g were kept in the animal house at 20 ± 2 °C in a 12-h light/dark cycle (light on at 07:00 hours). Tap water and food pellets were available ad libitum. All animal procedures were approved by University of Tartu Animal Care Committee in accordance with the European Communities Directive of 24 November 1986 (86/609/EEC). The animals were kept in the animal house at least 2 wk before the beginning of experiment.

Materials

Paroxetine, a selective 5-HT reuptake inhibitor, was provided by SmithKline and Beecham (UK). LY288,513 [*trans*-*N*-(4-bromophenyl)-3-oxo-4,5-diphenyl-1-pyrazolidinecarboxamide], an antagonist of CCK_B receptor, was kindly donated by Eli Lilly & Co. (USA). Paroxetine was dissolved in saline, whereas LY288,513 and diazepam (Sigma, USA) were suspended in 1% Tween-80 (Ferak, Germany) solution in saline. [Propionyl-^{3}H]propionylated-CCK-8-sulphated ([^{3}H]pCCK-8) was obtained from Amersham Radiochemicals (UK). The other chemicals for radioligand-binding studies (caerulein, Hepes (*N*-[2-hydroxyethyl]piperazine-*N*′-[-2-ethane-sulphonic acid]), NaCl, $MgCl_2$, KCl and EDTA ([ethylenediamine-tetraacetic acid]) were purchased from Sigma (USA).

Behavioural testing

The animals were brought into the experimental room 1 h before the experiment. The rats were new to handling and were not adapted to the experimental situation. Each rat was used only once. All experiments were carried out between 14:00 and 19:00 hours. Paroxetine, diazepam and LY288,513 were given 30 min before the beginning of studies.

Motility test

Exploratory activity of rats was measured by means of photoelectric motility boxes (448 × 448 × 450 mm) connected to a computer (TSE Technical & Scientific Equipment GmbH, Germany). Animals, new to the test situation were placed singly into the apparatus. Time in exploration (s), distance of exploration (in metres), number and duration of rearing were registered in 5-min intervals during the 15-min observation period.

Elevated plus-maze

The method initially suggested by Handley and Mithani (1984) for the measurement of exploratory activity was employed in rats with some modifications (Pellow et al., 1985). The apparatus consisted of two opposite open arms (50 × 10 cm) without side walls and two enclosed arms (50 × 10 × 40 cm) with side walls and end wall, extending from a central square (10 × 10 cm). The maze was elevated to the height of 50 cm, and placed in a lit room. During a 5 min observation session the following measures were taken by an observer: (1) time spent in exploring of open part (central square and open arms) of plus-maze; (2) time spent in open arm; (3) number of closed and open arm entries; (4) number of line crossings in open part; and (5) ratio between open and total arm entries. At the beginning of experiment an animal was placed into the centre of plus-maze, facing towards a closed arm. An arm entry was counted only when all four limbs of the rat were within a given arm.

[^{3}H]pCCK-8 binding assay

The animals were decapitated 30 min after the injection of various doses of paroxetine (0.5–16 mg/kg i.p.). After decapitation the brains were quickly dissected on ice. The binding studies were performed in the frontal cortex (also containing the anterior cingulate and frontoparietal cortex) and hippocampus. These brain structures were selected according to previous studies since the most prominent neurochemical changes due to the reduced exploratory behaviour have been found in these brain

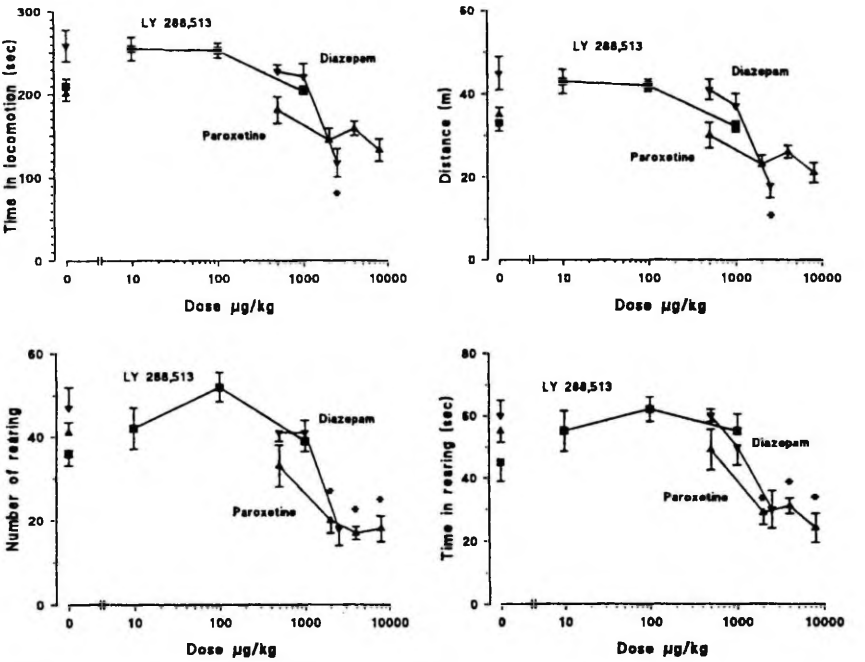

Figure 1. The effect of paroxetine (0.5–8 mg/kg), diazepam (0.5–2.5 mg/kg) and LY288,513 (0.01–1 mg/kg) on the exploratory activity of rat in the motility box. $^{*}p < 0.05$ compared to saline (paroxetine) or vehicle-treated (LY288,513, diazepam) rats, Tukey HSD test after the significant one-way ANOVA). Vehicle for LY288,513 and diazepam was 1% Tween-80 solution in saline.

regions (File et al., 1993a; Harro et al., 1995; Kõks et al., 1997; Rex et al., 1994). Brain tissues were homogenized in 20 volumes of ice-cold 50 mM Tris-HCl (pH 7.4 at 4 °C) using a Potter-S glass-Teflon homogenizer (1000 rpm, 12 passes). The membranes were washed twice in the same buffer by centrifugation (48000 *g* for 20 min) and resuspension. After the last centrifugation, the crude brain membranes were homogenized in Hepes buffer (10 mM Hepes; 130 mM NaCl; 5 mM KCl; 1 mM $MgCl_2$; 1 mM EDTA; pH 6.5 adjusted with 1 N NaOH) containing bovine serum albumin (0.5 mg/ml). The parameters of CCK receptors were determined in the presence of 0.05–2.4 nM [^{3}H]pCCK-8 (specific activity 79 Ci/mmol) at 23 °C in a total incubation volume of 0.5 ml. Caerulein (100 nM) was added to determine the nonspecific binding. The incubation was terminated after 120 min by the rapid filtration over Whatman GF/B filters presoaked with the bovine serum albumin (0.5 mg/ml). The filters were washed with 3 × 3 ml of ice-cold Hepes buffer. In the separate study paroxetine (0.01–1 mM) was added to the incubation medium to reveal the direct interaction of paroxetine with CCK-binding sites in the cerebral cortex. The protein content was measured according to a dye-binding assay (Bradford, 1976). Saturation curves were analysed using nonlinear least squares regression (Leatherbarrow, 1987).

Statistics

Results are expressed as mean values ± S.E.M. The behavioural studies were analysed using one-way analysis of variance (ANOVA). Post hoc comparisons between individual groups were performed by means of Tukey HSD test using the Statistica for Windows software. The data of radioligand-binding experiments were assessed by means of Student's *t* test.

Results

The administration of paroxetine (0.5, 2, 4, 8 mg/kg) induced a dose-dependent reduction of exploratory activity of rats in the motility test (Figure 1). Paroxetine significantly reduced the frequency of rearing ($F_{4,51} = 3.50$, $p < 0.05$) and time spent in rearing ($F_{4,51} = 3.02$,

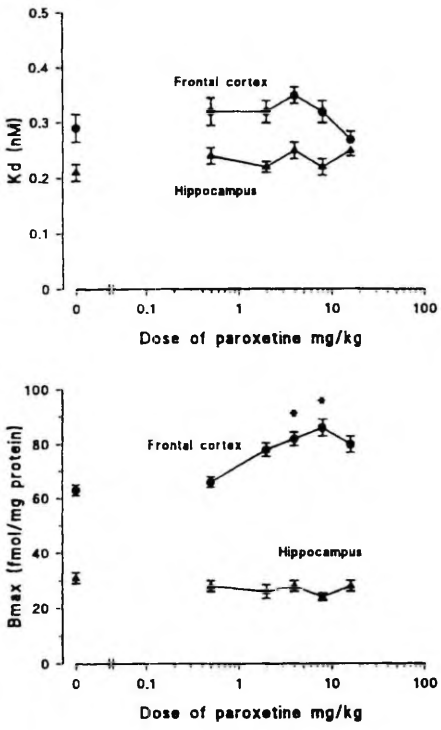

Figure 2. The effect of paroxetine (0.5–16 mg/kg) on the parameters of CCK binding in the frontal cortex and hippocampus. K_d, dissociation constant in nM; B_{max}, the apparent number of binding sites in fmol/mg protein. *$p < 0.05$ (compared to saline-treated rats, Student's t test).

$p < 0.05$). Paroxetine also tended to inhibit the other parameters of exploratory activity in the motility test. However, these changes were not statistically significant. The same doses of paroxetine tended to elevate the density of CCK-binding sites in the frontal cortex (also including the cingulate and frontoparietal cortex), but not in the hippocampus (Figure 2). The addition of paroxetine (0.01–1 mM) to the brain membranes did not modify CCK binding in the cerebral cortex (data not shown). Differently from the motility test, only 8 mg/kg of paroxetine inhibited the exploratory behaviour of rats in the elevated plus-maze. Paroxetine decreased time spent in open part ($F_{5,43} = 2.63$, $p < 0.05$), number of line crossings ($F_{5,43} = 2.97$, $p < 0.05$) and closed arm entries ($F_{5,43} = 3.18$, $p < 0.05$) (Table 1).

LY288,513 (0.01–1 mg/kg), a CCK_B receptor antagonist, did not affect the behaviour of rats in the motility test (Figure 1). Nevertheless, the pretreatment of rats with LY288,513 dose-dependently antagonized the anti-exploratory action of paroxetine (2 mg/kg; time in locomotion $F_{4,35} = 2.8$, $p < 0.05$; frequency of rearing $F_{4,35} = 3.83$, $p < 0.05$; time in rearing $F_{4,35} = 4.13$, $p < 0.01$) (Figure 3). The combination of CCK_B receptor antagonist with the higher dose of paroxetine (8 mg/kg) did not reverse the behavioural suppression induced by 5-HT reuptake inhibitor (Figure 4). In addition, LY288,513 (0.01–1 mg/kg) did not antagonize the anti-exploratory action of paroxetine (8 mg/kg) in the elevated plus-maze (data not shown).

The administration of the benzodiazepine agonist diazepam (0.5–2.5 mg/kg) also suppressed the exploratory activity of rats at the highest dose (2.5 mg/kg) (Figure 1, time spent in exploration $F_{3,32} = 3.33$, $p < 0.05$; the exploration distance $F_{3,32} = 2.97$, $p < 0.05$).

Table 1. The effect of paroxetine (0.5–16 mg/kg) on the exploratory activity of rats in the elevated plus-maze

Treatment	Time in open part (s)	No. of line crossings	No. of open arm entries	Time in open arm (s)	No. of closed arm entries	Ratio open:total arm entries
Saline	124±13	24±2.7	1.6±0.5	18±6	6.0±0.5	18±5
Paroxetine (0.5 mg/kg)	114±18	21±3.8	1.4±0.3	21±5	5.1±0.9	19±4
Paroxetine (2 mg/kg)	131±17	25±5.5	1.3±0.6	19±9	6.0±1.2	10±4
Paroxetine (4 mg/kg)	114±15	19±3.9	1.3±0.7	13±8	4.4±0.7	12±7
Paroxetine (8 mg/kg)	66±16*	8±1.9*	0.3±0.3	4±3	1.9±0.4*	6±4
Paroxetine (16 mg/kg)	85±13	14±4	0.5±0.4	18±13	4.0±1.2	7±5

*$p < 0.05$ (Tukey HSD test after the significant one-way ANOVA, compared to saline-treated rats).

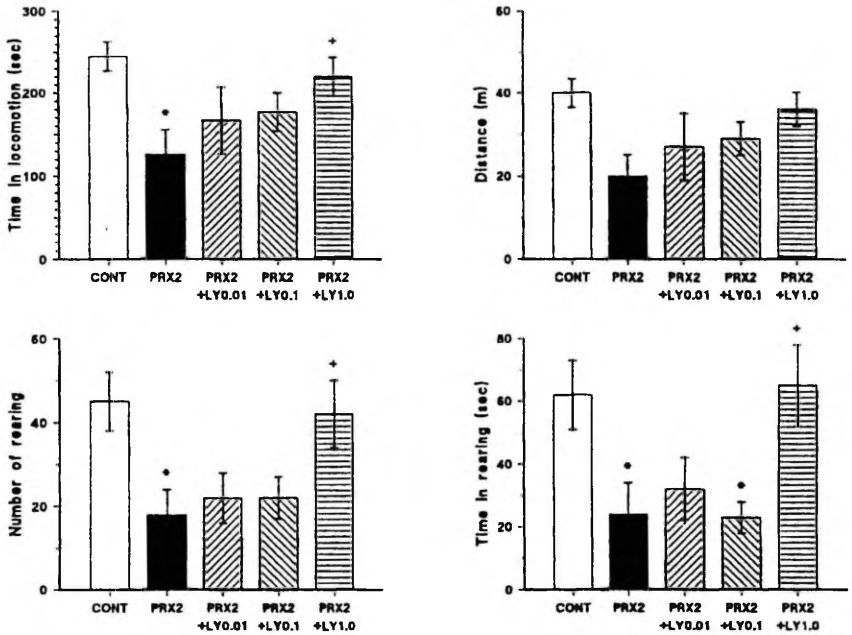

Figure 3. The effect of LY288,513 (0.01–1 mg/kg) on the anti-exploratory action of paroxetine (2 mg/kg). $^{*}p < 0.05$ (compared to saline + vehicle-treated rats); +, $p < 0.05$ (compared to paroxetine-treated rats, Tukey HSD test after the significant one-way ANOVA). CONT, saline + vehicle-treated rats; PRX2, paroxetine 2 mg/kg; LY, LY288,513.

However, the combination of diazepam at doses not suppressing exploratory behaviour (0.5–1.0 mg/kg) with paroxetine (2 mg/kg) did not modify the anti-exploratory action of 5-HT reuptake inhibitor (Figure 5).

Discussion

The administration of paroxetine, a selective inhibitor of 5-HT reuptake, induced a significant suppression of exploratory activity of rats in the motility test. Already 2 mg/kg of paroxetine inhibited the frequency of rearing and time spent in rearing. However, the selectivity of paroxetine in inhibition of rearing depends on the basal exploratory activity of control animals. In the experiments where the activity of rats was higher, this dose of paroxetine also reduced the other parameters of exploratory behaviour, namely time spent in exploration and distance of exploration. Nevertheless, the frequency of rearing and time spent in rearing are the most sensitive parameters in revealing the anti-exploratory action of paroxetine in the motility test. Moreover, paroxetine was apparently less potent in the reduction of exploratory activity of rats in the elevated plus-maze. Only 8 mg/kg of paroxetine decreased the exploratory behaviour of animals. The anti-exploratory action of paroxetine in the elevated plus-maze was accompanied by the decreased number of closed arm entries, reflecting the suppression of locomotor activity. The existing experimental data support the role of 5-HT in the regulation of anxiety. Handley and McBlane (1993) have shown that the administration of fluoxetine, the 5-HT reuptake inhibitor, induces the anxiogenic-like action in the rat elevated plus-maze. The administration of paroxetine produced an anxiogenic-like profile in the rat two-compartment exploration test (Sanchez and Meier, 1997). The exposure of rats to the aversive environment clearly increases the release of 5-HT in the frontal cortex and hippocampus (File et al., 1993; Rex et al., 1994). Nevertheless, the present behavioural study is in disagreement with the data since the anti-exploratory action of paroxetine is related to the decrease of exploratory drive rather than to the increase in anxiety.

Rosén et al. (1995) have shown that 5-HT reuptake inhibitor alaproclate increases the levels of CCK in the cingulate cortex and periaquaductal grey. The direct application of 5-HT to the neurons of cerebral cortex and nucleus accumbens evoked the release of CCK (Raiteri et al., 1993). In the present study paroxetine elevated the

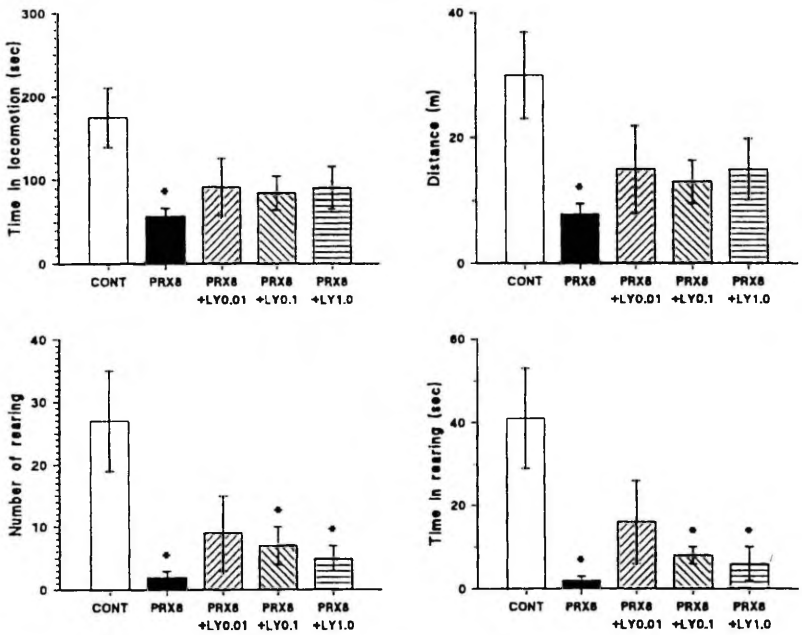

Figure 4. The effect of LY288,513 (0.01–1 mg/kg) on the anti-exploratory action of paroxetine (8 mg/kg). $^{*}p < 0.05$ (compared to saline + vehicle-treated rats, Tukey HSD test after the significant one-way ANOVA). CONT, saline + vehicle-treated rats; PRX8, paroxetine 8 mg/kg; LY, LY288,513.

number of CCK-binding sites in the frontal cortex, but not in the hippocampus. The stressful manipulations in rats are shown to increase the density of CCK receptors and mRNA levels of preproCCK in the frontal cortex (Harro et al., 1995; Pratt and Brett, 1995). The social isolation of rats for 7 d induced an anxiogenic-like action and elevated the number of CCK receptors in the frontal cortex, but not in the hippocampus (Vasar et al., 1993). Therefore, it is not surprising that the pretreatment of rats with the CCK_B receptor antagonist LY288,513 dose-dependently reversed the anti-exploratory action of paroxetine (2 mg/kg). This in line with the study of Matto et al. (1996) showing the ability of the CCK_B receptor antagonist L365,260 to antagonize the anti-exploratory effect of the 5-HT reuptake inhibitor citalopram in the elevated plus-maze. The data support the hypothesis that the administration of 5-HT reuptake inhibitors increases CCK-mediated neurotransmission in the brain. The antagonism of LY288,513 against paroxetine seems to be a specific since the CCK_B receptor antagonist does not increase the exploratory activity per se. Nevertheless, LY288,513 did not block the effect of the higher dose of paroxetine (8 mg/kg) showing a difference in the action of two doses of 5-HT reuptake inhibitor. This discrepancy could be explained by the fact that paroxetine at higher doses induces a more pronounced increase in the concentration of 5-HT in the synaptic cleft, masking a possible interaction of a drug with CCK. Differing from the present study, Harro et al. (1997) did not find changes in the density of CCK receptors or in the content of CCK-related peptides after long-term treatment with various antidepressant drugs, including 5-HT reuptake inhibitors. However, they analysed the action of long-term administration of antidepressant drugs, whereas the effect of acute treatment was not examined.

In conclusion, the selective serotonin reuptake inhibitor paroxetine induces a clear anti-exploratory effect in the motility boxes. However, the above-described behavioural studies do not reflect the increase in anxiety after the acute treatment with paroxetine. This statement is supported by the findings that paroxetine did not cause the anxiogenic-like action in the elevated plus-maze and that diazepam, a potent anxiolytic drug, did not antagonize the anti-exploratory action of paroxetine in the motility test. Therefore, it is likely that paroxetine reduces the exploratory drive in rats. This effect of paroxetine seems to be mediated via the increase in CCK-ergic neurotransmission.

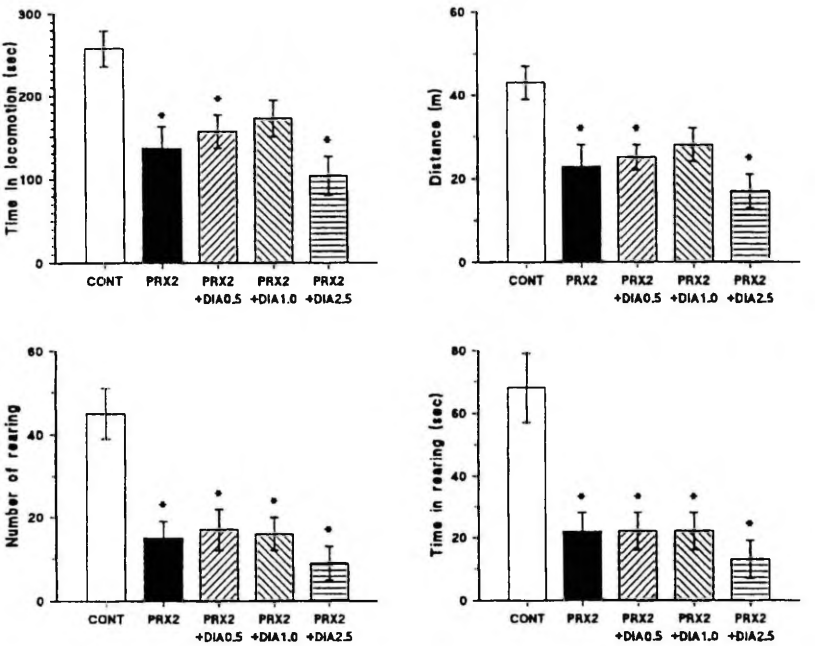

Figure 5. The effect of diazepam (0.5–2.5 mg/kg) on the anti-exploratory action of paroxetine (2 mg/kg). $^{*}p < 0.05$ (compared to saline + vehicle-treated rats, Tukey HSD test after the significant one-way ANOVA). CONT, saline + vehicle-treated rats; PRX2, paroxetine 2 mg/kg; DIA, diazepam.

Acknowledgements

Paroxetine and LY288,513 were kindly provided by SmithKline Beecham, and Eli Lilly & Co., respectively. This study was also supported by grant no. 1409 from the Estonian Science Foundation.

References

Agnati LF, Fuxe K, Benfenati F, Celani MF, Battistini N, Mutt V, Cavicchioli L, Galli G, Hökfelt T (1983). Differential modulation by CCK-8 and CCK-4 of [^{3}H]spiperone binding sites linked to dopamine and 5-hydroxytryptamine receptors in the brain of the rat. *Neuroscience Letters 3*, 179–183.

Bradford MM (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry 72*, 248–254.

Den Boer JA, Westenberg HGM (1996). Involvement of serotonin receptor subtypes in panic disorder: a critical appraisal of the evidence. In: Westenberg HGM, Den Boer JA, Murphy DI (Eds.), *Advances in the Neurobiology of Anxiety Disorders* (pp. 139–172). John Wiley & Sons Ltd.

File SE, Zangrossi JR, Andrews N (1993). Social interaction and elevated plus-maze tests: changes in release and uptake of 5-HT and GABA. *Neuropharmacology 32*, 217–221.

Gacsalyi I, Schmidt E, Gyertyan I, Vasar E, Lang A, Haapalinna A, Fekete M, Hietala J, Syvälahti E, Tuomainen P, Männistö PM (1997). Receptor binding profile and anxiolytic-like activity of deramciclane (EGIS 3886) in animal models. *Drug Development Research 40*, 333–348.

Handley SL, McBlane JW (1993). 5-HT drugs in animal models of anxiety. *Psychopharmacology 112*, 13–20.

Handley SL, Mithani S (1984). Effects of alpha-adrenoreceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behavior. *Naunyn-Schmiedeberg's Archives of Pharmacology 327*, 1–5.

Harro J, Lõfberg C, Pähkla R, Matto V, Rägo L, Oreland L, Allikmets L (1997). Different molecular forms of cholecystokinin and CCKB receptor binding in the rat brain after chronic antidepressant treatment. *Naunyn-Schmiedeberg's Archives of Pharmacology 355*, 57–63.

Harro J, Vasar E, Koszycki D, Bradwejn J (1995). Cholecystokinin in panic and anxiety disorders. In: Panksepp J (Ed.), *Advances in Biological Psychiatry* (pp. 235–262). Greenwich, CT: JAI Press Inc.

Kõks S, Vasar E, Soosaar A, Lang A, Volke V, Võikar V, Bourin M, Männistö PT (1997). Relation of exploratory

behavior of rats in elevated plus-maze to brain receptor binding properties and serum growth hormone levels. *European Neuropsychopharmacology 7*, 289–294.

Leatherbarrow RJ (1987). *Enzfitter, a non-linear regression data analysis program for the IBM PC*. Amsterdam: Elsevier.

Matto V, Harro J, Allikmets L (1996). The effects of cholecystokinin A and B antagonists, devazepide and L-365,260, on citalopram-induced decrease of exploratory behaviour in rat. *Journal of Physiology and Pharmacology 47*, 661–669.

Pellow S, Chopin P, File SE, Briley M (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods 14*, 149–167.

Poeschla B, Gibbs J, Simansky KY, Smith GP (1992). The 5-HT_{1A} agonist 8-OH-DPAT attenuates the satiety action of cholecystokinin. *Pharmacology, Biochemistry and Behavior 42*, 541–543.

Pratt JA, Brett RR (1995). The benzodiazepine inverse agonist FG 7142 induces cholecystokinin gene expression in rat brain. *Neuroscience Letters 184*, 197–200.

Raiteri M, Paudice P, Vallebuona F (1993). Release of cholecystokinin in the central nervous system. *Neurochemistry International 22*, 519–527.

Rex A, Fink H, Marsden CA (1994). Effects of BOC-CCK-4 and L-365,260 on cortical 5-HT release in guinea-pigs on exposure to the elevated plus-maze. *Neuropharmacology 33*, 559–565.

Rex A, Marsden CA, Fink H (1997). Cortical 5-HT-CCK interactions and anxiety-related behaviour in guinea-pigs: a microdialysis study. *Neuroscience Letters 228*, 79–82.

Rosén A, Franck J, Brodin E (1995). Effects of acute systemic treatment with the 5-HT-uptake blocker alaprocalate on tissue levels and release of substance P in rat periaquaductal grey. *Neuropeptides 28*, 317–324.

Sanchez C, Meier E (1997). Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? *Psychopharmacology 129*, 197–205.

Shlik J, Vasar E, Bradwejn J (1997). Cholecystokinin and psychiatric disorders. Role in aetiology and potential of receptor antagonists in therapy. *CNS Drugs 8*, 134–152.

Vasar E, Peuranen E, Harro J, Lang A, Oreland L, Mannisto PT (1993). Social isolation of rats increases the density of cholecystokinin receptors in the frontal cortex and abolishes the anti-exploratory effect of caerulein. *Naunyn-Schmeideberg's Archives of Pharmacology 348*, 96–101.

Voigt JP, Sohr R, Fink H (1998). CCK-8S facilitates 5-HT release in the rat hypothalamus. *Pharmacology, Biochemistry and Behavior 59*, 179–182.

IV

Kõks S., Soosaar A., Võikar V., Bourin M., Vasar E.
BOC-CCK-4, CCKB receptor agonist, antagonizes anxiolytic-like
action of morphine in elevated plus-maze.
Neuropeptides 1999; 33 (in press).

Neuropeptides (1999) 33 (1), 000–000

BOC-CCK-4, CCK_B receptor agonist, antagonizes anxiolytic-like action of morphine in elevated plus-maze

S. Kõks,[1,2] A. Soosaar,[1] V. Võikar,[1] M. Bourin,[3] E. Vasar[1]

[1]Department of Physiology, University of Tartu, Estonia [2]Institute of Molecular and Cell Biology, University of Tartu, Estonia [3]Laboratory of Pharmacology, University of Nantes, France

Summary This study investigated a role of cholecystokinin (CCK) in the anxiolytic-like action of morphine, an agonist of μ-opioid receptors, in the rat plus-maze model of anxiety. The acute administration of morphine (1 mg/kg) induced a significant increase of exploratory activity in the plus-maze, but did not affect the locomotor activity in the motility test. The higher dose of morphine (2.5 mg/kg) tended to decrease the locomotor activity and, therefore, did not cause the anxiolytic-like action in the plus-maze. The other drugs (naloxone, BOC-CCK-4, L-365,260) and their combinations with morphine (0.5–1 mg/kg) did not affect the locomotor activity of rats. The opioid antagonist naloxone itself (0.5 mg/kg) did not change the exploratory activity in the plus-maze, but potently antagonized the anxiolytic-like action of morphine (1 mg/kg). An agonist of CCK_B receptors BOC-CCK-4 (1–50 μg/kg) induced a dose-dependent anxiogenic-like action in the plus-maze. Nevertheless, only one dose of BOC-CCK-4 (10 μg/kg) completely reversed the action of morphine. Also, one dose of CCK_B receptor antagonist L-365,260 (10 μg/kg) was effective to modify the behaviour of rats in the elevated plus-maze. Namely, this dose of L-365,260 increased the ratio between open and total arm entries, a behavioural measure believed to reflect the anxiolytic-like action in the elevated plus-maze. The combination of L-365,260 (100 μg/kg) with the sub-effective dose of morphine (0.5 mg/kg) caused the anxiolytic-like action in the plus-maze not seen if the drugs were given alone. In conclusion, morphine induces a potent anxiolytic-like action in the elevated plus-maze and CCK is acting as an endogenous antagonist of this effect of morphine.

INTRODUCTION

Recent studies suggest that the administration of opioid receptor agonists potently decreases anxiety in the rat plus-maze model. Motta and Brandao[1] have shown that the systemic treatment with morphine, an agonist of μ-opioid receptors, at low doses or its injection into the dorsal periaquaductal gray induced anxiolytic-like action in the plus-maze. An antagonist of opioid receptors naloxone at low doses reversed the anxiolytic-like effect of morphine. Privette and Terrian[2] have found that the administration of κ opioid agonists, U-50, 488H and U-69, 593, induces a strong anxiolytic-like action in the elevated plus-maze. This action of κ opioid agonists is reversed by naloxone, also suggesting an opioid receptor site of action[2].

The behavioural effectiveness of opioid receptor agonists seems to be dependent on the activity of cholecystokinin (CCK), a neuropeptide widely distributed in the mammalian brain. The distribution of CCK in the brain parallels that of endopioid peptides in the various brain regions[3,4]. Moreover, CCK and endopioid peptides are colocalized in the same neurons in discrete brain areas.[5,6] Two different CCK receptor subtypes, respectively CCK_A (peripheral subtype) and CCK_B (brain subtype) receptors, are described in the central nervous system.[7,8] It is suggested that CCK might function via the interaction with CCK_B receptors as an endogenous opioid antagonist in various behavioural models.[9,11] CCK_B receptor antagonists, but not CCK_A antagonists,

Received 18 November 1998
Accepted 10 February 1999

Correspondence to. Dr. Sulev Kõks, Department of Physiology, University of Tartu, 2 Näituse Street, EE2400 Tartu, Estonia. Tel. + 372 7 37433 Fax: + 372 7 374332; E-mail: sulev.koks@ut.ee

facilitate the antidepressant-like effect induced by opioid peptides in the conditioned suppression of motility test in mice[9]. Moreover, the selective CCK_B receptor antagonist PD-134,308 is shown to potentiate the rewarding effect of morphine in the place preference paradigm[10] and to augment antinociception induced by endopiod peptides.[12] The CCK_B receptor antagonists L-365,260 and PD-134,308 reversed the place aversion induced by naloxone in morphine dependent rats, whereas the CCK_A receptor antagonist devazepide was ineffective.[13]

CCK is also implicated in the neurobiology of anxiety.[14] Recently, we have found that the pretreatment with the opioid antagonist naloxone potentiates the anxiogenic-like action of CCK agonists in the plus-maze.[15] Therefore, in the present study an attempt was done to show that CCK acts as an antagonist of the anxiolytic-like action of morphine. The interaction of BOC-CCK-4, a CCK_B receptor agonist, and L-365, 260, a CCK_B receptor antagonist, with morphine was studied in the elevated plus-maze model of anxiety. Simultaneously, the locomotor activity of animals was measured in the motility boxes to exclude the unspecific interaction with the locomotor activity in rats.

MATERIAL AND METHODS

Animals

Male Wistar (Han/Kuo: WIST) rats (National Animal Centre, Kuopio, Finland) weighing 250–300 g were kept four per cage in the animal house at 20 ± 2C in 12 h light/dark cycle (light on at 07:00). Tap water and food pellets were available ad libitum. All subjects were experimentally naive and, apart from routine husbandry, were not specifically handled prior to testing.

The elevated plus-maze

The method initially suggested by Handley and Mithani[16] for the measurement of exploratory activity was employed in rats with some modifications.[17] The apparatus consisted of two opposite open arms (50 × 10 cm) without side walls and two enclosed arms (50 × 10 × 40 cm) with side walls and end wall, extending from a central square (10 × 10 cm). The maze was elevated to the height of 50 cm, and placed in a lit room. During a 5 min observation session the following measures were taken by an observer: 1) time spent in exploring of open part and open arms of plus-maze; 2) number of closed and open arm entries; 3) number of line crossings in open part; and 4) ratio between open and total arm entries. At the beginning of experiment an animal was placed into the center of plus-maze, facing toward an open arm. An arm entry was counted only when all four limbs of the rat were within a given arm. The animals were brought into the experimental room one hour before the experiment. Each animal was used only once.

Motility test

Immediately after the plus-maze exposure the rats were placed into the motility boxes. The locomotor activity was measured by means of photoelectric motility boxes (448 × 448 × 450 mm) connected to a computer (TSE Technical & Scientific Equipment GMBH, Germany). Time in exploration (s), distance of exploration (in metres), number and duration of rearing were registered during a 5 min observation period.

Drugs

Morphine sulphate (Boehringer-Ingelheim), BOC-CCK-4 (N-tert-butoxy-carbonyl-CCK-4, Sigma Co) and naloxone hydrochloride (Sigma Co) were dissolved in physiological saline (0.9 % sodium chloride solution). L-365,260 (Merck Sharp & Dohme) was suspended in saline with the help of few drops of Tween-80 (Sigma Co). L-365,260, BOC-CCK-4 and naloxone were administered intraperitoneally, whereas morphine was given subcutaneously. L-365,260 was administered 30 min before the experiment, naloxone and BOC-CCK-4 were injected 20 min and morphine 15 min prior to the study.

Statistics

Results are expressed as mean values ± S.E.M. The data of behavioural studies were analysed using one-way analysis of variance (ANOVA). Post hoc comparisons between individual groups were performed by means of Duncan multiple range test using the Statistica for Windows software.

RESULTS

The subcutaneous administration of morphine (1 mg/kg), a μ-opioid receptor agonist, induced a significant increase of exploratory behaviour in the elevated plus-maze (Figure 1). Morphine increased the number of open arm entries ($F_{3,77} = 5.18$, $P < 0.01$), time spent in open arm ($F_{3,77} = 5.53$, $P < 0.01$) and number of closed arm entries ($F_{3,77} = 5.60$, $P < 0.01$). However, the increase in the ratio between open and closed arm entries was not statistically significant ($F_{3,77} = 2.56$, $P = 0.06$, Duncan multiple range test $P = 0.056$,). This is probably due to the fact that morphine increased not only the number of open part entries, but also the number of closed arm entries. Despite the increase of close arm entries the administration of morphine (1 mg/kg) did not change the locomotor activity of rats in the motility box (Fig. 2). The

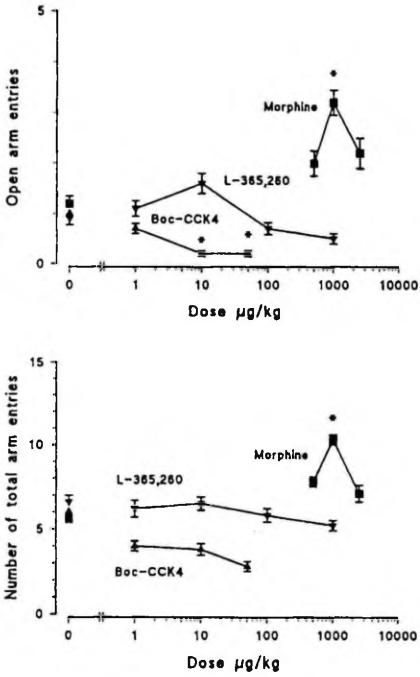

Fig. 1 The effect of morphine (0.5–2.5 mg/kg), BOC-CCK-4 (1–50 µg/kg) and L-365,260 (1–1000 µg/kg) on the exploratory behaviour of rats in the plus-maze.* – $P < 0.05$ (compared to vehicle-treated rats, Duncan multiple range test after the significant one-way ANOVA). Vehicle: saline in the case of morphine and BOC-CCK-4 and few drops of Tween-80 in saline in the case of L-365,260.

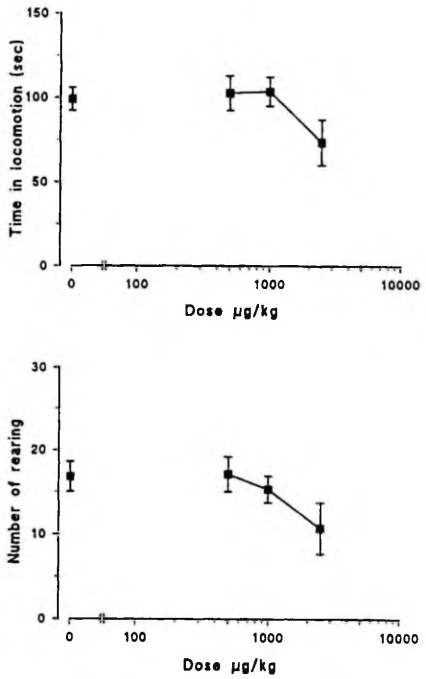

Fig. 2 The effect of morphine (0.5–2.5 mg/kg) on the locomotor activity of rats in the motility box.* – P < 0.05 (compared to saline-treated rats).

lower(0.5 mg/kg) and higher (2.5 mg/kg) doses of morphine did not modify the exploratory behaviour of rats in the elevated plus-maze (Fig. 1). Nevertheless, the higher dose of morphine (2.5 mg/kg) tended to reduce the locomotor activity in the motility test (Fig. 2; rearing time $F_{3,74} = 1.80$, $p = 0.16$; Duncan multiple range test, $P = 0.037$). The other tested drugs (naloxone, BOC-CCK-4 and L-365, 260) and their combinations with morphine (0.5–1 mg/kg) did not modify the locomotor activity of rats.

The pretreatment of rats with naloxone (0.5 mg/kg), an opioid receptor antagonist, did not change the exploratory behaviour of rats, but antagonized the anxiolytic-like action of morphine (Fig. 3). Naloxone antagonized morphine-induced increase of the number of open arm entries ($F_{3,41} = 4.30$, $p < 0.01$), time spent in open arm ($F_{3,41} = 4.61$, $P < 0.01$) and the number of closed arm entries ($F_{3,41} = 5.93$, $P < 0.01$). However, the antagonism of naloxone against the increase in the ratio between open and total arm entries was not statistically significant ($F_{3,41} = 1.65$, $P = 0.19$, Duncan multiple range test $P = 0.056$). The intraperitoneal injection of BOC-CCK-4, a selective CCK_B receptor agonist, induced a dose-dependent reduction of exploratory behaviour of rats in the plus-maze paradigm. One µg/kg of BOC-CCK-4 did not modify the behaviour of rats if compared to control rats (Fig. 1). However, the higher doses of BOC-CCK-4 (10–50 µg/kg) induced the statistically significant reduction in the behavioural measures believed to reflect anxiety in rodents – the number of open arm entries ($F_{3,69} = 4,52$, $P < 0.01$) and ratio between open and total arm entries ($F_{3,69} = 3.54$, $P < 0.05$) (Fig. 1). Ten µg/kg of BOC-CCK-4 blocked the anxiolytic-like action of morphine (the number of open arm time: $F_{4,57} = 2.72$, $P < 0.05$; time spent in open arm: $F_{4,57} = 2.65$, $P < 0.05$; the ratio between open

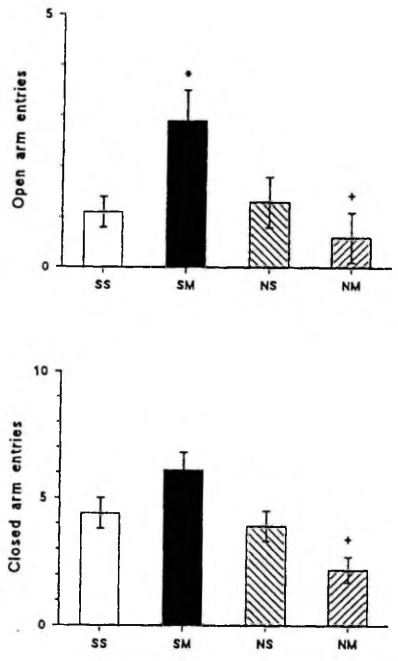

Fig. 3. The effect of naloxone (0.5 mg/kg) on the anxiolytic-like action of morphine (1 mg/kg) in the elevated plus-maze. SS (saline + saline); SM (saline + morphine); NS (naloxone + saline); NM (naloxone + morphine).* – $P < 0.05$ (compared to saline-treated rats);+ – $P < 0.05$ (compared to morphine-treated rats).

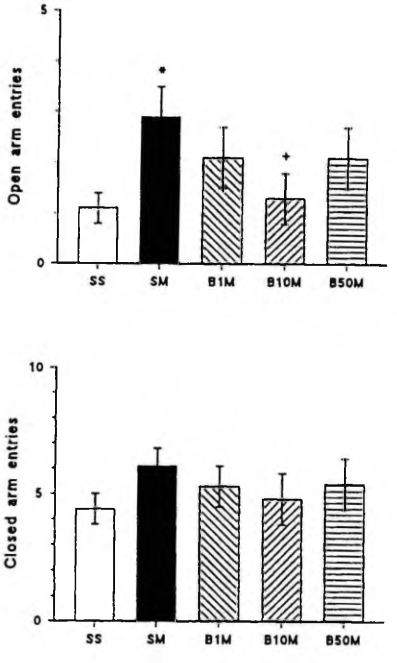

Fig. 4 The action of BOC-CCK-4 (1–50 μg/kg) on the anxiolytic-like action of morphine (1 mg/kg) in the elevated plus-maze. SS (saline + saline); SM (saline + morphine); B1M (BOC-CCK-4 1 μg/kg + morphine); B10M (BOC-CCK-4 10 μg/kg + morphine); B50M (BOC-CCK-4 50 μg/kg + morphine).* – $P < 0.05$ (compared to saline-treated rats);+ – $P < 0.05$ (compared to morphine-treated rats).

and total arm entries: $F_{4,57} = 1.36$, $P = 0.25$, Duncan multiple range test: $P = 0.076$), whereas the other doses of BOC-CCK-4 (1 and 50 μg/kg) were ineffective (Fig. 4). Only one dose (10 μg/kg) of L-0365,260, an antagonist of CCK_B receptors, tended to increase the exploratory behaviour of rats (Fig. 1). Namely, this dose of L-365,260 increased the ratio between open and total arm entries ($F_{4,78} = 3.05$, $P < 0.05$) and time spent in exploration of open arm ($F_{4,78} = 2.62$, $P < 0.05$). The combination of L-365,260 (100 μg/kg) with the sub-effective dose of morphine (0.5 mg/kg) induced a significant anxiolytic-like action in the elevated plus-maze. This dose of CCK receptor antagonist in combination with morphine increased the ratio between open and total arm entries ($F_{4,57} = 2.72$, $P < 0.05$; Fig. 5). The other doses of L-365,260 (10 and 1000 μg/kg) did not modify the action of morphine.

DISCUSSION

An acute treatment with morphine, an agonist of μ-opioid receptors, induced at a low dose (1 mg/kg) a significant anxiolytic-like action in the elevated plus-maze. Morphine increased not only the number of open arm entries, but also the number of closed arm entries. Nevertheless, morphine (1 mg/kg) did not modify the locomotor activity in the motility box showing that the action of μ-opioid receptor agonist was specific in the elevated plus-maze. The anxiolytic-like action of morphine was completely reversed by the opioid antagonist

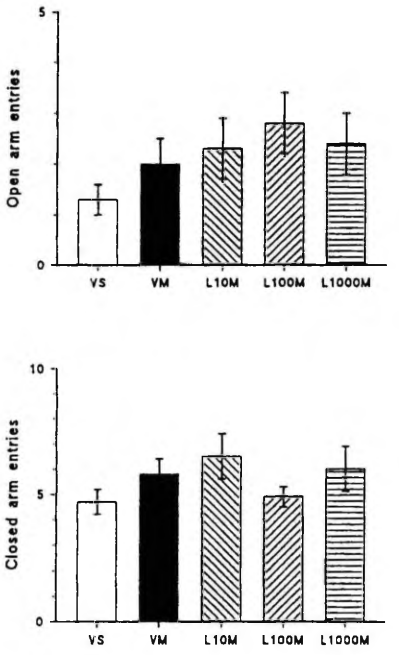

Fig. 5 The interaction of L-365,260 (1–1000 μg/kg) with the sub-effective dose of morphine (0.5 mg/kg) in the plus-maze. VS (vehicle + saline); VM (vehicle + morphine); L10M (L-365,260 10 μg/kg + morphine); L100M (L-365,260 100 μg/kg + morphine); L1000M (L-365,260 1000 μg/kg + morphine). Vehicle – few drops of Tween-80 suspended in saline.* – $P < 0.05$ (compared to vehicle-treated rats);* – $P < 0.05$ (compared to morphine-treated rats).

naloxone. This is in a good accordance with a recent study of Motta and Brando.[1] The higher dose of morphine (2.5 mg/kg) did not cause the anxiolytic-like action. However, this dose of morphine seems to reduce the locomotor activity of rats. According to our previous studies 2.5 mg/kg) of morphine was able to block apomorphine-induced aggressiveness in rats.[18] This behavioural effect of apomorphine is probably mediated via dopamine D_2 receptors in the mesolimbic structures.[19] The inhibition of the dopaminergic neurotransmission in the mesolimbic structures seems to be a possible explanation why the higher doses of morphine are reducing the locomotor activity of rats.

The administration of BOC-CCK-4, an agonist of CCK_B receptors, induced a dose-dependent reduction of exploratory behaviour in rats. According to our previous studies CCK agonists produced the anxiogenic-like action only in rats not acclimatized to the experimental situation.[20] BOC-CCK-4 antagonized the anxiolytic-like effect of morphine, but CCK agonist possessed U-shaped action against morphine in the plus-maze. Namely, 10 μg/kg of BOC-CCK-4 reversed the anxiolytic-like effect of morphine, whereas the other doses of CCK agonists were ineffective. The antagonistic interaction between the opioid agonist and CCK agonist is in accordance with our previous study where the opioid antagonist naloxone potentiated the anxiogenic-like action of CCK agonists (BOC-CCK-4 and caerulein).[15] Differently from BOC-CCK-4 only one dose (10 μg/kg) of L-365,260, an antagonist of CCK_B receptors, modified the exploratory activity of rats. Namely, this dose of L-365,260 increased the exploratory behaviour of rats. The reversed U-shaped action of L-365,260 has been demonstrated also in our previous studies.[21] The background of such action of L-365,260 is not clear and will need the further clarification. On the other hand, the combination of L-365,260 with the sub-effective dose of morphine (0.5 mg/kg) induced an anxiolytic-like action. However, again L-365,260 was effective only at one dose 100 μg/kg. It is worthy to note that this dose of L-365,260 did not change the exploratory activity of rats per see showing that the interaction between L-365,260 and morphine was specific. The potentiation of the action of morphine by the CCK_B receptor antagonist could also be explained in the light of recent finding that morphine increased the release of CCK in the frontal cortex of rat.[22] Moreover, this dose of L-365,260 (100 μg/kg), but not the lower doses, antagonized the anxiogenic-like action of caerulein, an agonist of CCK receptors.[20] Altogether these data re in favour of the antagonistic interaction between morphine and CCK in the regulation of exploratory behaviour.

There are several studies showing the negative interaction between CCK and endopioid peptides in the regulation of pain sensitivity. However, like in our studies the action of CCK was evident in rats not adapted to the experimental environment. Wiertelak et al.[23] have shown that CCK antagonizes morphine-induced antinociception only in the novel, but not in the familiar environment. This finding was confirmed by Lavigne et al.[24] demonstrating that CCK antagonists devazepide and L-365,260 enhance morphine-induced analgesia only in non-acclimatized rats exposed to the novel environment. Therefore, it is likely that even in the case of pain regulation the interplay between CCK and endopioid peptides actually results on the level of anxiety. This suggestion is supported by the recent study of Benedetti et al.[25]

Namely, they have found that nocebo induced hyperalgesia was reversed dose-dependently by CCK antagonist proglumide, but not by naloxone. Since the nocebo procedure represents an anxiogenic stimulus it is likely that nocebo hyperalgesia may be due to a CCK-dependent increase of anxiety.[25]

According to the hypothesis of Wiertelak et al.[23] the environmental stimuli that signal the occurrence of aversive or dangerous events activate endogenous opioid analgesia systems. The signals for safety (the nonoccurrence of aversive events) produce the opposite and inhibit environmentally produced analgesia. Wiertelak et al.[23] believe that CCK is a mediator of that kind of safety signals. However, the present study as well as our previous study[15] are not in favour of that statement since CCK apparently increases anxiety in the novel environment and reverses the anxiolytic-like action of morphine. Therefore, CCK is rather signalling that the novel environment is aversive and unsafe.

In conclusion, morphine potently increased the exploratory behaviour of rats in the elevated plus-maze. Namely, morphine increased the number of open arm entries and time spent in exploring of open part – the behavioural measures believed to reflect the anxiolytic-like activity in the plus-maze. The stimulation of CCK_B receptors with BOC-CCK-4 apparently antagonized the anxiolytic-like action of morphine. The combination of the subeffective dose of morphine with L-365,260, a drug blocking the CCK_B receptors, potentiated the effect of μ-opioid receptor agonist. Consequently, CCK is acting as the endogenous antagonist of anxiolytic-like action of morphine.

ACKNOWLEDGMENTS

This study was granted by the Estonian Science Foundation (grant No 1409).

REFERENCES

1. Motta V, Brandao ML. Aversive and anti-aversive effects of morphine in the dorsal periaquaductal gray of rats submitted to the elevated plus-maze test: Pharmacol Biochem Behav 1993; 44: 119–125.
2. Privette TH, Terrian DM. Kappa opioid agonists produce anxiolytic-like behavior on the elevated plus-maze. Psychopharmacol 1995; 118: 444–450.
3. Stengaard-Pedersen K, Larsson LI. Localization and opiate receptor binding of enkephalin, CCK, and ACTH/β-endorphin in the rat central nervous system. Peptides (suppl) 1981; 2: 3–19.
4. Skinner K, Busbaum AI, Fields HL. Cholecystokinin and enkephalin in brain stem pain modulating circuits. NeuroReport 1997; 8: 2995–2998.
5. Gall C, Lauterborn J, Burks D, Seroogy K. Co-localization of enkephalin and cholecystokinin in discrete areas of rat brain. Brain Res 1987; 403: 403–408.
6. Pohl M, Benoliel JJ, Bourgoin S et al. Regional distribution of calcitonin gene-related peptide-, substance P-, cholecystokinin-, Met^5-enkephalin-, and dynorphin A (1–8)-like material in the spinal cord and dorsal root ganglia of adult rats. Effects of dorsal rhizotomy and neonatal capsaicin. J Neurochem 1990; 55: 1122–1130.
7. Moran TH, Robinson PH, Goldrich MS, McHugh PR. Two brain cholecystokinin receptors: implications for behavioural actions. Brain Res 1986; 362: 175–179.
8. Wank SA. Cholecystokinin receptors. Am J Physiol 1995; 269: G628–646.
9. Smadja C, Maldonado R, Turcaud S, Fournie-Zaluski MC, Roques BP. Opposite role of CCK_A and CCK_B receptors in the modulation of endogenous enkephalin antidepressant-like effects. Psychopharmacol 1995; 120: 400–408.
10. Valverde O, Fournie-Zaluski MC. Roques BP, Maldonado R. The CCK_B antagonist PD-134,308 facilitates rewading effects of endogenous enkephalins but does not induce place preference in rats. Psychopharmacol 1996; 123: 119–126.
11. Valverde O, Smadja C, Roques BP, Maldonado R. The attenuation of morphine place preference following chronic mild stress reversed by a CCK_B receptor antagonist. Psychopharmacol 1997; 131: 79–85.
12. Valverde O, Maldonado R, Fournie-Zaluski MC, Roques BP. Cholecystokinin B antagonists strongly potentiate antinociception mediated by endogenous enkephalins. J Pharmacol Exp Ther 1994; 270: 77–88.
13. Valverde O, Roques BP. Cholecystokinin modulates the aversive component of morphine withdrawal syndrome in rats. Neurosci Lett 1998; 244: 37–40.
14. Shlik J, Vasar E, Bradwejn J. Cholecystokinin and Psychiatric Disorders. Role in Aetiology and Potential of Receptor Antagonists in Therapy. CNS Drugs 1997; 8: 134–152.
15. Kõks S, Soosaar A, Võikar V et al. Opioid antagonist naloxone potentiates anxiogenic-like action of cholecystokin agonists in elevated plus-maze. Neuropeptides, 1998; 32: 235–240.
16. Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear' – motivated behaviour. Naunyn-Schmiedeberg's Arch Pharmacol 1984; 327: 1–5.
17. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety ib the rat. J Neurosci Meth 1985; 14: 149–167.
18. Allikmets L, Vasar E. Sensitization of male rats to aggressive behaviour. Zh. Vyssh. Nerv. Deiat. 1982; 32: 130–135.
19. Lang A, Harro J, Soosaar A et al. Role of N-methyl-D-aspartic acid and cholecystokinin receptors in apomorphine-induced aggressive behaviour in rats. Naunyn-Schmiedeberg's Arch Pharmacol 1995; 351: 363–370.
20. Vasar E, Kõks S, Volke V, Võikar V. Cholecystokinin in animal models of anxiety. Biol Psychiatry 1997; 42, 1S: 196–197.
21. Harro J, Vasar E. Evidence that CCK_B receptors mediate the regulation of exploratory behaviour in the rat. Eur J Pharmacol 1991; 193: 379–381.
22. Benoliel JJ, Becker C, Mauborgue A, Bourgoin S, Hamon M, Cesselin F. Interactions between central opioidergic and cholecystokininergic systems in rats: possible significance for the development of opioid tolerance. Bull Acad Natl Med 1998; 182: 311–324.
23. Wiertelak EP, Maier SF, Watkins LR. Cholecystokinin antianalgesia: safety cues abolish morphine analgesia. Science 1992; 256: 830–833.

24. Lavinge GJ, Millington WR, Mueller GP. The CCK-A and CCK-B receptor antagonists, devazepide and L-365,260, enhance morphine antinociception only in non-acclimated rats exposed to a novel environment. Neuropeptides 1992; 21: 119–129.

25. Benedetti F, Amanzio M, Casadio C, Oliaro A, Maggi G. Blockade of nocebo hyperalgesia by the cholecystokinin antagonist proglumide. Pain 1997; 71: 135–140.

V

Kõks S., Männistö P. T, Bourin M., Shlik J., Vasar V., Vasar E.
Cholecystokinin (CCK)-induced anxiety in rats: relevance
of pre-experimental stress and seasonal variations.
J Psychiatry Neurosci (re-submitted).

CHOLECYSTOKININ (CCK)-INDUCED ANXIETY IN RATS: RELEVANCE OF PRE-EXPERIMENTAL STRESS AND SEASONAL VARIATIONS

S. Kõks[1, 2*], P. T. Männistö[3], M. Bourin[4], J. Shlik[5], V. Vasar[5], E. Vasar[1]

[1]Department of Physiology, University of Tartu, 2 Näituse Street, 50409 Tartu, Estonia; [2]Institute of Molecular and Cell Biology, University of Tartu, 23 Riia Street, 50050 Tartu, Estonia; [3]Department of Pharmacology and Toxicology, University of Kuopio, P. O. Box 1627, FIN-70211 Kuopio, Finland; [4]Department of Pharmacology, University of Nantes, Nantes Cedex, 44035, France; [5]Department of Psychiatry, University of Tartu, 31 Raja Street, 50417 Tartu, Estonia

ABSTRACT

In experiments on male Wistar rats the influence of pre-experimental stress on the anxiogenic-like action of caerulein, an agonist of CCK receptors, was studied. Caerulein (5 μg/kg s.c.) caused the strongest action in animals brought to the experimental room immediately before the experiment and kept in isolation after the administration of caerulein. Caerulein did not cause any reduction of exploratory activity in rats made familiar to the experimental room and kept in the home-cage after the injection of the CCK agonist. The anti-exploratory action of caerulein in stressed rats was reversed by the CCK antagonists L-365,260 (100 μg/kg i.p.) and devazepide (100 μg/kg i.p.), demonstrating the involvement of CCK_B receptor subtype. In addition, seasonal fluctuations occur in the exploratory activity of rats. The exploratory activity of rats was much lower in July compared to the study performed in November. The rats displaying the reduced exploratory activity had an increased number of CCK receptors in the frontal cortex and hippocampus. Simultaneously, the density of serotonin 5-HT_2 receptors in the frontal cortex, but not that of dopamine D_2 receptors in the striatum, was elevated. The blood levels of growth hormone were also higher in July. Accordingly, the anti-exploratory action of caerulein is dependent on the pre-experimental stress of rats. Moreover, the seasonal variations of exploratory behavior of rats are evident in the plus-maze model of anxiety. The reduced exploratory activity in summer appeared to be related to the elevated density of CCK and 5-HT_2 receptors in the brain. The results of present study have been discussed in the light of recent findings obtained from the human studies and some parallels between the animal and human data have been established.

Running title: CCK in a rat model of anxiety

Key words: CCK; anxiety; elevated plus-maze; exploratory activity; seasonal variations; neurohormonal changes

INTRODUCTION

Cholecystokinin octapeptide (CCK-8) is a widely distributed neuropeptide in the mammalian brain (Rehfeld and Nielsen, 1995). Recent evidence suggests that CCK-8 is implicated in the neurobiology of anxiety (Harro and others, 1993). The anxiogenic-like action of CCK agonists is established in various animal species including the monkey, guinea-pig, rat, mouse and cat (Shlik and others, 1997a). CCK_B receptor (brain subtype) antagonists are effective in antagonizing the anxiogenic-like action of CCK agonists, but they also cause an anxiolytic-like effect in various animal models of anxiety (Harro and others, 1993). There is a growing body of evidence that the administration of CCK_B receptor agonists CCK-4 and pentagastrin induces panic-like attacks in healthy volunteers and patients suffering from panic disorder (De Montigny, 1989; Bradwejn and others, 1991; Van Megen and others, 1994). The sensitivity of panic patients to the panicogenic action of CCK_B agonists is significantly higher compared to that of healthy subjects (Bradwejn and others, 1994). The striking similarity between CCK-induced panic-like attacks and natural attacks has been established in panic patients. The augmented response to CCK agonists is also described in patients suffering from the other anxiety disorders (Shlik and others, 1997a). Accordingly, increased sensitivity to CCK_B receptor agonist-induced panic-like attacks seems to be a common feature of anxiety disorders. The pretreatment of healthy subjects with CCK_B receptor antagonists blocks the panicogenic action of CCK agonists, demonstrating the implication of CCK_B receptors (Bradwejn and others, 1995).

There is some evidence that the response of rats to the anxiogenic-like action of CCK may be dependent on the level of pre-experimental stress (Harro and others, 1993). Biro and others (1993) have demonstrated that the anxiogenic-like action of CCK is related to the release of corticotropin-releasing hormone (CRH). CRH is obviously playing a key role in the behavioural and hormonal mechanisms of stress (Chrousos and Gold, 1992). Recent studies have shown that mice lacking CRH1 receptor display impaired stress response and reduced anxiety in the light/dark compartment test (Timpl and others, 1998; Smith and others, 1998). The results obtained from the clinical studies provide some evidence for an involvement of neuronal CRH in anxiety disorders, but not to the same extent observed in the case of depression (Jolkkonen and others, 1993; Fossey and others, 1996; Smith and others, 1989; Arborelius and others, 1999). Patients suffering from panic disorder display increased sensitivity to CRH-induced ACTH and cortisol release (Curtis and others, 1997). The administration of the CCK_B receptor agonists CCK-4 and pentagastrin is shown to activate the hypothalamic-pituitary-adrenal axis (De Montigny 1989; Abelson and others, 1994; Koszycki and others, 1996). In their recent study Koszycki and others (1998) have described that the administration of CCK-4 significantly enhanced ACTH secretion in healthy volunteers responding with panic-like attacks compared to non-responders. Therefore, in the present study an attempt was made to clarify the significance of pre-experimental stress in the anxiogenic-like action of CCK. For that purpose one half of the rats was habituated to the experimental situation, but the others were not.

Several investigators have described the seasonal fluctuations in anxiety. Recent reports have described a higher summer incidence in the occurrence of the first panic attack (Cameron, 1989; Lelliott and others, 1989; Lepine and others, 1991) irrespective

of the hemisphere in which the study was carried out. Lelliott and others (1989) have found that of 57 patients with panic disorder with agoraphobia more had their first panic in late spring and summer than in fall and winter, and in warm weather than in cold weather. Therefore, the second major goal for the present study was the revealing of differences in anxiety of rats in summer and winter, and the role of CCK in these behavioral alterations. Simultaneously, changes in the density of serotonin 5-HT_2 and dopamine D_2 receptors, but also in the blood levels of anterior pituitary hormones (prolactin, growth hormone and thyrotropin), were studied.

METHODS

Animals

Male Wistar (Han/Kuo: WIST) rats (National Animal Center, Kuopio, Finland) weighing 250–300 g were kept 4 per cage in the animal house at 20±2C in a 12h light/dark cycle (light on at 7.00 a.m.). Tap water and food pellets were available *ad libitum.*

The behavioural studies

Two different studies have been performed. In the first part (study was performed in November and December) an attempt was made to reveal the significance of pre-experimental stress on CCK-induced anxiety in the elevated plus-maze. The male Wistar rats were divided into four different groups. Two groups of rats were handled in the experimental room on three consecutive days (twice daily) before the experiment. The other two groups of animals were brought to the experimental room immediately before the beginning of experiment. The handled and non-handled rats were divided into two groups after the injection of caerulein (5 µg/kg s.c., Sigma), an agonist of CCK receptors. Caerulein or saline was injected 15 min before the beginning of the plus-maze study. One half of animals was isolated after the injection, whereas the other half was placed back into the home-cage. The action of the CCK_B antagonist L-365,260 (1–100 µg/kg i.p., Merck Sharp & Dohme) and CCK_A antagonist devazepide (1–100 µg/kg i.p., Merck Sharp & Dohme) on the anxiogenic-like action of caerulein was also studied. CCK antagonists or vehicle (2% Tween-85 in physiological saline) were injected 30 min before the plus-maze study.

In the second half of the experiment, possible seasonal differences in the exploratory activity of rats were studied. Two studies were performed - one study was conducted at the beginning of July 1993 (summer) and the other one in late November of 1994 (winter). This study was performed in 40 handling-naive rats (in both experiments). The animals were decapitated immediately after the plus-maze exposure and the blood and brain samples were taken for neurohormonal studies.

The elevated plus-maze

The method initially suggested by Handley and Mithani (1984) for the measurement of exploratory activity was employed with some modifications (Pellow and others, 1985). The apparatus consisted of two opposite open arms (50×10 cm) without side walls and two enclosed arms (50×10×40 cm) with side walls and an end wall, extending from a

central square (10×10 cm). The maze was elevated to the height of 50 cm, and placed in a lit room. During a 5 min observation session the following measures were taken by an observer: 1) latency of first open part entry; 2) time spent in exploring of open part and open arms of plus-maze; 3) number of closed and open arm entries; 4) number of lines crossed and 5) ratio between open and total arm entries. At the beginning of experiment an animal was placed into the centre of plus-maze, facing toward a closed arm. An arm entry was counted only when all four limbs of the rat were within a given arm. Time spent in open arms, number of open arm entries and ratio between open and total arm entries are the "classical" measures of anxiety in the elevated plus-maze (Rodgers and Johnson, 1995). By contrast, the number of closed arm entries and number of line crossings are the measures reflecting the locomotor activity of rats (Rodgers and Johnson, 1995).

Hormonal studies

The animals were killed by decapitation immediately after the plus-maze exposure. In all cases the truncal blood was collected, serum was separated by centrifugation and samples were stored at –20°C until prolactin, thyrotropin and growth hormone concentrations were determined from duplicate samples (0.1 ml) by specific radio-immunoassays. The rat prolactin, thyrotropin and growth hormone kits were gifts from NIH. Prolactin results are expressed in ng/ml of NIDKK-rPRL-RP-2 standard. Thyrotropin data are expressed in ng/ml of NIDKK-TSH-RP-2 standard. Growth hormone results are expressed in ng/ml of NIDKK-GH-RP-2 standard. Each hormone was assayed in a single session. The intra-assay coefficient of variation was less than 15%.

Radioligand binding studies

For the radioligand binding studies 28 rats from the study performed in summer and 28 animals from the experiment conducted in winter were used. After decapitation the brains were quickly dissected on ice. The binding studies were performed in various brain structures of rats. The striatum was used to determine the density and affinity of dopamine D_2 receptors, the frontal cortex for 5-hydroxytryptamine 5-HT_2 and CCK receptors, and the hippocampus for CCK receptors (Table 1). These brain structures were selected according to previous studies since in these brain regions the most significant neurochemical changes due to the elevated plus-maze exposure had been established (Rägo and others, 1988, 1991; Harro and others, 1990; Rex and others, 1993; File and others, 1993). The brain tissue was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl (pH 7.4 at 4C) using a Potter-S glass-teflon homogenizer (1000 rpm, 12 passes). The membranes were washed twice in the same buffer by centrifugation (48000×g for 20 min) and resuspension. After the last centrifugation the crude brain membranes were suspended in the incubation buffer for the appropriate binding assay.

The protein content was measured according to a dye-binding assay (Bradford, 1976). Saturation curves were analyzed using non-linear least squares regression (Leatherbarrow, 1987).

Statistics

Results are expressed as mean values ± S.E.M. The behavioural studies with caerulein and CCK antagonists were analysed using one-way analysis of variance (ANOVA). *Post hoc* comparisons between individual groups were performed by means of the Newman-Keuls test using the Statistica for Windows software. The comparison of experiments performed in July and November was assessed by means of the Student's *t*-test.

RESULTS

The anti-exploratory action of caerulein (5 μg/kg) was dependent on the pre-experimental handling and isolation. Caerulein did not cause any reduction of exploratory activity in animals subjected to the handling and kept in the home-cage after the injection of the CCK agonist (Table 2). Caerulein tended to reduce the activity of rats who were not handled but kept in the home-cage. However, this change was not statistically significant. In the third group where the rats were handled but kept separated after the treatment, caerulein apparently reduced the exploratory activity of rats in the elevated plus-maze. Caerulein displayed the strongest action in rats not subjected to the handling and kept in isolation after the treatment with the CCK agonist (Table 2).

The anti-exploratory effect of caerulein in these stressed animals (no handling + isolation) was dose-dependently antagonized by L-365,260 (1–100 μg/kg), an antagonist of CCK_B receptors (Table 3, time spent in open part $F_{4,66}=2.90$, $p<0.05$; number of line crossings $F_{4,66}=3.18$, $p<0.05$; number of open arm entries $F_{4,66}=2.53$, $p<0.05$; time spent in open part $F_{4,66}=2.57$, $p<0.05$; number of total arm entries $F_{4,66}=2.94$, $p<0.05$; ratio between open and total arm entries $F_{4,66}=2.63$, $p<0.05$). One μg/kg of L-365,260, if administered together with caerulein, tended to reduce the locomotor activity since this combination decreased the number of line crossings and total arm entries. However, 10 μg/kg of L-365,260 tended to antagonize the anti-exploratory action of caerulein, whereas 100 μg/kg of the CCK antagonist completely reversed the effect of the CCK agonist. Ten μg/kg of L-365,260 increased *per se* the exploratory activity of stressed (no handling + isolation) rats in the elevated plus-maze (time spent in exploring of open arms, $F_{3,61}=2.62$, $p<0.05$; ratio between open and total arm entries $F_{3,61}=3.05$, $p<0.05$), whereas the lower (1 μg/kg) and higher doses (100 μg/kg) of CCK_B antagonist were ineffective in this respect (Table 4). Devazepide, an antagonist of CCK_A receptors, also antagonized the action of caerulein in the plus-maze (Table 5, time spent in open part $F_{4,35}=4.79$, $p<0.01$; number of line crossings $F_{4,35}=4.88$, $p<0.01$; number of total arm entries $F_{4,35}=3.02$, $p<0.05$). The highest dose of devazepide (100 μg/kg) counteracted the anti-exploratory effect of the CCK agonist. The administration of devazepide as a single treatment did not affect the exploratory activity of rats in the plus-maze (Table 6).

The exploratory activity of rats in November was significantly higher compared to the similar study conducted in July (Table 7). All the studied plus-maze parameters were significantly different if the data from these two studies were subjected to the statistical analysis. In summer the rats displaying the reduced exploratory activity in the plus-maze had the higher densities of CCK receptors in the frontal cortex and hippocampus compared to the rats in study performed in winter (Table 8). The number of $5\text{-}HT_2$ receptors in the frontal cortex, but not dopamine D_2 receptors in the striatum, was also

increased in summer. The animals from the studies in the different seasons also differed in levels of growth hormone, but not of thyrotropin and prolactin, in the blood (Table 9). The decreased exploratory activity of rats in summer appeared to be related to the increased levels of growth hormone in serum.

DISCUSSION

In the present study a clear dependence of the anti-exploratory effect of caerulein on pre-experimental stress was revealed. In animals made familiar with the experimental situation and kept in the home-cage after the injection of caerulein, the CCK agonist did not increase anxiety in the elevated plus-maze. On the other hand, in rats brought into the experimental room immediately before the study and kept in the isolation after the treatment with caerulein, the CCK agonist caused a significant anxiogenic-like action. This finding is in good accordance with previous studies showing the presence of an anxiogenic-like action of CCK only in a novel environment (Daugé and others, 1989). Moreover, CCK antagonized morphine-induced analgesia in rats in novel, but not in familiar, experimental conditions (Wiertelak and others, 1992). Accordingly, caerulein seems to potentiate neophobia in rats. However, the novelty is not the only factor determining the action of caerulein in the plus-maze. The keeping of rats in social isolation after the injection of CCK also contributes to the action of caerulein. In our previous studies we have found that the social isolation of rats for 7 days induced anxiety in animals, but also increased the density of CCK, but not benzodiazepine, receptors in the frontal cortex (Vasar and others, 1993). Therefore, it is likely that the social isolation of rats sensitizes the animals to the anxiogenic-like effect of caerulein. It has been shown that the pre-experimental stress also increases the effectiveness of anxiolytic drugs in the plus-maze showing that the endogenous tone is an important factor in studying of anxiety in rodents (Rodgers, 1997). It is important to point that patients suffering from anxiety disorders are also more sensitive to CCK-4- and pentagastrin-induced panic attacks than healthy volunteers (Bradwejn and others, 1994; Shlik and others, 1997b). The characteristic feature of anxiety disorders is the increased serum level of stress hormones (ACTH, cortisol) (Abelson and others, 1994; Arborelius and others, 1999). Collectively, the level of pre-experimental stress is a factor determining a potential response both in animals and in man to the anxiogenic-like action of CCK agonists.

The potentiation of neophobia induced by caerulein was dose-dependently antagonized by the CCK antagonists L-365,260 and devazepide. The highest dose (100 μg/kg) of L-365,260 and devazepide completely reversed the action of caerulein. The nearly equal potency of L-365,260 and devazepide against caerulein makes it unlikely that this effect of CCK antagonists is mediated primarily via CCK_A receptors. This statement is based on the knowledge that devazepide has very high affinity for CCK_A receptors and much better penetration, compared to L-365,260, into the brain (Hargreaves and Lin, 1992). Therefore, the CCK_B receptor subtype is a likely target for the anxiogenic-like action of caerulein. This is in agreement with the previous studies showing a key role of CCK_B receptors in CCK-induced anxiety both in the human and animal studies (Harro and others, 1995). The single treatment with L-365,260, but not with devazepide, induced the anxiolytic-like action in rats. However, the action of

L-365,260 was not dose-dependent since only one dose of the CCK_B receptor antagonist (10 μg/kg) was effective, and the lower and higher doses did not change the exploratory behaviour of rats. The U-shape action of L-365,260 has been also established in our previous studies (Harro and Vasar, 1991), but the background of this peculiar action remains to be established.

The present study also provides a strong evidence of seasonal variations in the exploratory activity of rats. The comparison of exploratory activity of rats in two experiments, conducted in July (summer) and in November (winter), revealed a clear difference, namely that the exploratory activity of rats was much lower in summer compared to winter. Similar results showing a significant variation in the exploratory activity of rats in two studies, in November and in June, were obtained by Harro and others (1997). Moreover, it is important to stress that the anxiogenic-like action of caerulein is much weaker in summer compared to the studies performed in winter (our unpublished data). This was a reason why the influence of pre-experimental stress to the anxiogenic-like action of CCK agonist was studied in winter, but not in summer. Also, these data are interesting in the light of recent reports describing significantly higher summer incidence in the occurrence of the first panic attack compared to winter (Cameron, 1989; Lelliott and others, 1989; Lepine and others, 1991, Marriott and others, 1994). The hypothetical reason for such seasonality is the increased activity of people which leads to overcrowding of public places (Lelliott and others, 1989). In rats the increased level of anxiety in summer could be explained by the higher pressure from the surrounding nature since in summer the number of potential predators is much higher than is winter when the life in nature is apparently ceasing down. This statement is supported by the recent findings showing the seasonal fluctuations in the serum levels of ACTH in a sand rat with the maximum concentration during the late spring and summer (Amirat and Brudieux, 1993).

The reduced exploratory activity of rats in summer appears to be related to the increased number of CCK receptors in the frontal cortex and hippocampus. Harro and others (1997) have reached the same conclusions, i.e. that in June when the exploratory activity was lower the density of CCK receptors was higher in the frontal cortex, hippocampus and striatum, but not in the hypothalamus. In the light of these data, it is likely that the tone of CCKergic transmission is higher in summer compared to winter. This could be a possible explanation why the anxiogenic-like action of caerulein is much weaker in summer since CCK receptors seems to be occupied by the endogenous ligand. In addition, we have noted an increased density of serotonin $5\text{-}HT_2$ receptors in the frontal cortex of rats having decreased exploratory activity. By contrast, the number of dopamine D_2 receptors in the striatum remained unchanged. Accordingly, increased anxiety in rats seems to be related to the increased density of CCK and $5\text{-}HT_2$ receptors in the forebrain.

In summer rats also had significantly increased levels of growth hormone, whereas the levels of prolactin and thyrotropin remained unchanged. A recent study has shown that healthy subjects respond to CCK-4 challenge with an increased level of growth hormone, which was higher in those who panicked after CCK-4 than in non-panickers (Koszycki and others, unpublished observations). In a previous study we have also investigated the release of anterior pituitary hormones in relation to the exploratory behavior of rats (Kõks and others, 1997). In that study, neither the exposure of rats to the plus-maze nor distinctive exploratory activity correlated with the serum levels of the

anterior pituitary hormones prolactin or thyrotropin. Only the concentrations of growth hormone were significantly different in the rats selected according to the exploratory behavior, that is the "non-anxious" rats had markedly lower levels of growth hormone compared to the "anxious" and intermediate groups. Anterior pituitary hormones are regulated by a multitude of classic neurotransmitters like 5-HT and dopamine but also by neuropeptides (Tuomisto and Männistö, 1985). It has been shown that 5-HT stimulates the baseline release of growth hormone and may be involved in the stress-induced growth hormone elevation in man (Charney and others, 1987). CCK also participates in the regulation of growth hormone. The activation of CCK_A receptors inhibits growth hormone secretion while the activation of CCK_B receptors caused an opposite effect (Männistö and others, 1994; Peuranen and others, 1994). Therefore, the augmented levels of growth hormone in the "anxious" animals might be caused by the increased activity of 5-HT and CCK in the brain.

In conclusion, the anxiogenic-like action of caerulein was dependent on pre-experimental stress in animals. The CCK agonist caused the strongest action in rats not adapted to the experimental conditions. In addition, seasonal variations of exploratory activity were revealed in the present study. The rats were more active in winter than in summer. The reduced exploratory activity of rats was apparently related to the increased density of CCK and $5\text{-}HT_2$ receptors in the brain. Also, the levels of growth hormone were markedly higher in animals displaying reduced exploratory activity. There is a striking similarity between the animal and human studies. The obvious relation to stress, increased sensitivity to CCK and existence of seasonal variations are also apparent in the case of human anxiety.

REFERENCES

Abelson JL, Nesse RM. 1994. Pentagastrin infusions in patients with panic disorder. I. Symptoms and cardiovascular responses. Biol Psychiatry 36: 73–83.

Amirat Z, Brudieux R. 1993. Seasonal changes in in vivo cortisol release to ACTH and plasma and pituitary concentrations of ACTH in a desert rodent, the sand rat (Psammomys obesus). Comp Biochem Physiol Comp Physiol 104: 29–34.

Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. 1999. The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocinol 160: 1–12.

Biro E, Sarnyai Z, Penke B, Szabo G, Telegdy G. 1993. Role of endogenous corticotropin-releasing factor in mediation of neuroendocrine and behavioral responses to cholecystokinin octapeptide sulfate ester in rats. Neuroendocrinology 57: 340–345

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–54.

Bradwejn J, Koszycki D, Shriqui C. 1991. Enhanced sensitivity to cholecystokinin tetrapeptide in panic disorders. Clinical and behavioral findings. Arch Gen Psychiatry 48: 603–10.

Bradwejn J, Koszycki D, Cou toux du Tetre A, van Megen H, den Boer J, Westenberg H, Annable L. 1994. The panicogenic effects of cholecystokinin tetrapeptide are antagonized by L-365,260, a central cholecystokinin receptor antagonist, in patients with panic disorder. Arch Gen Psychiatry 51: 486–93.

Bradwejn J, Koszycki D, Paradis M, Reece P, Hinton J, Sedman A. 1995. Effect of CI-988 on cholecystokinin tetrapeptide-induced panic symptoms in healthy volunteers. Biol Psychiatry 38: 742–746.

Cameron OG. 1989. Frequency of panic disorder in summer. Am J Psychiatry 146: 123.

Charney DS, Woods SW, Goodman WK, Heninger GR. 1987. Serotonin function in anxiety: II. Effects of the serotonin agonist mCPP in panic disorder patients and healthy subjects. Psychopharmacology 92: 14–24.

Chrousos GP, Gold PV. 1992. The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. JAMA, 267: 1244–1252.

Curtis GC, Abelson JL, Gold PW. 1997. Adrenocorticotropic hormone and cortisol responses to corticotropin-releasing hormone: changes in panic disorder and effects of alprazolam treatment. Biol. Psychiatry, 41: 76–85.

Daugé V, Dor A, Feger J, Roques BP. 1989. The behavioral effects of CCK8 injected into the medial nucleus accumbens are dependent on the motivational state of the rat. Eur J Pharmacol 163: 25–32.

De Montigny C. 1989. Cholecystokinin tetrapeptide induces panic-like attacks in healthy volunteers. Arch Gen Psychiatry 46: 511–7.

File SE, Zangrossi JR, Andrews N. 1993. Social interaction and elevated plus-maze tests: changes in release and uptake of 5-HT and GABA. Neuropharmacology 32: 217–21.

Fossey MD, Lydiard RB, Ballenger JC, Laraia MT, Bissette G, Nemeroff CB. 1996. Cerebrospinal fluid cortricotropin-releasing factor concentrations in patients with anxiety disorders and normal comparison subjects. Biol Psychiatry 39: 703–707.

Hargreaves RJ, Lin JH. 1992. Blood-brain transfer of the CCK antagonists L-365,260 and devazepide. In: Dourish CT, Cooper SJ, Iversen SD, Iversen LL, editors. Multiple Cholecystokinin Receptors in the CNS. New York: Oxford University Press. Pp 107–114.

Handley SL, Mithani S. 1984. Effects of alpha-adrenoreceptor agonists and antagonists in a maze-exploration model of fear -motivated behavior. Naunyn-Schmiedeberg's Arch Pharmacol 327: 1–5.

Harro J, Kiivet RA, Lang A, Vasar E. 1990. Rats with anxious or non-anxious type of exploratory behavior differ in their brain CCK-8 and benzodiazepine receptor characteristics. Behav Brain Res 39: 63–71.

Harro J, Vasar E. 1991. Evidence that CCKB receptors mediate the regulation of exploratory behaviour in the rat. Eur J Pharmacol 193: 379–81.

Harro J, Vasar E, Bradwejn J. 1993. Cholecystokinin in animal and human research of anxiety. Trends Pharmacol Sci 14: 244–9.

Harro J, Oreland L, Vasar E, Bradwejn J. 1995. Impaired exploratory behavior after DSP-4 treatment in rats: implications for the increased anxiety after noradrenergic denervation. Eur Neuropsychopharmacol 5:447-55

Harro J, Löfberg C, Pähkla R, Matto V, Rägo L, Oreland L, Allikmets L. 1997 Different molecular forms of cholecystokinin and CCKB receptor binding in the rat brain after chronic antidepressant treatment. Naunyn-Schmiedeberg's Arch Pharmacol 355: 57–63.

Jolkkonen J, Lepola U, Bissette G, Nemeroff CB, Riekkinen P. 1993. CSF corticotropin-releasing factor is not affected in panic disorder. Biol Psychiatry 33: 136–138.

Koszycki D, Zacharko RM, Le Melledo JM, Young SN, Bradwejn J. 1996. Effect of acute tryptophan depletion on behavioral, cardiovascular, and hormonal sensitivity to cholecystokinin-tetrapeptide challenge in healthy volunteers. Biol Psychiatry 40: 648–655.

Koszycki D, Zacharko RM, Le Melledo JM, Bradwejn J. 1998. Behavioral, cardiovascular and neuroendocrine profiles following CCK-4 challenge in healthy volunteers: a comparison of panickers and nonpanickers. Depress Anxiety 8: 1–7.

Kõks S, Vasar E, Soosaar A, Lang A, Volke V, Võikar V, Bourin M, Männistö PT. 1997. Relation of exploratory behavior of rats in elevated plus-maze to brain receptor binding properties and serum growth hormone levels. Eur Neuropsychopharmacol 7: 289–94.

Marriott PF, Greenwood KM, Armstrong SM. 1994 Seasonality in panic disorder. J Affect Disord 31: 75–80.

Lang A, Vasar E, Soosaar A, Harro J. 1992. The involvement of sigma and phencyclidine receptors in the action of antipsychotic drugs. Pharmacol Toxicol 71: 132–8.

Lang A, Harro J, Soosaar A, Ktks S, Volke V, Oreland L, Bourin M, Vasar E, Bradwejn J, Männistö PT. 1995. Role of N-methyl-D-aspartic acid and cholecystokinin receptors in apomorphine-induced aggressive behavior in rats. Naunyn-Schmiedeberg's Arch Pharmacol 51: 363–70

Leatherbarrow RJ. 1987. Enzfitter, a non-linear regression data analysis program for the IBM PC. Elsevier, Amsterdam.

Lelliott P, Marks I, McNamee G, Tobe a A. 1989. Onset of Panic Disorder with Agoraphobia. Toward an integrated model. Arch Gen Psychiatry. 46: 1000–1004

Lepine JP, Chignon JM, Teherani M. 1991. Suicidal behavior and onset of panic disorder. Arch Gen Psychiatry 48: 668–669.

Männistö PT, Lang A, Harro J, Peuranen E, Bradwejn J, Vasar E. 1994. Opposite effects mediated by CCK_A and CCK_B receptors in behavioral and hormonal studies in rats housed in social groups. Naunyn-Schmiedeberg's Arch Pharmacol 349: 478–84.

Pellow S, Chopin P, File SE, Briley M. 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14: 149–67.

Peuranen E, Lang A, Harro J, Vasar E, Männistö PT. 1994. Effect of CCK-agonists and antagonists on hypophyseal hormone secretion in single-housed and group-housed male rats. In: Seredenin S, Longo VG, Gavirachi G, editors. Biological Basis of Individual Sensitivity to Psychotropic Drugs (Proc. 2nd Intern. Golden Ring Confer.) Graffham Press, Edinburgh. p. 63–72.

Rehfeld JF, Nielsen FC. 1995. Molecular forms and regional distribution of cholecystokinin in the central nervous system. In: Bradwejn J, Vasar E, editors. Cholecystokinin and anxiety: from neuron to behavior. Austin: Springer Verlag-R.G. Landes Company. pp 33–56.

Rex A, Marsden CA, Fink H. 1993. Effect of diazepam on cortical 5-HT release and behavior in the guinea-pig exposure to the elevated plus-maze. Psychopharmacology 110: 233–6.

Rodgers RJ, Johnson NJT 1995. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol Biochem Behav 52: 297–303.

Rodgers RJ 1997. Animal models of anxiety: an ethological perspective. Braz J Med Biol Res 30: 289–304.

Rägo L, Kiivet RA, Harro J, Ptld M. 1988. Behavioral differences in an elevated plus-maze: correlation between anxiety and decreased number of GABA and benzodiazepine receptors in mouse cerebral cortex. Naunyn-Schmiedeberg's Arch Pharmacol 337: 675–8.

Rägo L, Adojaan A, Harro J, Kiivet RA. 1991. Correlation between exploratory activity in an elevated plus-maze and number of central and peripheral benzodiazepine binding sites. Naunyn-Schmiedeberg's Arch Pharmacol 343: 301–6.

Shlik J, Vasar E, Bradwejn J. 1997a. Cholecystokinin and Psychiatric Disorders. Role in Aetiology and Potential of Receptor Antagonists in Therapy. CNS Drugs 8: 134–52.

Shlik J, Aluoja A, Vasar V, Vasar E, Podar T, Bradwejn J. 1997b. Effects of Citalopram Treatment on Behavioral, Cardiovascular, and Neuroendocrine Response to Cholecystokinin Tetrapeptide Challenge in Panic Disorder Patients. J Psychiatry Neurosci 22: 332–40.

Smith MA, Davidson J, Ritchie JC, Kudler H, Lipper S, Chappel P, Nemeroff CB. 1989. The corticotropin-releasing hormone test in patients with posttraumatic stress disorder. Biol Psychiatry. 26: 349–355.

Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikjian LM, Gold LH, Chen R, Marchuk Y, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee KF. 1998 Corticotropin-releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 20: 1093–1102.

Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JMHM, Stalla GK, Blanquet V, Steckler T, Holsboer F, Wurst W. 1998. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. Nature Genet 19: 162–166.

Tuomisto J, Männistö PT. 1985. Neurotransmitter regulation of anterior pituitary hormones. Pharmacol Rev 37: 249–332.

Van Megen HJGM, Westenberg HGM, den Boer JA. 1994. Effect of the cholecystokinin-B (CCK-B) receptor antagonist L-365,260 on lactate induced panic attacks (PA). Psychopharmacology 10 (suppl. Pt II): 3s.

Van Megen HJGM, Westenberg HGM, den Boer JA, Kahn RS. 1996. The panic-inducing properties of the cholecystokinin tetrapeptide CCK4 in patients with panic disorder. Eur Neuropsychopharmacol 6: 187–194.

Vasar E, Peuranen E, Harro J, Lang A, Oreland L, Männistö PT. 1993. Social isolation of rats increases the density of cholecystokinin receptors in the frontal cortex and abolishes the anti-exploratory effect of caerulein. Naunyn-Schmiedeberg's Arch Pharmacol 348: 96–101.

Wiertelak EP, Maier SF, Watkins LR. 1992. Cholecystokinin antianalgesia: safety cues abolish morphine analgesia. Science 56: 830–3.

Table 1. Basic data about the receptor binding methodology used in the studies

Receptor	Ligand	Specific activity	Brain area	Nonspecific binding	Reference
Dopamine D_2	[^{3}H]-spiperone	105 Ci/mmole	Striatum	Raclopride 1 μM	Lang *et al.*, 1992
5-hydroxy-tryptamine 5-HT_2	[^{3}H]-spiperone	105 Ci/mmole	Frontal cortex	Ketanserin 1 μM	Lang *et al.*, 1992
CCK	[^{3}H]-pCCK-8	75 Ci/mmole	Frontal cortex and hippocampus	Caerulein 100 nM	Lang *et al.*, 1995

Table 2. The effect of handling and isolation on the anti-exploratory action of caerulein in the plus-maze

	Handling +	No isolation +	Handling +	Isolation +	No handling +	No isolation +	No handling +	Isolation +
Parameters	Saline	Caerulein	Saline	Caerulein	Saline	Caerulein	Saline	Caerulein
Number of line crosssings	19±4	15±3	39±5	· 24±3*	· 26±4	19±4	25±2.8	18±1.6
Number of open arm entries	1.6±0.6	1.1±0.4	3.6±0.7	1.7±0.5*	2.7±0.8	1.8±0.6	2.0±0.4	0.7±0.2*
Time spent in open arm (sec)	24±9	21±9	57±14	36±14	54±14	33±9	46±11	14±6*
Number of total arm entries	5.8±1.2	4.7±0.7	11.4±1.0	7.7±0.9*	8.1±1.1	6.4±1.4	7.1±0.7	5.5±0.5
Ratio between open/total arm entries	19±5	21±7	36±5	22±6	28±8	19±5	27±4	9±3*

* — $p<0.05$ (compared to the respective saline treated group, Newman-Keuls test after significant one-way ANOVA). Number of line crossings $F_{7,92}=4.58$, $p<0.01$; number of open arm entries $F_{7,92}=3.36$, $p<0.01$; time spent in open arm $F_{7,92}=2.31$, $p<0.05$; number of total arm entries $F_{7,92}=4.51$, $p<0.01$; ratio between open and total arm entries $F_{7,92}=2.79$, $p<0.05$). Number of animals is 12 in each group.

Table 3. The effect of L365,260 on the anxiogenic-like action of caerulein in the elevated plus-maze

Parameters	Vehicle+ saline	Vehicle+ caerulein 5 μg/kg	L365,260 1 μg/kg+ caerulein 5 μg/kg	L365,260 10 μg/kg+ caerulein 5 μg/kg	L365,260 100 μg/kg+ caerulein 5 μg/kg
Number of line crossings	22±1.9	17±1.5	14±2.3*	18±1.8	22±2.0
Time spent in open part (sec)	135±13	96±8	100±16	132±14	143±11**
Number of open arm entries	1.3±0.4	0.4±0.2*	0.4±0.2*	1.0±0.4	1.3±0.4**
Time spent in open arm (sec)	30±10	5±3*	8±4	21±7	31±11**
Number of total arm entries	6.3±0.7	5.5±0.6	4.0±0.7*	5.4±0.6	6.9±0.6
Ratio between open/ total arm entries ×100	19±5	6±2*	8±4	13±5	15±5

* — $p<0.05$ (compared to vehicle+saline treated rats; Newman-Keuls test after significant one-way ANOVA); ** — $p<0.05$ (compared to vehicle+caerulein treated rats). Number of animals is 14 in each group.

Table 4. The action of L-365,260 on the exploratory activity of rats in the plus-maze

Parameters	Vehicle	L-365,260 1 μg/kg	L-365,260 10 μg/kg	L-365,260 100 μg/kg
Number of line crossings	20±2.9	20±3.0	22±3.2	18±2.2
Time spent in open part (sec)	102±10	116±11	125±12	97±10
Number of open arm entries	0.9±0.27	1.1±0.33	1.6±0.40	0.7±0.24
Time spent in open arm (sec)	12±4	26±7	30±11*	8±3
Number of total arm entries	6.6±0.8	6.2±1.0	6.5±0.8	5.8±0.8
Ratio between open /total arm entries × 100	10±3	16±4	25±7*	8±3

* — $p<0.05$ (compared to vehicle treated group, Newman-Keuls test after significant one-way ANOVA). Number of animals is 16 in each group.

Table 5. The effect of devazepide on the anxiogenic-like action of caerulein in the elevated plus-maze

Parameters	Vehicle+ saline	Vehicle+ caerulein 5 μg/kg	devazepide 1 μg/kg+ caerulein 5 μg/kg	devazepide 10 μg/kg+ caerulein 5 μg/kg	devazepide 100 μg/kg+ caerulein 5 μg/kg
Number of line crossings	19±2.2	8±1.6*	11±1.8*	11±3.3	19±2.5**
Time spent in open part (sec)	175±11	102±17*	126±19	82±23*	158±13**
Number of open arm entries	1.0±0.4	0.3±0.2	0.4±0.4	0.5±0.3	0.8±0.4
Time spent in open arm (sec)	21±12	8±6	10±7	9±5	21±12
Number of total arm entries	5.4±0.7	2.5±0.5	3.0±0.7	3.4±0.9	5.5±0.5
Ratio between open/ total arm entries × 100	16±6	7±5	5±5	11±6	11±4

* — $p<0.05$ (compared to vehicle+saline treated rats, Newman-Keuls test after the significant one-way ANOVA); ** — $p<0.05$ (compared to vehicle+caerulein treated rats). Number of animals is 8 in each group.

Table 6. The action of devazepide on the exploratory activity of rats in the plus-maze

Parameters	Vehicle	devazepide 1 μg/kg	devazepide 10 μg/kg	devazepide 100 μg/kg
Number of line crossings	14±3.6	8±2.1	13±2.1	11±2.2
Time spent in open part (sec)	126±25	117±19	109±16	115±15
Number of open arm entries	0.8±0.31	0.6±0.32	0.3±0.16	0.3±0.16
Time spent in open arm (sec)	12±7	7±4	5±3	9±7
Number of total arm entries	4.6±1.1	2.9±0.7	3.8±0.8	4.0±0.9
Ratio between open/total arm entries x 100	10±4	13±7	6±4	4±3

Nunber of rats is 10 in each group.

Table 7. The comparison of the results of two distinct experiments, performed in July and in November: plus-maze exploration

Plus-maze parameters	July	November
Number of line crossings	10±1	8±2*
Time in open part (sec)	46±6	91±6*
Number of open arm enrtries	0.2±0.1	1.3±0.3*
Time in open arm (sec)	4±2	14±3*
Number of total arm entries	3.1±0.3	7.8±0.6*
Ratio between open and total arm entries (x100)	4±2	19±4*

* — $p<0.05$ (Student's *t*-test).

Table 8. The comparison of the results of two experiments, performed in July and in November: CCK, dopamine and 5-HT receptors in the brain

Radioligand binding in the brain structures		July	November
[^{3}H]pCCK-8 in the frontal	K_d (nM)	0.87±0.08	0.79±0.05
cortex	B_{max} (fmol/mg protein)	8.7±0.4	6.1±0.3*
[^{3}H]pCCK-8 in the	K_d (nM)	0.77±0.07	0.58±0.06*
hippocampus	B_{max} (fmol/mg protein)	5.3±0.3	3.7±0.3*
[^{3}H]spiperone in the striatum	K_d (nM)	0.59±0.06	0.75±0.10
	B_{max} (fmol/mg protein)	214±8	198±8
[^{3}H]spiperone in the frontal	K_d (nM)	1.52±0.25	1.11±0.11
cortex	B_{max} (fmol/mg protein)	140±18	106±5*

* — $p<0.05$ (Student's *t*-test).

Table 9. The comparison of the results from two experiments, performed in July and in November: blood levels of hormones

	TSH, ng/ml	Growth hormone, ng/ml	Prolactin, ng/ml
July	1.6±0.2	35±4	15±2
November	1.2±0.2	13±2*	11±1

* — $p<0.05$ (Student's *t*-test).

CURRICULUM VITAE

SULEV KÕKS

Citizenship: Estonian
Date and place of birth: January 20, 1971, at Pärnu, Estonia
Marital status: Married
Address: University of Tartu, Department of Physiology, 2 Näituse Street, Tartu 50409, Estonia
Phone: + 372 7 374 330; fax: + 372 7 374 332
E-mail: Sulev.Koks@ut.ee

Education

1978–1989	Pärnu Secondary School No 4
1989–1995	University of Tartu, Faculty of Medicine, graduated as M.D.
1995–1999	University of Tartu, Institute of Cell and Molecular Biology, Ph.D. student

Professional employment

1993–1996	Technician at the Department of Physiology, University of Tartu
1996–1999	Research scientist at the Department of Physiology, University of Tartu
1996	Visiting scientist at the Department of Pharmacology and Toxicology, University of Kuopio
1999–now	Research scientist at the Department of Physiology, University of Tartu

Scientific work

At the Department of Physiology I have studied the neurochemistry of anxiety and exploratory behaviour. I have performed behavioural and neurochemical studies. I have analysed the behaviour of animals in several tests, mostly measuring the exploratory behaviour (elevated plus-maze, zero-maze, light/dark compartment test, motility boxes, open-field). From the neurochemical studies I have experience with the radioligand binding studies, microdialysis, stereotactic lesioning. I have little experience with the electrophysiological studies

(patch-clamp studies, intracellular membrane potential recordings, *in vitro* voltammetry). At the Institute of Cell and Molecular Biology I have done the cloning of mouse CCK_B receptor.

My main scientific interest is the role of cholecystokinin in the Central Nervous System, especially in the generation of negative emotions. Also, the interaction between cholecystokinin and other neurotransmitters in the generation of "anxiety" network is under intense research.

CURRICULUM VITAE

SULEV KÕKS

Kodakondsus: Eesti Vabariik
Sünniaeg ja koht: 20. jaanuar 1971, Pärnu
Perekonnaseis: abielus
Aadress: Tartu Ülikool, füsioloogia instituut, Näituse 2, Tartu 50409, Eesti
Tel: + 372 7 374 330; faks: + 372 7 374 332
E-mail: Sulev.Koks@ut.ee

Haridus

1978–1989	Pärnu 4. Keskkool
1989–1995	Tartu Ülikool, arstiteaduskond, ravi eriala, arsti aste
1995–1999	Tartu Ülikooli molekulaar- ja rakubioloogia instituut, doktorant

Erialane teenistuskäik

1993–1996	Tartu Ülikooli füsioloogia instituudi laborant
1996–1999	Tartu Ülikooli füsioloogia instituudi teadur
1997	Kuopio Ülikooli farmakoloogia ja toksikoloogia instituudi külalisteadur
1999–	Tartu Ülikooli füsioloogia instituudi teadur

Teadustegevus

Tartu Ülikooli füsioloogia instituudi juures uurisin ärevuse ja uudistamiskäitumise neurokeemiat. Olen teinud hulgaliselt käitumiskatseid mitmesuguste, peamiselt uudistamiskäitumist hindavate mudelitega (plusspuur, nullpuur, hele/tume kahe kambri test, avarväli). Neurokeemilistest eksperimentidest olen teinud peamiselt radioligandretseptorsidumist, mikrodialüüsi narkoosis rottidel, stereotaktilisi purustusi. Mul on veidi kogemusi ka elektrofüsioloogiliste eksperimentidega (*patch-clamp*, membraanipotentsiaali intratsellulaarne registreerimine, *in vitro* voltameetria). Tartu Ülikooli molekulaar- ja rakubioloogia instituudis kloonisin hiire CCK_B-retseptori.

Minu peamine teaduslik huvi on koletsüstokiniini roll kesknärvisüsteemis, eeskätt negatiivsete emotsioonide kujunemises. Peale selle olen pööranud tähelepanu ka koletsüstokiniini interaktsioonile teiste neurotransmitteritega ärevusega seotud närviringide funktsioneerimisel.

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.
19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.

20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro*. Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous crassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.
40. **Arnold Kristjuhan.** Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu.** Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.
42. **Veljo Kisand.** Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.

43. **Kadri Põldmaa.** Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa.** Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik.** Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo.** Cuticular and stomatal antechamber conductance to water vapour diffusion in *Pice abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo.** Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots.** Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero.** Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees.** Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.

ISSN 1024–6479
ISBN 9985–56–417–0