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TARTU RIIKLIKU ÜLIKOOLI

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MOLECULAR PHARMACOLOGY OF RECEPTORS II

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Editor Lembit H. Allikmets

TARTU 1988

C o n t e n t s

Содержание

PREFACE

At Tartu University, the neuro-psychopharmacological research, including that done by the teams of pharmacology, psychiatry and biochemistry departments, has been going on for 25 years already. The Laboratory of Psychopharmacology both for experimental and clinical research was established in 1967. At Tartu University, the symposia on neuro-psychopharmacology have been performed regularly in every 2-3 years. The transactions (in Russian) on special fields of psychopharmacological research (GABAergic drugs, neuroleptics, novel psychoactive compounds, etc.) have also been published. The present publication is our first English edition, and it deals with the molecular mechanisms of psychotropics. This problem has been investigated at the Department of Pharmacology during past 8 years. The first volume of this series "Molecular Mechanisms of psychotropics" was issued in 1987 (Acta et comm. Univereitatis Tartuensis, N 766).

In this volume, the papers are mostly devoted to benzodiazepine receptors (binding sites in the brain and in peripheral tissues), to functional changes under stress and GABAergics. The other topic deals with D_1 and D_2 receptors, neuroleptic and cholecystokinin interactions, including the adaptation problem in pharmacology. We hope that this volume will be of use and value for those who are directly concerned with fundamental research in molecular pharmacology.

Lembit H. Allikmets

Professor and Chairman
Department of Pharmacology

PERIPHERAL-TYPE BENZODIAZEPINE BINDING SITES: FUNCTIONAL RECEPTORS OR ACCEPTORS

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Abstract:

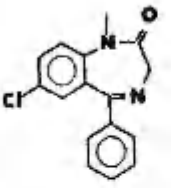
The central-type benzodiazepine (BZ) binding site, often referred to as the BZ receptor, is an integral part of the $GABA_A$ receptor-chloride channel in the mammalian CNS by means of which benzodiazepine and nonbenzodiazepine compounds produce either positive or negative allosteric modulation of the channel gaiting function with subsequent characteristic pharmacological effects. So far BZ receptors have not been established outside the CNS. In the contrary, the peripheral-type BZ binding sites are widely distributed in many organs and tissues outside the CNS but they can be found also in the CNS. Although the peripheral type BZ binding sites have been known for ten years already, the main efforts of the researchers were concentrated on central-type BZ binding sites. However, during the past few years considerable progress has been made in clarifying the multiple functions and properties of peripheral-type BZ binding sites. In our review the distribution, development, physiological regulation and functions, as well as the problem of possible exogeneous and endogeneous ligands of peripheral-type BZ recognition sites are discussed to determine whether they are functional receptors or acceptors.

1. INTRODUCTION

1,4-Benzodiazepines (BZ) belong to the most widely used drugs. They are primarily used as anxiolytics, being also effective as anticonvulsants, hypnotics, and muscle relaxants. Their site of action, specific benzodiazepine (BZ) receptors, was discovered a decade ago (69,95). When the binding of tritiated diazepam on various tissues in vitro was studied, diazepam appeared to attach saturably, specifically and with a high affinity also to the tissues not belonging to the central nervous system (22). This binding to the peripheral BZ binding sites has been demonstrated also in vivo (11). The binding sites in the peripheral organs, however, differ in several respects from those in the CNS, and many BZ compounds show selectivity in their binding to these two receptor groups.

Until recently, it was considered that the binding of the peripheral recognition sites would not, unlike that to the central receptors, be stereospecific (104). This, obviously, is the case with cultured erytholeukemia cells, while binding to several rat tissues, as well as to human granulocytes shows definite stereospecificity (19,33).

The molecular structure of BZ compounds determines the affinity of each compound. For a benzodiazepine compound to have a high affinity for the peripheral site, methyl substituent must be in position 1 (104). In contrast, clonazepam lacks the substituent in position 1, and it has a very high affintiy to the central receptor, but it only very weakly displaces the specific ligands from the peripheral BZ binding sites. On the other hand, Ro 5-4864, a chloro-substituted diazepam, displaces diazepam from peripheral BZ recognition sites approximately 30 thousand times more potently than from the central BZ receptors (22). Besides 1,4 -benzodiazepines some isoquinoline carboxamides, such as PK 11195, have also high affinity for the peripheral BZ binding sites (14). However, a great part of the research into the peripheral BZ binding sites has been carried out using tritiated diazepam or flunitrazepam, which nonselectively bind both to central and peripheral binding sites (22,78). The availability of such peripheral BZ receptor ligands as Ro 5-4864 (22, 90) and PK 11195 (14) has stimulated research into these binding sites.



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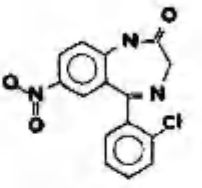

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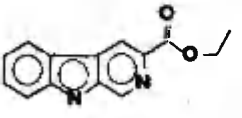

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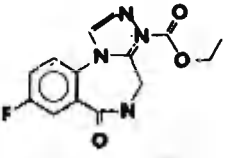
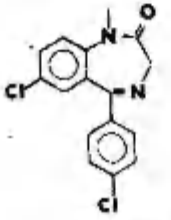
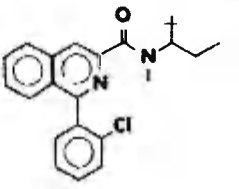


RO 15-1788



RO 5-4864



PK 11195

Fig. 1. Compounds with a high affinity for the peripheral-type benzodiazepine binding sites (Ro 5-4864, diazepam, PK 11195). Clonazepam, ß-carbolines such as 3-carboethoxy-ß-carboline and imidazobenzodiazepine Ro 15-1788 bind selectively to the central-type receptor only.

2. DISTRIBUTION OF PERIPHERAL-TYPE BINDING SITES.

Originally the classification of BZ receptors was made according to the localization of receptors in various organs. While the central BZ receptors are situated in the neuronal tissue only, the peripheral BZ binding sites occur in several tissues, e.g. in heart (28,42,100), lungs and adrenals (22,31), in kidneys (3,8,30,31,42,100), in testes, liver, spleen, the epithelian tissue of nose, tongue and in the salivary glands (31), in human placenta (34), lymphocytes (66) mast cells (98), granulocytes (19) and platelets (15,103). So far only few muscles have been investigated in the case of the human: iris/ciliary-body preparations (111) and myometrium (81) both contain high affinity peripheral-type BZ binding sites. Also the rat diaphragm and the guinea-pig ileal muscle contain these binding sites (48,110), although the striated muscle and gastrointestinal tissues have been said to lack any peripheral BZ receptors (1).

The peripheral-type BZ binding sites are situated in brain (3,14,31,42,56,89) and in spinal cord (29,113). The olfactory bulb (90) and the pineal gland (54,75,108) show particularly high density of these recognition sites. In general, their density in the neuronal tissues is lower than that in peripheral organs, but in several animal species as well as in man the distribution of the peripheral-type BZ binding sites in brain shows a distinctive pattern different from the relative heterogeneity of the distribution of the central-type binding sites (1,42). Autoradiographic localization of peripheral BZ receptors has shown that specific ^{3}H-Ro 5-4864 binding sites can also be observed in the choroid plexus and ependyma cells within the rat brain (42). Intrastriatal injection of excitotoxic compounds has been demonstrated to provoke a dose-dependent increase in the levels of peripheral BZ binding sites with the following potency order: kainate > (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) > N-methyl-D-aspartate (NMDA) > quisqualate (9). The authors concluded that peripheral BZ binding sites constitute a more sensitive indirect index of neuronal damage than neuronal enzymes, choline acetyltransferase or glutamate decarboxylase. Although intrastriatal kainate is known to cause neuronal damage and glial proliferation, a further characte-

rization of the localization of peripheral BZ binding sites to different types of glia is necessary. Similarly to the experimental neuronal damages the peripheral-type BZ binding sites appear to be elevated also in the case of senile dementia of the Alzheimer type and Huntington's disease (72, 91).

However, recent autoradiographic studies in human brain with ^{3}H-PK 11195 suggest a neuronal rather than glial localization of peripheral BZ binding sites found in forebrain structures and the localization was not limited to any functional system, nor did it resemble any previously described transmitter system (32).

The tissues that contain high densities of the peripheral BZ binding sites produce their energy by oxidative phosphorylation. For example, in kidneys the highest densisities of these receptors are in the neighbourhood of the ascending part of the loop of Henle and the collecting ducts - these structures contain the highest amount of mitochondria. Also the oytochromal activity is the highest in the areas which contain the highest densities of peripheral BZ binding sites (3).

Subcellularly, the peripheral BZ binding sites occur most abundantly in the nuclear (P1) and mitochondrial fractions, while central-type receptors are predominant in the synaptosomal and P2 fractions (22,56,90). The most striking species difference is that in the rat ^{3}H-PK binding is highly enriched in the nuclear fraction, whilst this nuclear enrichment is not seen in the cat or human (32). It has been suggested that the peripheral-type BZ recognition sites are located in the outer membrane of mitochondria (4,112).

3. DEVELOPMENT OF THE PERIPHERAL-TYPE BZ BINDING SITES.

The ontogenetic development of the peripheral BZ binding sites resembles that of the central receptors: both types of BZ receptors can be detected already on the 16th - 17th day of conception (3,21,77). Phylogenetically, the peripheral BZ binding sites appear later than the central BZ receptors; they are found in mammals only, while the latter are also detectable in fish, reptiles and birds (20,71).

4. ENDOCRINE REGULATION OF PERIPHERAL-TYPE BZ BINDING SITES

The endocrine functions seem to alter the characteristics of the peripheral-type BZ binding sites: adrenalectomy causes a decrease in the number of these binding sites, in kidney this change can be reversed with exogeneous aldosterone (6). Hypophysectomy also tends to decrease the number of the BZ binding sites in adrenals and in testes, and this change is more marked than the decrease in the weight of these organs - in the kidneys, brain, heart and lungs the characteristics of the peripheral-type BZ binding sites remain unaltered after the hypophysectomy (2). Ro 5-4864 inhibits ß-endorphine secretion from AtT-20 (anterior pituitary-derived tumor cell line) cells through the blocade of the voltage-dependent membrane Ca^{2+} channels (18). Pituitary stalk transection has been shown to increase markedly ^{3}H-Ro 5-4864 binding in this organ (23). The deprivation of neural stimulation to the pineal gland by constantly keeping the animal in illuminated conditions causes a selective diminishment of the peripheral BZ binding sites, while the central-type receptors in the pineal gland are not altered (108). In diabetes insipidus, the number of the peripheral BZ binding sites increased in the kidneys of Brattleboro rats (30). Exogeneous hormons also affect the peripheral BZ binding sites: thyroxine alters their density (38) and estradiol reduces their number in testis and increases it in the kidneys (40).

5. PERIPHERAL-TYPE BZ BINDING SITES AND CALCIUM CHANNELS

At micromolar concentrations the calcium antagonists nifedipine and nitrendipine competitively displace Ro5-4864 from the peripheral BZ binding sites in brain, kidneys and heart. These drugs do not affect the binding of flunitrazepam to the central-type BZ receptors (24,87). This affinity for the peripheral BZ binding sites is not common for all calcium antagonists: diltiazepam and verapamil lack the ability to displace the peripheral-type BZ receptor ligands (87). Nitrendipine inhibits potassium-induced cellular uptake of calcium in the hypophysis of rats, and Ro 5-4864 at micromolar concentrations causes a similar effect, which

can be counteracting with PK 11195 (12). In the rabbit aorta PK 11195 in a micromolar range of concentration behaves as an antagonist of voltage-operated calcium channels, but it does not interact with receptor-operated channels (62).

In glia-cells, BZs displace nitrendipine from its binding sites, exhibiting the same rank order of potency as in displacing diazepam from glia-cells. The BZ binding sites in the glia-cells are of the peripheral type (16). BZs in micromolar concentrations also inhibit Ca^{2+} conductance in neurons (49). Recently it was demonstrated that ^{3}H-Ro 5-4864 binds specifically and saturably to an apparently homogene - ous, univalent type of binding site on the calmodulin molecule (K_D 644 $\pm$ 121 nM). Such binding of ^{3}H-Ro 5-4864 was protein-, pH- and temperature-dependent and demonstrated pharmacological selectivity (68).

All these findings suggest that the peripheral BZ binding sites might be linked with calcium channels (63) and other calcium mediated processes (68). However, it has been shown (47) that Ro 5-4864 at concentrations under 3 µm does not alter the calcium influx in the ventricles of heart during depolarization. At higher concentrations, Ro 5-4864 decreased the ion-influx while causing negative inotropic effect on the papillary muscle. As the concentration needed to influence the movements of calcium is so high, the connection between the peripheral BZ binding sites in heart and in calcium channels does not seem entirely convincing.

6. LIGANDS FOR PERIPHERAL-TYPE BZ BINDING SITES

Several compounds with BZ structure show high affinity (Ki values at nanomolar level) for the peripheral BZ binding sites, while pharmacologically active non-BZs usually lack this affinity, e,g. acetylcholine, atropine, carbacho - line, adrenaline, phentolamine, propranolol and serotonin (98,99,103). On the other hand, in addition to nifedipine and nitrendipine, some vasodilators, such as cyclandelate or prazosin exhibit a moderate affinity (Ki against flunitrazepam in heart 7.1 and 30 µM, respectively) (87). Dipyridamole - a drug with vasodilating and anti-aggregatory properties , exhibits affinity with Ki value being 0.1 µM (28). Also some anti-inflammatory analgesics possess affinity for peripheral

BZ binding sitee: proquazone has a rather high affinity, Ki 0.14 μM, and also azapropazone, diflunisal, fenoprofen, mefenamic acid, sulindac and tolfenamic acid show affinity, Ki values ranging from 5-25 μM (87). It has been demonstrated that several anion transport inhibitors, like 9-anthroic acid, furosemide, bumetanide, hydrochlorothiazide and SITS inhibit ^{3}H-Ro 5-4864 binding to renal membranes with Ki values from 30 to 130 μM which were consistent with their actione as anion transport inhibitors (5). The effects of long-term drug treatment have been less thoroughly investigated. In schizophrenics medicated with antipsychotic drugs, a 30 % decrease in the number of peripheral BZ binding sites in platelets has been demonstrated (41). An increased number of renal peripheral BZ binding sites and diuresis was observed after five days administration of hydrochlorothiazide or Ro 5-4864 (5).

7. PERIPHERAL EFFECTS OF THE PERIPHERAL-TYPE BZ BINDING SITE LIGANDS.

One of the major reasons for considering the peripheral BZ binding sites as pharmacologically meaningless acceptor sites has been the relative absence of the BZs effects on the peripheral organs with high affinity for these receptors. One such example is diazepam. In therapeutic use, BZs produce their central effects at less than mg/kg dosage level, and hardly ever can any other effects be as important as those on the CNS. However, diazepam has been shown to affect the cardiovascular system by increasing the coronary flow and by decreasing blood pressure and heart rate (27). Ro 5-4864 has negative inotropic action and it can specifically counteract with PK 11195 which also is a potent ligand for the peripheral BZ binding sites (64). These two ligands show opposite properties also in their effects on behaviour (65).

Some reports have demonstrated changes in the charac - teristics of the peripheral BZ binding sites in kidneys correlating with hypertension (78,98,101), while in some studies the changes have not been observed. In a majority of cases, the affintiy of antihypertensive compounds for the binding sites mentioned, however, is very low (87), which

suggests that the peripheral-type BZ binding sites hardly represent the pharmacological site of action of these drugs, although they may be the mediators of the regulatory processes concerned with blood pressure. Recently, the data indicating that the peripheral BZ binding sites may have an active role in the regulation of aldosterone secretion have been presented (92).

BZs also exert a variety of slowly appearing effects on cultured cells. These compounds can induce cell differentiation in the Friend erythroleukemia cells and block mitogenesis in the 3T3 cells (26). Melanoma cells possess the peripheral-type BZ binding sites, and the melanogenesis in these cells can be enhanced with Ro 5-4864 (58). In pinealocytes , BZs increase the production of melatonin (59). BZs also inhibit the proliferation of cultured thymoma cells; these cells have also the peripheral BZ binding sites (102). Ro 5-4864 stimulates the phospholipid methylation in the C6 astrocytoma cells (97). BZs appear to induce the chemotaxis of human monocytes (82) and stimulate humoral immune response (115). Peripheral BZs have been reported to enhance the respiratory burst of macrofage-like $P388D_1$ cells stimulated by arachidonic acid in a PK 11195 reversible manner (114). As the O_2 production is an important biochemical indicator of the defensive activity of macrofages against infection and malignant cells, the potential immunopharmacological usefulness of peripheral ligands remains to be elucidated.

Dipyridamole, a non-BZ with high affinity for peripheral BZ receptors, has been used as an anti-melanoma agent with some success (79); dipyridamole also enhances the cytotoxic action of metotrexate (51). These findings may indicate a pharmacological effect possibly related to interaction with peripheral BZ binding sites. At least in some cases, however, the potency in effect does not correlate with the affinity for the peripheral BZ binding sites (e.g. inhibition of neurite outgrowth in PC12 cell cultures (69)).

8. CENTRAL EFFECTS OF THE PERIPHERAL-TYPE BZ BINDING SITE LIGANDS.

Attempts to modify the benzodiazepine structure in order to develop new compounds with more selective spectrum of

activity led to the synthesis of Ro 5-4864, now recognized as a specific ligand for the peripheral BZ binding sites. In preliminary studies, this compound was shown to be effective against the electroshock-induced convulsions, to cause tranquilization, drowsiness and occasional ataxia being only slightly less effective than chlordiazepoxide (116). However later it was demonstrated that Ro 5-4864 exhibits convulsive and anxiogenic actions in animal tests (73) that cannot be antagonized with the central-type BZ receptor antagonist Ro 15-1788 (37). Moreover, the convulsive effect of Ro 5-4864, can be counteracted with PK 11195, which also is a ligand for the peripheral BZ binding sites (10, 35). On the other hand, Ro 5-4864 in a PK 11195 reversible manner blocked the anticonvulsant action of carbamazepine but not that of diazepam on amygdala-kindled seizures (105). According to neurochemical and pharmacological studies, it has been proposed that carbamazepine may exert some of its anticonvulsant effects by acting as a partial agonist at adenosine receptors (94). Recently it has been suggested that Ro 5-4864 has both antagonistic and potentiative interactions with adenosine on the cerebral cortical neurons (74). This effect of Ro 5-4864 may partly explain the described interactions with carbamazepine. The Ro 5-4864 induced convulsions were blocked by diazepam and pentobarbital, but they were not blocked by the central BZ antagonist Ro 15-1788 (76). The activation of GABA receptors by amino-oxyacetic acid, the GABA-T inhibitor or GABA agonist, muscimol, protected, while the subconvulsive doses of bicuculline, a direct GABA receptor antagonist enhanced the convulsant action of Ro 5-4864 (76,106).The examination of a series of compounds that are structurally related to Ro 5-4864 revealed a good correlation between their potencies as convulsants and their capacities to inhibit the ^{35}S-TBPS binding (107). These results support the proposal that a significant aspect of convulsant actions of Ro 5-4864 is of central origin, and most likely the site of action appears to be the picrotoxin site of GABA-BZ receptor complex. However, it is important to take into consideration that the described neuropharmacological effects of the peripheral BZ binding site ligands have been obtained in rodents, and in the light of large differences between the regional and subcellular distribution of peripheral BZ binding sites in rat

and in human (32) their relevance to human neuronal function does not become obvious.

9. REGULATION OF PERIPHERAL-TYPE BZ BINDING SITES BY GABA AND STRESS.

The majority of the central-type BZ binding sites in the CNS are linked to the GABA-receptor and chloride ion channel (44,46). The connection between the peripheral- type BZ binding sites and GABA receptors is not so clear yet. In vitro, GABA does not alter the binding affinity or the density of the peripheral BZ binding sites, and neither does the chloride ion (56,90). Moreover, there are no ^{3}H-muscimol binding sites, no GABA-ergic regulation, no barbiturate regulation nor the ^{35}S-TBPS binding present in the purified peripheral BZ recognition sites (57). Nevertheless, the apparent molecular weight of solubilized peripheral- and central BZ receptors is quite similar: 215,000 and 260,000-270,000 , respectively (13,52). In contrast to these data, in vivo $GABA_A$ and $GABA_B$ agonists have been shown to alter both central and peripheral BZ binding sites (84). Acute treatment of rats with muscimol and (-)baclofen resulted in a marked increase in the affinity of BZ recognition sites not only in cerebral cortex but also in kidneys. In addition to that the (-)baclofen treatment also lowered the number of BZ binding sites in both structures studied (85). Several lines of evidence demonstrate that central BZ and GABA receptors are affected by stress (17,60). However, little is known about how stress can influence the peripheral-type BZ binding sites. A comparative study of naive (stressed) and handling-habituated (unstressed) rats showed that also the number of both central and peripheral BZ binding sites was higher in naive rats (83). After forced swimming stress a significant increase in the density and a certain decrease in the affinity of the ^{3}H-flunitrazepam binding sites in the rat cerebral cortex and kidneys were observed (86). Thus, the central and peripheral-type BZ binding sites seem to be regulated analogously by at least two stress models. Considering the above-mentioned results, it was of great interest to study the action of stress on the peripheral-type BZ binding sites in blood platelets. According to our recent data (Table 1)

Table 1

The effect of acute swimming stress (at 20 ± 1°C) on peripheral-type BZ binding sites on intact blood platelets in rat.
^{3}H-Ro 5-4864 binding was carried out by incubation of the ligand at concentrations ranging from 0.75-48 nM with ul of washed platelet preparation in a modified Hanks solution (pH 7.4) for 30 min at +4°C. Each value is the mean ± SEM of four separate experiments, each carried out using pooled blood from 8 rats.

Experimental group	^{3}H-Ro 5-4864 binding			
	B_{max} (pmol/ 10^8 cells)	%	K_D (nM)	%
Control	6.8 ± 1.2	100	6.8 ± 1.6	100
Stress	4.1 ± 0.7*	60	11.3 ± 2.7	166

* - $P < 0.05$ as compared with control rats

swimming stress also enhanced the number and had a tendency to decrease the affinity of ^{3}H-Ro 5-4864 binding sites on the intact blood platelets of rats. In contrast to this, in man, the binding capacity of peripheral-type BZ binding sites on the platelets of anxious patients has shown a certain reduction in comparison with normal controls (109). A surprising similarity between the in vivo regulation of the central and peripheral BZ binding sites by GABA agonists and stress is difficult to explain. Recently, a new neuropeptide called DBI (diazepam binding inhibitor) that possesses a unique genetic code, which is located in neurons and can be released by depolarization, has been isolated (43, 45). The DBI mRNA is expressed not only in neurons but also in glial cells, in liver, kidney and heart and its concentration has some features that are related to emotional state

(45). Similar regulation of central- and peripheral-type binding sites by GABA agonists and stress seems to support the idea that there must be at least one common endogenous ligand for both types of BZ receptors. The localization of relatively high concentrations of peripheral BZ recognition sites in pituitary and adrenals (31) may indicate the possibility that these binding sites could be involved in the neuroendocrinological regulation of emotional behavior. Indirectly this suggestion is supported by the data demonstrating that the hyperthyroidism produced by chronical T_4 treatment increases the number of both central and peripheral BZ receptors (39). There is no doubt that hyperthyroidism can also change emotional behavior.

In conclusion, although the mechanism by which GABA and stress can modulate peripheral BZ binding sites remains to be elucidated, a possibility to use peripheral BZ binding sites on blood cells (platelets for example) as the potential markers of the functional activity of central BZ receptors can be suggested.

10. ENDOGENOUS LIGANDS AND THE PHYSIOLOGICAL ROLE OF THE PERIPHERAL-TYPE BZ BINDING SITES.

Freezing and thawing the membrane preparation from the adrenals of the rat increases the affinity of Ro 5-4864 binding sites without affecting the number of these sites. A similar change can be brought about by detergent treatment. It has been suggested that the increase in the affinity may be due to the displacement of endogenous ligands from the peripheral BZ binding sites (39). Beaumont et al. (7) have detected compounds with Ro 5-4864 displacing activity from the blood and urine of the man. In another study, acidified methanol extracts from stomach, kidney and lungs were found to inhibit only ^{3}H-Ro 5-4864 binding to the peripheral BZ binding sites but not that of ^{3}H-diazepam to central BZ receptors (55). BZ binding to kidneys can be augmented by perfusion, possibly through washing out the endogenous ligand (90). In the guinea-pig ileum, BZs inhibit the effect of cholecystokinin, but not that of neurotensin, 5-HT or substance P (61). In a recent study, Verma et al. (112) have convincingly shown that porphyrins exhibit a ve-

ry high affinity (Ki values less than 50 nM) for peripheral, or as they call them, mitochondrial BZ receptors. They support the hypothesis about a possible physiological role of this receptor as a regulator of the voltage-dependent anion-channel already presented by Anholt (1). Porphyrins would thus be the endogenous ligands for peripheral BZ receptors at least in the steroid forming tissues. The role of these receptors in nonendocrine tissues (e.g. in ventricles of heart, brain) is still obscure. However, recently the peripheral BZ binding sites labeled by ^{11}C-PK 11195 have been characterized in the living heart of dog and human being by means of the positron emission tomography (25). Since it has recently been proposed that the peripheral BZ binding sites could be coupled to the calcium channel in heart (63), positron emission tomography opens way to the investigation of this receptor in clinical situations. In brain, the role of the peripheral-type BZ binding sites is even more obscure than in the oregans outside the CNS. Recently, it has been reported that the number of these binding sites increased as a result of neuronal damage, which makes them a sensitive indicator of the diseases involving brain, e.g. Parkinson's or Huntigton's disease (9). Moreover, the presence of high concentration of peripheral BZ binding sites on glial tumors suggests that human primary central nervous system tumors could be imaged and diagnosed using the peripheral BZ ligands labeled with positron- or γ-emitting isotopes (96).

11. RECEPTORS OR ACCEPTORS?

In spite of the lack of overwhelming convincing evidence, the peripheral-type BZ binding sites are often referred to as peripheral BZ receptors. Originally the peripheral-type BZ binding sites were established using a radioligand binding method. However, although a radioligand may bind to a site, this site may not be a neurotransmitter receptor or another receptor of interest. Until the binding of a radioligand can be shown to be associated with a pharmacological or physiological event, the mere fact of the existence of an even saturable specific high affinity binding cannot be quated with a receptor (53,80). A true receptor involves signal sensing and transduction, i.e. the following

binding with the receptor allosteric modulation of signal detection (which happens after the binding of a ligand with the central-type BZ receptors) and/or the signal transduction causes postreceptor events (receptor-effector coupling, second and third messengers) that lead to the pharmacological or physiological response. Thus, a receptor involves 3 consequent processes, while an acceptor site is only a binding site without a following signal transduction and physiological response. When defining a binding site as a receptor it is important to satisfy a number of criteria (Table 2).

Table 2

Specificity criteria for defining a receptor (modified by Laduron, 1984).

1. Drug displacement (agonists and antagonists belonging to different chemical or pharmaceutical classes)
2. Binding (drug affinity) in vitro can be shown to be associated with a pharmacological or physiological event
3. Regional distribution or tissue specificity
4. Subcellular distribution
5. Saturability (finite number of sites)
6. High affinity
7. Reversibility
8. Stereospecificity

The fact that many of the criteria for defining a binding site as a receptor (Table 2) are satisfied by are satisfied by peripheral-type BZ binding sites, is intriguing. Nevertheless, mainly the lack of a definitive drug effect following the attachment of drug molecules on these recognition sites does not enable to determine whether they are indeed functional receptors or merely acceptor sites. In conclusion, in spite of the gained success, the role of the peripheral-type BZ binding sites in mediating drug effects or physiological processes remains still obscure.

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BIOCHEMICAL AND BEHAVIORAL CHARACTERIZATION OF NAIVE AND HANDLING-HABITUATED RATS

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Abstract:

Chronical habituation of rats to the handling procedures preceding sacrifice led to changes in binding characteristics of ^{3}H-flunitrazepam, ^{3}H-GABA, ^{3}H-muscimol, ^{3}H-spiperone and ^{3}H-diprenorphine. Differences were also registered in the blood concentration of growth hormone and in the amount of conjugated diene in frontal cortex. In the model of elevated plus-maze the habituated rats showed diminished exploratory behaviour. The differences in the binding parameters of several receptors and in the behaviour of the rat provide evidence that the habituated animals showed greater emotionality.

Introduction

The effects of various models of stress and anxiety on the CNS neurotransmission have been extensively studied in animals, using biochemical and behavioural techniques. There is substantial literature which shows that the environmental novelty, in various forms, activates the pituitary-adrenal axis of stress-response. For example, simply moving rats and their housing cages from one place to another leads to a rapid rise in the corticosterone, prolactin and thyroid stimulating hormone (6,8). Similarly, transporting mice or rats to places different from the housing cage leads to the release of epinephrine, norepinephrine and adre-

nal corticosteroids, these responses being resistant to habituation (11).

In a series of experiments it has been shown (1,2) that GABA receptors in different brain areas may vary with the state of animals before sacrifice. Namely, the rats habituated to the handling procedures preceding sacrifice presented a higher number of GABA receptors than naive animals. The lack of resistance of habituated animals during handling procedures and the in vitro binding studies led the authors to the conclusion that the handling-habituated rats could be termed as unstressed animals (1,2).

However, on the other hand, chronic intermittent exposure of rats to a variety of unpredictable stressors has been widely used (15,22,9) as an animal model of depression inducing a variety of behavioral, neuroendocrine and neurochemical alterations.

In addition, handling prior to experimentation was reported (25) to diminish the behavioral and hormonal differences in response to the novel situation between normal rats and those with decreased emotional reactivity. Reduced emotional reactivity in adulthood is also one of the most obvious effects of handling infant rats during their preweaning period (24). These contradictory data prompted us to further characterize the biochemical and behavioral parameters of naive and handling-habituated rats.

Materials and Methods

Male albino laboratory rats (Rappolovo farm, Leningrad) were divided into two groups, termed as naive and handling-habituated animals. The handling-habituated rats were habituated twice daily for 10-12 days to the handling manoeuvres preceding sacrifice by decapitation. Naive animals were left in their home cages for that period. To determine the exploratory activity of naive and habituated rats, an elevated plus-maze was used as described recently (20). During a 4 min test session the latency period of the first open part entry, the number of sectors crossed in open arms, and total time spent in open arms were measured.

For binding studies the animals were killed by decapitation without any previous experiments. Dissected and ho-

mogenized brain regions, ren and adrenal were washed twice in ice-cold Tris-HCl buffer (pH 7.4) by centrifugation and resuspension. Five additional washes of membranes with Tris-HCl buffer were carried out for the $GABA_A$ receptor binding assays.

The binding of ^{3}H-flunitrazepam (81 Ci/mmol, Amersham) was carried out in the presence of 0.25-32 nM of the labeled ligand and 10 μM flunitrazepam. After 60 min incubation at 4° G the reaction was stopped by rapid filtration over GF/B Whatman filters, followed by three washes of 5 ml each with ice-cold buffer.

$GABA_A$ receptors were assayed in extensively washed membranes using 2-60 nM ^{3}H-muscimol (18 Ci/mmol, Amersham) and 4-80 nM ^{3}H-GABA (52 Ci/mmol, Amersham). The incubation was carried out at 4° for 15 min and nonspecific binding was determined in the presence of 10 μM muscimol and 100 μM GABA respectively. The reactions were stopped by centrifugation at 10 000 g for 5 min.

The binding of 0.1-4 nM of ^{3}H-spiperone (72 Ci/mmol, Amersham) was determined after 30 min incubation at 37° 0 in the presence and absence of 10 μM haloperidol. Opiate receptors were labelled with 0.2-6 nM ^{3}H-diprenorphine (34 Ci/mmol, Amersham) using 5 μM naloxone to determine nonspecific binding. The reaction was carried out at 20°C for 45 min and stopped by centrifugation at 10 000 g for 5 min.

The values for the number of binding sites (B_{max}) and the apparent dissociation constant (K_D) were determined for each individual binding experiment from the Scatchard plots.

Growth hormone was measured in the serum of the trunk blood of decapitated animals using a radioimmunoassay kit (CIS). The conjugated diene assay of rat cortex was carried out according to the heptane-izopropanol method at 233-234 nm (26).

Results and Discussion

Binding studies with ^{3}H-flunitrazepam revealed clear differences between naive and habituated animals. From various tissues studied, reduction in the density of ^{3}H-flunitrazepam binding sites of the habituated rats reached statistical significance in cerebral cortex, ren and adrenal

(Table 1). There was no significant change in the affinity of benzodiazepine binding sites. The diminished number of central benzodiazepine receptors in habituated rats resembles the decrease observed by other authors after acute (18) and chronic stress-situations (5).

Table 1

^{3}H-flunitrazepam binding parameters derived from various tissues of naive and habituated rats. Each value is the mean ± SEM of 5-8 separate experiments.

Brain region, Group	^{3}H-flunitrazepam binding	
	B_{max} (fmol/mg protein)	K_D (nM)
C E R E B R A L C O R T E X		
Naive	1670±120	1.32±0.14
Habituated	1260±100^{x}	1.15±0.11
H I P P O C A M P U S		
Naive	1060±80	1.12±0.12
Habituated	920±70	1.02±0.10
C E R E B E L L U M		
Naive	960±70	1.27±0.10
Habituated	1020±60	1.42±0.17
R E N		
Naive	3560±190	11.6±1.2
Habituated	2670±230^{x}	10.0±1.3
A D R E N A L		
Naive	18300±1100	27.6±3.2
Habituated	13800±1200^{x}	21.4±2.5

^{x} $P < 0.05$ as compared to naive animals

Reduction in the number of the peripheral benzodiazepine binding sites of habituated rats (Table 1) may be related to the recent findings (7) of a decrease in the density of peripheral benzodiazepine binding sites in a strain selectively bred for a high degree of "fearfulness". Thus, our results of ^{3}H-flunitrazepam binding demonstrate some neurochemical signs of anxiety in habituated rats, an effect observed simultaneously in the central and peripheral type benzodiazepine receptors.

Table 2

Binding parameters of brain $GABA_A$ receptors of naive and habituated rats. ^{3}H-muscimol (^{3}H-MUSC) and ^{3}H-GABA were used to characterize $GABA_A$-receptors. Each value is the mean ± SEM of 3-6 separate experiments.

Brain region / Group	Ligand	B_{max} (fmol/mg protein)	K_D (nM)
C E R E B R A L C O R T E X			
Naive	^{3}H-MUSC	3160±180	25.3±1.7
Habituated		2520±130^{x}	22.7±1.8
Naive	^{3}H-GABA	3650±280	220±29
Habituated		2520±260^{x}	180±15
H I P P O C A M P U S			
Naive	^{3}H-MUSC	1730±120	23.5±3.0
Habituated		1250±110^{x}	18.3±1.1
C E R E B E L L U M			
Naive	^{3}H-MUSC	1860±140	18.6±2.3
Habituated		1970±160	21.3±2.7

^{x} $P < 0.05$ as compared to naive animals

The data presented in Table 2 indicate that the number of $GABA_A$ receptors is lower in the cerebral cortex and hippocampus of habituated animals, but not in the cerebellum. These results are in conflict with the results of Biggio (2) who registered a higher number of low-affinity ^3H- GABA binding sites in cortical membranes of handling- habituated rats using slightly different experimental conditions. The discrepancies can be explained in at least two ways. First, it is well known (16) that the differences in the tissue preparation and buffers used for ^3H-GABA binding assay can lead to different binding characteristics. Secondly, it has been recently suggested (10) that stress can affect the sensitivity of the components of the GABA-benzodiazepine supermolecular complex to chloride ions. Thus, the results in Table 2 can reflect a change in the coupling between chloride ionophore and GABA receptor rather than a direct effect on the GABA receptor.

With the intention to find the behavioral correlates to the changes in benzodiazepine and GABA binding sites, an elevated plus-maze model was used, reported to be a specific measure of anxiety in the rat (20).

Table 3

Exploratory activity of rats in an elevated plus-maze. Results are expressed as a mean ± SEM

	Latency of first open arm entry(s)	N of open arm sectors crossed	Total time spent in open arms (s)	Defecation (N of boluses)
Naive	12.2±2.1	13.6±1.4	123±13	0.4±0.2
Habituated	18.8±2.8	8.5±0.8^x	82±13^x	2.1±0.5^x

x P 0.05 as compared to naive animals

Table 3 shows that in our experiments the handling-habituated animals showed lower explorstory activity and higher defecation rate than naive rats. Diminished exploratory activity after various chronic stressors has been reported by several authors (9,22,5) and differences in open field defecation have been interpreted as an indicator of emotionality (3). Therefore, it can be assumed that actually

chronic exposure to the stressors in our experiments resulted in a decreased exploratory activity and defection, and, thus, the habituated rats behaved like "stressed" animals, e.g showed greater emotionality in the test model used.

Peroxidation of unsaturated lipids has become an attractive hypothesis for the explanation of various pathological conditions, including stress (4). Since lipid peroxidation is related to oxygen metabolism in biological systems, it is assumed that lipid peroxidation can be set into motion whenever the conditions of the increased oxidative stress (increased production of free radicals and/or decreased antioxidant defences) occur in the cell (14).

Table 4

Values of conjugated diene and growth hormone of naive and habituated rats. Results are expressed as a mean ± SEM for groups of 9 rats.

	Conjugated diene (unit/100 mg tissue)	Growth hormone (ng/ml serum)
Naive	114.5±16.3	0.84±0.12
Habituated	130.0±22.8	1.13±0.18

From several products of lipid peroxidation reactions, the amount of conjugated diene was determined in this study from the cerebral cortex of naive and habituated rats.(see Table 4), In the habituated rats the amount of conjugated diene was found to be higher than in the naive animals, although the differences were not statistically significant . However, the increase of conjugated diene described here is in agreement with the hypothesis on the protective adapta - tion in lipid metabolim during the initial stages of stress (14).Benzodiazepines, GABA and various stress situations stimulate the growth hormone secretion in the man and in the rat (for review, see 23).In our studies the blood-level of growth hormone was higher in the handling-habituated rats (Table 4), thus indirectly indicating more stressful expe- riences of habituated animals compared with naive ones.

The stress-induced increase in the level of growth- hormone has been reported using several stressors including electric shock (17) but these models are not comparable to the handling-habituation procedure used in this study. Moreover, the differences in the blood-level of the growth-hormone of the naive and habituated rats in our studies was not statistically significant and therefore, no conclusive decisions should yet be drawn.

In a further study an attempt was made to investigate the effects of handling on the receptors of catecholamines using ^{3}H-spiperone as a labelled ligand and haloperidol to determine nonspecific binding.

Table 5

^{3}H-spiperone (^{3}H-SPI) and ^{3}H-diprenorphine (^{3}H-DIP) binding parameters derived from brain tissue of naive and habituated rats. Each value is the mean ± SEM of 3 separate experiments.

Brain region, Group	Ligand	B_{max} (fmol/mg protein)	K_D (nM)
1	2	3	4
C E R E B R A L C O R T E X			
Naive	^{3}H-SPI	170±15	0.67±0.05
Habituated		210±25	0.85±0.10
Naive	^{3}H-DIP	260±20	0.48±0.05
Habituated		180±16*	0.52±0.07
S T R I A T U M			
Naive	^{3}H-SPI	360±41	0.35±0.03
Habituated		420±38	0.30±0.04
Naive	^{3}H-DIP	340±32	0.69±0.10
Habituated		280±47	0.75±0.07
H Y P O T H A L A M U S			
Naive	^{3}H-SPI	160±17	0.32±0.04
Habituated		105±10*	0.28±0.03

Table 5 continued

1	2	3	4
Naive	^{3}H-DIP	280±13	0.86±0.10
Habituated		210±20^x	1.27±0.11^x

^{x} $P < 0.05$ as compared to naive animals

Of the three brain regions studied (Table 5), statistically significant differences were registered only in the mesolimbic region (e.g., structures ventral to striatum). Namely, the number of ^{3}H-spiperone binding sites was smaller in the mesolimbic region of the habituated rats. It is difficult to interpret these results because the ligand combination used for this binding assay labels not only dopamine receptors but also some subgroups of serotonin- and α-adrenoreceptors. Moreover, the majority of the reports investigating the influence of stress on the brain catecholamines deal with the release and metabolism of these biogenic amines. To our knowledge, there is only one previous report concerning the effect of the chronic low-level stress on the brain ^{3}H-spiperone binding (21) and it was found that chronic prenatal stress increased the ^{3}H-spiperone binding in the cerebral cortex and hippocampus. In our studies, a statistically insignificant increase in the number of binding sites of ^{3}H-spiperone in the cerebral cortex of the habituated rats was found (Table 5). Nevertheless, our binding data suggest that handling procedures can influence the ^{3}H-spiperone binding characteristics. Whether this influence is caused by the changes in dopamine, serotonin or α-adrenoreceptors, remains to be established.

The role of the endogenous opiate system in stress has been investigated frequently (for review, see 19). Several authors have also provided evidence that the persistent activation of opiate receptors by the endogenous opioids released during restraint-stress leads to the receptor "down-regulation" (12,13). In our studies, the reduction in the number of the ^{3}H-diprenorphine binding sites of the handling-habituated animals could be registered in all brain re-

gions studied (Table 5), reaching statistical significance in the cerebral cortex and mesolimbic structures. A decrease in the apparent affinity for ^{3}H-diprenorphine was also observed in the mesolimbic structures of the habituated rats. To decide whether these effects were also secondary to the stress-induced release of endogeneous opioids or not, more complete studies are required.

The present study provides the evidence that handling manoeuvres yield marked changes in the binding parameters of several receptors in the CNS and in peripheral organs and in the behavior of the rat.

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CHANGES IN GABA AND BENZODIAZEPINE RECEPTORS AFTER FOOT-SHOCK IN THE RAT: INFLUENCE OF DIAZEPAM

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Abstract:

The binding studies revealed that foot-shock decreases the number of ^{3}H-flunitrazepam and ^{3}H-muscimol binding sites in frontal cortex and hippocampus. A decrease was also registered in the peripheral ^{3}H-flunitrazepam binding sites in ren and adrenals. This effect was of the same range both in the naive rats and in those chronically habituated to the handling procedures preceding sacrifice. The effect of foot-shock on binding parameters was not attenuated by in vivo diazepam (2.5 mg/kg) administration.

Introduction

Stressful situations are known to produce numerous neurochemical and hormonal changes. Since benzodiazepines (BD) can counteract several of the effects produced by stress (3, 10), and the GABA-ergic system is reported to play a regulatory role in stress-induced hormone release (5,9), investigation of the possible involvement of the BD and GABA receptors in stress is of great importance.

The information concerning the changes of the BD or GABA receptors following stress is rather contradictory, probably due to the great variability in the stress situations used. Stressful manipulations are reported both to increase (17) and decrease the number of the brain BD receptors (12,14). It has been shown (1) that the GABA receptors in different brain areas may vary with the state of animals before sac-

rifice: the rats habituated to the handling procedures preceding sacrifice presented a higher number of GABA receptors than naive animals. On the basis of this evidence, naive and handling-habituated rats were proposed (2) as a useful model to study the effect of stress on GABA receptors.

In the present study, we report that foot-shock decreases the number of ^{3}H-flunitrazepam and ^{3}H-muscimol binding sites in various tissues of both naive and habituated rats - an effect not attenuated by the in vivo diazepam administration.

Materials and Methods

Male albino laboratory rats (Rappolovo farm, Leningrad) were divided into two groups, termed as naive and handling-habituated animals. The handling-habituated rats were habituated twice daily for 10-12 days to the handling manoeuvres preceding sacrifice by decapitation. The naive animals were left in their home cages for that period. Immediately before sacrifice half of the rats from both groups were treated with a 3 min. intermittent foot-shock (60 Hz, 0.5 mA, 10 s on every 15 s). In a separate study, diazepam (2.5 mg/kg) or vehicle 60 min. prior to the foot-shock was administered intraperitoneally to the naive and habituated animals.

After decapitation cerebral cortex, hippocampus, ren and adrenal were rapidly diosected and homogenized in 30 volumes of ice-cold Tris-HCl buffer (pH 7.4). For ^{3}H-flunitrazepam binding the homogenate was centrifuged twice at 48 000 g for 20 min. and resuspended finally in the Tris-HCl buffer containing 0.1-0.3 mg of protein per sample. Binding of ^{3}H-flunitrazepam (81 Ci/mmol, Amersham Radiochemicals) was carried out in the presence of 0.25-32 nM of the labelled ligand and 10 μM flunitrazepam. The incubation, performed at 4° C was terminated 60 min. later by filtration under vacuum through GF/B Whatman filters, followed by 3 washes of 5 ml each with ice-cold buffer.

For ^{3}H-muscimol binding, the tissues under study were washed 3 times in Tris-HCl buffer (48,000 g for 20 min.), stored at -20° C for at least 24 hours and washed 4 addi-

tional times immediately before the binding assay. ^{3}H-muscimol (18 Ci/mmol, Amersham Radiochemicals) binding was carried out in the presence of 2-60 nM of the labelled ligand and 10 µM muscimol in a total incubation volume of 500 µl containing 0.3-0.4 mg of protein. After 15 min. of incubation at 4° C the reaction was stopped by centrifugation at 10,000 g for 5 min. The characteristics of the binding data (B_{max} and K_D) were obtained with the help of the Scatchard analysis. The significance of the differences between groups was calculated using the Student's t-test.

Results and Discussion

An intermittent foot-shock decreased the number of binding sites of ^{3}H-flunitrazepam and ^{3}H-muscimol in the membranes of cerebral cortex and hippocampus of the naive rats (Table 1). In this experiment the binding of the two ligands studied vary in the same direction without any changes in affinity. The alterations of the BD receptors were of the opposite direction to those observed in the convulsive states produced by electroshock (15), but resembling the decrease in the number of BD receptors after forced swimming (14).

In our studies, the foot-shock was able to attenuate also the number of ^{3}H-muscimol binding sites in naive rats (see Table 1), the effect that was not found in naive rats by others (2) when ^{3}H-GABA was used to label the $GABA_A$ receptors. The discrepancies in the binding studies using ^{3}H-muscimol or ^{3}H-GABA as a ligand have been reported in various situations (8,13) and for explanation it has been suggested that ^{3}H-muscimol does not label all, but mainly the postjunctional $GABA_A$ receptors (4). On the other hand, the decreased number of the binding sites of ^{3}H-muscimol after a foot-shock in habituated rats (Table 2) is in good correlation with the results obtained with the help of ^{3}H- GABA (2).

The foot-shock-induced reduction in the density of the GABA and BD receptors in the cerebral cortex and hippocampus of habituated animals (Table 2) corresponds to the decrease in the ^{3}H-muscimol and ^{3}H-flunitrazepam binding sites that we observed in naive animals (Table 1). So, in our experiments there were no clear differences between the naive and the habituated animals in the foot-shock-induced effects

Table 1

Effect of foot-shock on the characteristics of ^{3}H-flunitrazepam (^{3}H-FNZ) and ^{3}H-muscimol (^{3}H-MUSC) binding to washed membranes of naive rats.
Each value is the mean ± SEM of 3 separate experiments.

Animal group	Ligand	B_{max} (fmol/mg prot.)	K_D (nM)
		C E R E B R A L C O R T E X	
Naive	^{3}H-FNZ	1760±100	1.45±0.18
Shock	^{3}H-FNZ	1410± 80*	1.09±0.14
Naive	^{3}H-MUSC	3230±320	27.6 ±4.2
Shock	^{3}H-MUSC	2870±210	24.2 ±4.7
		H I P P O C A M P U S	
Naive	^{3}H-FNZ	1080± 80	1.06±0.12
Shock	^{3}H-FNZ	800± 60*	0.96±0.12
Naive	^{3}H-MUSC	1630±120	21.7 ±4.0
Shock	^{3}H-MUSC	1210± 80*	18.3 ±3.2

* $P < 0.05$ as compared to naive animals

Table 2

Effect of foot-shock on the characteristics of ^{3}H-flunitrazepam (^{3}H-FNZ) and ^{3}H-muscimol (^{3}H-MUSC) binding to washed membranes of handling-habituated rats.
Each value is the mean ± SEM of 3 separate experiments.

Animal group	Ligand	B_{max} (fmol/mg protein)	K_D (nM)
1	2	3	4
		C E R E B R A L C O R T E X	
Habituated	^{3}H-FNZ	1380± 80	1.17±0.16
Shock		1060± 60*	1.02±0.09
Habituated	^{3}H-MUSC	2690±160	28.5 ±3.7
Shock		2020±180*	21.6 ±4.3

Table 2 continued

1	2	3	4
		HIPPOCAMPUS	
Habituated	^{3}H-FNZ	960± 70	1.16±0.10
Shock		920± 50	1.28±0.08
Habituated	^{3}H-MUSC	1460±130	20.7 ±2.9
Shock		1020± 70	18.6 ±2.3

* $P < 0.05$ as compared to habituated animals

on central BD and GABA receptors. Thus, it can be suggested that handling procedure has no effect on the direction and size of the BD and GABA receptor alterations after foot - shock. However, it is important to note that the handling procedure was able to decrease the basal density of both ^{3}H-flunitrazepam and ^{3}H-muscimol binding sites without significant changes in the affinity.

Recently we observed (16) similar modulation of central and peripheral BD receptors by different GABA agonists and these findings led us to the investigation of the possible changes in the peripheral BD receptor binding characteristics after foot-shock. We found that the number of ^{3}H-flunitrazepam binding sites was decreased after the foot-shock in the washed membranes of ren (Fig. 1) and adrenal (Fig. 2) in both naive and habituated rats. The changes of the peripheral BD receptors were parallel to those observed in the brain. The attenuating effect of the handling procedure on the number of peripheral binding sites of ^{3}H-flunitrazepam was registered also in the peripheral tissues studied.

These observations suggest that the peripheral BD binding sites are modulated similarly to the central BD receptors: the density of the peripheral BD binding sites is decreased, following a stressful situation. Recently, it has been reported that (6) the exposure of rats to an inescapable tailshock produced a reduction in the density of the peripheral BD binding sites in heart and kidney but not in the other tissues surveyed (e.g., CNS and adrenal gland) . Such discrepancies in the stress-induced changes can be ex-

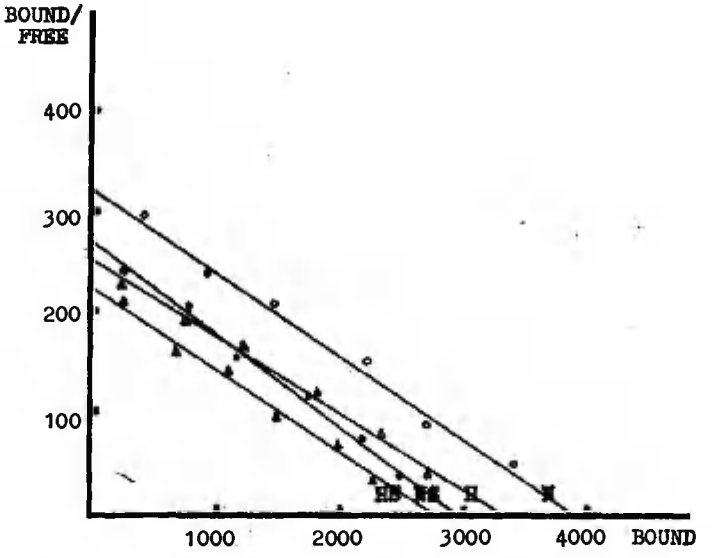

Fig.1. Scatchard analysis of a representative experiment of ^{3}H-flunitrazepam binding to washed membranes of rat ren after foot-shock.

	B_{max} (fmol/mg protein)	K_D (nM)
N - naive	3860 ± 280	12.2 ± 1.3
NS - naive + shock	2900 ± 220 *	10.8 ± 1.0
H - habituated	3310 ± 140	13.6 ± 1.6
HS - habituated + shock	2730 ± 160 **	11.8 ± 1.4

* $P < 0.05$ as compared to naive animals
** $P < 0.05$ as compared to habituated animals

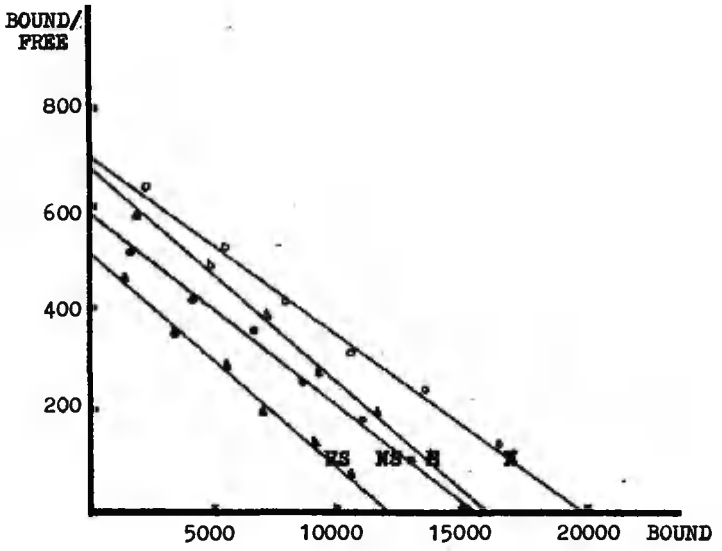

Fig.2. Scatchard analysis of a representative experiment of ^{3}H-flunitrazepam binding to washed membranes of rat adrenal after foot-shock.

	B_{max} (fmol/mg protein)	K_D (nM)
N - naive	19,600 ± 1,200	29,3 ± 4.5
NS - naive + shock	15,100 ± 1,100*	25.6 ± 4.3
H - habituated	15,500 ± 900 *	23.7 ± 2.8
HS - habituated + shock	12,200 ± 800 **	24.8 ± 4.5

* $P < 0.05$ as compared to naive animals

** $P < 0.05$ as compared to habituated animals

plained with different ligands and stress models used and the differences in the tissue preparation for binding studies. Nevertheless, a systematic investigation of the hormonal and neural regulation of peripheral organs may shed some light on the etiology of the stress-induced reduction in the density of peripheral BD binding sites in various tissues.

The next series of experiments was designed to study the effect of diazepam on the foot-shock-induced changes of BD and GABA receptors.

Table 3

Influence of acute treatment with diazepam (2.5 mg/kg) and foot-shock on the binding of ^{3}H-flunitrazepam to the washed membranes of rat cerebral cortex.
Each value is the mean ± SEM of 3 separate experiments.

Animal group	Treatment	B_{max} (fmol/mg protein)	K_D (nM)
	NAIVE ANIMALS		
Control	vehicle	1560 ± 120	1.15 ± 0.12
Control	diazepam	1410 ± 120	1.23 ± 0.09
Shock	vehicle	1120 ± 90*	1.06 ± 0.15
Shock	diazepam	1180 ± 70*	1.11 ± 0.12
	HABITUATED ANIMALS		
Control	vehicle	1360 ± 100	1.20 ± 0.15
Control	diazepam	1180 ± 80	1.06 ± 0.18
Shock	vehicle	1020 ± 60*	1.23 ± 0.10
Shock	diazepam	950 ± 100*	1.11 ± 0.12

* $P < 0.05$ as compared to control + vehicle group

Table 3 shows an example from these studies. Diazepam has been shown to attenuate the biological consequences of exposure to a stressor (2,7,10,14). Surprisingly, in our studies diazepam (2.5 mg/kg) was unable to modulate the stress-induced changes in either ^{3}H-flunitrazepam or ^{3}H-muscimol binding characteristics in all tissues studied and in both

naive and habituated animals. On the other hand, using other models it has been shown that diazepam potentiated some effects of a stressor (11) or prevented the stress-induced biochemical changes only in relatively high doses, e.g. 10 mg/kg (7). Therefore, a too small dose of diazepam used could be the most probable explanation for the lack of the effect of diazepam in our studies.

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THE FUNCTIONAL ROLE OF DOPAMINE D_1 RECEPTORS IN THE CNS

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Abstract:

In experiments with rat brain membranes the binding of selective D1 receptor agonist SKF 38393 has been studied. It has been found that [^{3}H] SKF 38393 binding is largely dependent on the buffer used and on incubation conditions. The maximal ratio specific to nonspecific binding was achieved by using phosphate buffer and incubation at 0° C. Since glass-fibre filters were found to adsorb radioligand in a great extent, the bound radioligand was separated by microcentrifugation. Under these conditions, ^{3}H SKF 38393 binds in striatum to the single receptor population with B_{max} = 384±43 fmol/mg protein and K_D = 5.8±1.0 nM. The regional distribution of D1 sites well corresponded to the distribution of D2 sites labeled by ^{3}H spiperone in the rat forebrain.

Several neuroleptic drugs and dopamine receptor agonist varied in their binding affinities for both D1 and D2 receptors. Comparing the ability of neuroleptics to induce catalepsy and their affinity for D1 sites, it is proposed that the blockade of D1 receptors might among other factors account for the cataleptic properties of these drugs.

Introduction

Up to the present, at least two different types of dopamine receptors have been described (9), which can be identified on the basis of their linkage to adenylate cyclase. Thus, the dopamine D_1 receptor is associated with the dopamine sensitive adenylate cyclase, whereas the D_2 receptor is independent of it or is even involved into the inhibitory control of adenylate cyclase (17).

There is enough evidence to suggest that the majority of the behavioral and biochemical effects of dopamine agonists and antagonists are mediated via the D_2 receptors, whereas the role of the D_1 receptors remains unknown.

The recent introduction of a novel selective D_1 receptor agonist SKF 38393 and antagonist SCH-23390 has provided an invaluable tool for studying the functional role of these receptors (6,13,14).

The behavioral and neurochemical effects of SKF 38393 in many aspects differed from the other dopamine receptor agonists. Thus, SKF 38393 did not induce stereotyty and did not influence dopamine release (11,16). However, similarly to the D_2 receptor agonists, SKF 38393 induced contralateral rotations in the 6-hydroxydopamine lesioned rats (1).Recently, the tritium labeled SKF 38393 with high specific activity was introduced that opened a possibility to study directly the interaction of various dopamine receptor agonists with the D_1 receptor thus enabling to elucidate the precise role of these receptors in the dopamine-mediated behavior. In the present study, an attempt has been made to study the potentials of several drugs of the binding of the D_1 and D_2 receptors labeled by /^{3}H/ SKF 38393 and /^{3}H/ spiperone and to compare with their behavioral effects in rats

Material and Methods

Animals: In our experiments male rats and male mice weighing 170-250 g and 20-25 g, respectively, were used.

Drugs: The following drugs were dissolved in saline: SKF 38393 (2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-7,8 diol, hydrochloride) obtained as a gift from the Smith, Kline and French Laboratories (Philadelphia, USA); Ly 171 555

(trans-(-)- 4 aR-4, 4a,5,6,7,8,8a,9-octahydro-5-propyl-; 1H (oR 2H)-pyrozo-(3,4-g)quinoline, monohydrochloride) as a gift from the Lilly Research Laboratories (Indianopolie,USA); Chlorpromazine (USSR); Carbidine (USSR); Asaailane (Institute of Organic Chemistry, Latvian Academy of Sci., Riga); Raclopride (S)-(-)-3,5-dichloro-N-(1-ethyl-2-pyrrolidinyl) methyl-6-methoxysalicylamide) as a gift from Astra AB (Sweden); Remoxipride (S-(-)-3-bromo-2,6-dimethoxy-N-/(1-ethyl-2-pyrrolidinyl)methyl/-benzamide) as a gift from Astra AB (Sweden); SCH 23390 I(R)-(+)-8-chloro-2,3,4,5-tetrahydro- 3 methyl-5-phenyl-1H-3-benzasepine as a gift from Schering Corporation (Kenilworth, USA); Clozapine (Sandoz, Basle, Switzerland); Azaperone (Janssen Farmaceutica, Beerse,Belgium); (+) and (-) Sulpiride (Ravizza, Italy); Butaclamol (Abott Labs., USA); Haloperidol (Janssen Farmaceutica,Beerse, Belgium) were dissolved in hydrochloric acid before dilution with water. Apomorphine HCl (USSR) was dissolved in saline containing 0.05 % ascorbic acid.

Receptor binding; "In vitro" ^{3}H-spiperone binding was performed according to the method of Muller and Seeman (12). The rats were sacrificed by the cervical dislocation. The brains were rapidly removed, the striata were dissected out on the ice-cold plate and weighed. Fresh tissues were homogenized with a glass-teflon homogenizer (1000 RPM, 9 passages) in 10 vol of 50 mM TRIS HCl buffer (pH = 7.4), the homogenate was centrifuged twice at 40,000 g for 20 min with intermediate resuspension in a fresh buffer and finally homogenized in 750 vol of the TRIS HCl buffer containing 120 mM NCl, 5 mM KCl, 1 mM Mg Cl_2 and 2 mM $CaCl_2$. Test tubes received 0.8 ml of membrane suspension (0.1-0.15 mg of protein), 100 ul of buffer or 1 uM of haloperidol and 100 ul of ^{3}H-spiperone (77 Ci/mmol "Amersham", England), final conc. 0.25 nM. The samples were incubated at 37° C for 30 min and rapidly filtered through GF/B (Whatman) filters under vacuum. Following three 5 ml washes with incubation buffer filters were placed in scintillation vials, 10 ml of scintillation cocktail were added. The samples were counted with a Beckman LS-6800 counter with the 45-47 % efficiency. Specific binding of ^{3}/H/-spiperone was defined as that displaceable by 1 uM haloperidol.

"In vitro" ^{3}H-SKF 38393 binding was performed accord-

ing to the method of Gredal and Nielsen (4) in our modification. The fresh striatal tissue was homogenized in 10 vol of ice-cold 50 mM of potassium phosphate buffer (pH = 7.4) using a glass-teflon homogenizer (1000 RPM, 9 passages). The homogenate was centrifuged twice at 30000 g for 10 min with an intermediate rehomogenization in fresh cold buffer between centrifugations. The final pellet was resuspended in potassium phosphate buffer in a 500 V containing 40 mM of NaCl. Test tubes received 0.8 ml of membrane suspension (0.1 mg of protein), 100 ml of buffer or 10 uM SCH 23390 and 100 ml of /^{3}H/SKF 38393) 50 Ci/mmol "Amersham", England). The samples were incubated at 0° C for 60 min and were centrifuged in a Microfuge 12 (Beckman) 12,000 RPM for 4 min. The buffer solution was aspirated and the tube was washed twice with the same buffer. The samples were counted with a Beckman LS-6800. The specific binding of ^{3}H-SKF 38393 was defined as that displaceable by 10 uM SCH 23390. Proteins were determined by the method of Lowry (10).

Behavioral Studies

Catalepsy was assessed in rats according to the method of Honma, Fukushima (5). Briefly, at various times after drug treatment the animals were placed by their forepaws on the horizontal bar positioned 8 cm above the floor and the time in sec (max 300 sec) during which animals remained in such a position was recorded.

For the stereotyped behavior measurement the rats were placed into individual square Plexiglas boxes (15x15x15 cm). The intensity of stereotyped behavior was assessed according to the system described by Costall and Naylor (3). For the assessment of the yawnings, the rats were placed into individual boxes. The number of episodes of yawnings was recorded during a 60 min test using a hand-held counter.

Statistics

The results were expressed as mean ± SEM. IC_{50} were calculated from log-dose response curves. Statistical comparisons of behavioral data were calculated using the Dunnet t-test. Statistical comparisons of binding data were calculated using Student's t-test.

Results

Binding characteristics of /^{3}H/-SKF 38393. The binding of /^{3}H/ SKF 38393 showed a great dependency between the composition of incubation buffers and temperature. The highest specific binding was obtained if the incubation was performed at 37°C in the TRIS HCl buffer containing 120 mM of NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$ or in the potassium phosphate buffer containing 40 mM of HaCl at 0°C (see Table 1). However, the specific binding of /^{3}H/ SKF 38393 in the TRIS HCl buffer was not saturable and increased with increasing of the concentrations of ligand (data not shown). Furthermore, it has been found that a considerable amount of radioactivity was retained on the tubes and GF/B filters if the binding was performed in the TRIS HCl buffer (Table 2). When the binding was performed in the potassium phosphate buffer at 0°6 in microfuge tubes, a minimal amount of radioactivity was recovered from the surface of the tubes. Therefore, these conditions were used in the further experiments. The binding of /^{3}H/ SKF 38393 was saturable in concentrations ranging from 0.25 to 16 nM (Fig. 1) and reached the equilibrium within 30-60 min at 0°C. As revealed by the Scatchard analysis, in the striatal tissue /^{3}H/ SKF 38393 binds with a single population of sites with K_D = 5.8 ± 1.0 nM and maximal capacity (B_{max}) of 384 ± 43 fmol/mg of protein, while the bound ligand was separated by centrifugation and K_D = 5.7 ± 1.0 nM and B_{max} = 591 ± 236 fmol /mg of protein if the binding was stopped by the filtration through the GF/B filters (Fig. 2).

Regional distribution of /^{3}H/ SKF 38393 and /^{3}H/ spiperone binding. As revealed by the binding studies, the regional distribution of the D1 sites closely correlated (r = 0.9) $P < 0.05$ to the distribution of the D2 sites (Table 3).

The effect of various DA receptor agonists and antagonists on the /^{3}H/ SKF 38393 and /^{3}H/ spiperone binding and on the behavior of rats. There were considerable differences between the DA receptor agonists in the binding affinities for the D1 receptors labelled by the /^{3}H/ SKF 38393 and D2 receptors labelled by /^{3}H/ spiperone (Table 4). The order of potencies for /^{3}H/ SKF 38393 binding were SKF 38393 > apomorphine ~ dopamine > Ly 171555. In contrast to

Table 1

Binding of /^{3}H/ SKF 38393 (2 nM) to striatal membranes in TRIS-HCl and potassium phosphate buffers. Nonspecific binding was determined in the presence of 10^{-5} M of SCH 23390

Assay conditions	/^{3}H/ SKF 38393 bound, CPM			
	Total	Nonspecific	Specific	% of total
Phosphate buffer				
(filters) 37°C	3631	3618	13	0.4
TRIS-HCl buffer				
(filters) 37°C	10878	3568	7310	67
(tubes) 37°C	7183	1752	5431	76
Phosphate buffer				
(filters) 0°C	1981	987	994	50
(tubes) 0°C	1934	916	1018	53
TRIS-HCl buffer				
(tubes) 0°C	3335	3037	298	9

that, the order of potencies for /^{3}H/ spiperone binding was as follows: apomorphine > dopamine > Ly 171555 > SKF38393. However, the ratio D1/D2 was the highest for Ly 171555 and the lowest for SKF 38393 suggesting high specificity of Ly 171555 and SKF 38393 for D2 and D1 receptors, respectively (Table 4). The affinities of Ly 171555 and apomorphine for /^{3}H/ SKF 38393 or /^{3}H/ spiperone binding did not correspond to their ability to induce yawning behavior (Table 3). The effect of neuroleptics on the /^{3}H/ SKF 38393 and /^{3}H/ spiperone binding is presented in Table 5. The most potent inhi - bitory activity for /^{3}H/ SKF 38393 binding possessed SCH 23390 > (+) butaclamol > haloperidol, the least potent -sulpiride, raclopride, carbidine. (+) Butaclamol and haloperidol were also the potent inhibitors of /^{3}H/ spiperone binding, whereas SCH 23390 was not. It should, however, be pointed out that the potency of a neuroleptic to compete for the D1 sites corresponded well to their ability to induce cata-

Table 2

Binding of /^{3}H/ SKF 38393 (4 nM) to filters and microfuge tubes in various buffers and conditions of incubation

Assay conditions	/^{3}H/SKF 38393 bound, (CPM/filter, CPM/tube)	
	Total	+ 10 uM SCH 23390
Filter (CF/B Whatman)	2898	2057
TRIS-HCl buffer 37°C	2898	2057
Phosphate buffer 37°C	556	484
Phosphate buffer 0°C	407	316
Microfuge tube		
TRIS-HCl buffer 37°C	470	221
TRIS-HCl buffer 0°C	1444	1377
Phosphate buffer 0°C	126	89

lepsy (Table). An exception was clozapine which potently inhibited /^{3}H/ SKF 38393 binding but lacked any cataleptic action.

Table 3

Binding of /^{3}H/ SKF 38393 (4 nM) and /^{3}H/ spiperone (0.25 nM) in various structures of the rat brain. Nonspecific binding was determined in the presence of 10^{-5} M SCH 23390 and 10^{-6} baloperidol respectively. Values are means ± SEM of the data from two separate experiments.

Structure	fmol/g tissue	
	/^{3}H/ SKF 38393 D-1	/^{3}H/ spiperone D-2
Striatum	10.8±0.3	14.7±0.5
N. acumbens	9.7±3.0	8.4±5.5
Septum+tuberculum olfactorium	3.4±1.3	7.3±2.5
Hippocampus	1.8±0.5	1.5±0.15
Cortex	1.6±0.1	4.7±0.1
Cerebellum	0.4±0.4	1.1±0.3

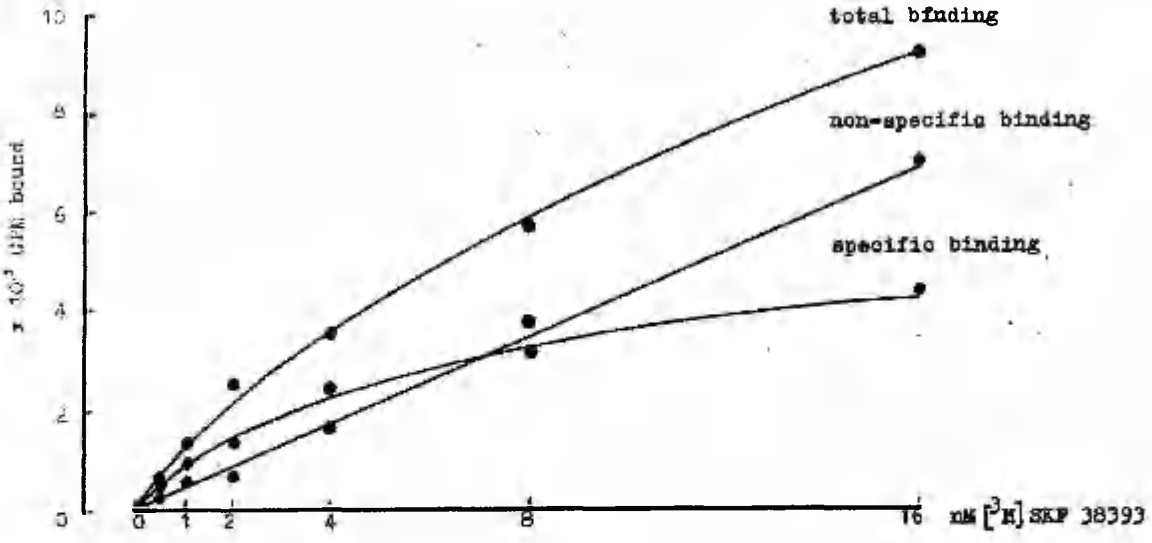

Fig. 1. Saturation of /^{3}H/ SKF 38393 binding in rat striatum as a function of increasing concentration of /^{3}H/ SKF 38393 (0.25-32 nM), nonspecific binding was defined as the binding in the presence of 10 uM SOH 23390-

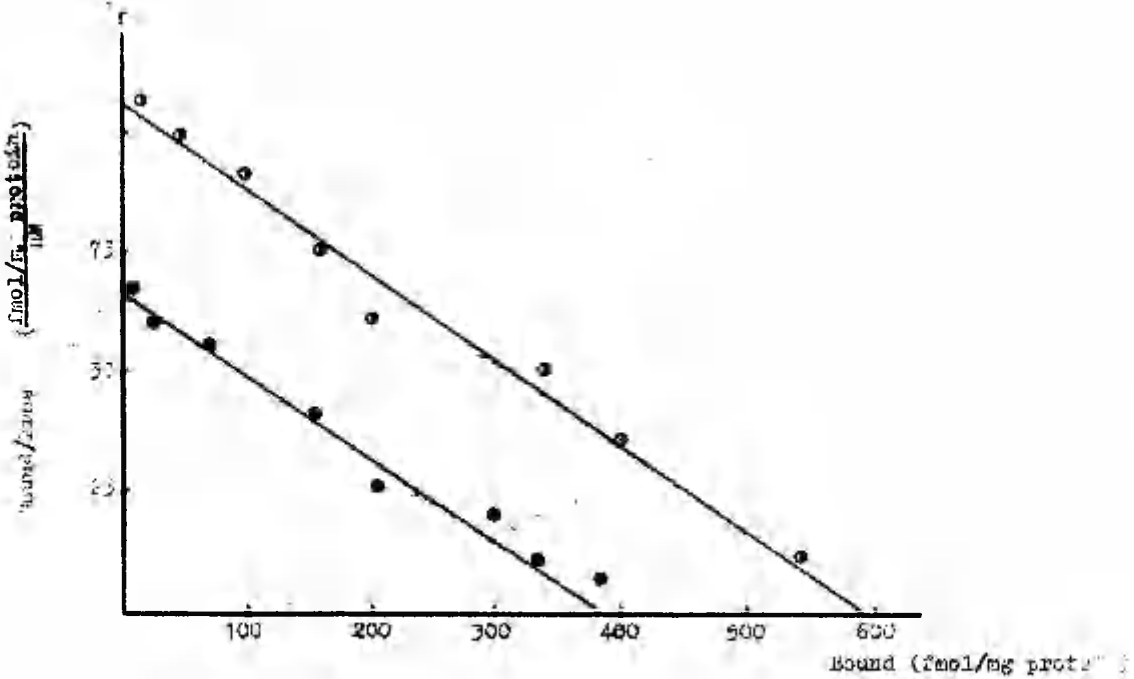

Fig. 2. Scatchard analysis of /^{3}H/ SKF 38393 binding (0.25-32 nM) in potassium phosphate buffer. ◑ - filtration, ● - centrifugation.

Table 4

Inhibition of /^{3}H/ SKF 39393 (2 nM) and /^{3}H/ spiperone (0.25 nM) binding to striatal membranes by dopamine agonists and ED_{50} for induction of yawning in rats. Values are means ± SEM of the data from 2-4 separate experiments.

Compound	IC_{50} (nM)			
	Dopamine D_1 /^{3}H/ SKF 38393	Dopamine D_2 /^{3}H/ spiperone	Ratio D_1/D_2	Yawning ED_{50} mg/kg
Apomorphine	111±37	30±30	1.2	0.015
Ly 171555	100,000	3,600±750	28	0.01
SKF 38393	15±7.6	31,000±6,500	0.0005	-
Dopamine	4,600±1,200	2,500±900	1.8	-

Table 5

Inhibition of /^{3}H/ SKF 38393 (2 nM) and /^{3}H/ spiperone (0.25 nM) binding to striatal membranes by dopamine antagonists and ED_{50} for induction of catalepsy in rats.
Values are mean ± SEM of the data from 2-4 separate experiments.

Compound	IC_{50} (nM)			Catalepsy
	Dopamine D_1 /^{3}H/SKF 38393	Dopamine D_2 /^{3}H/spiperone	Ratio D_1/D_2	ED_{50} mg/kg
Haloperidol	89±20	5.5±2.5	16	0.18
(+)-Butaclamol	8.5±20	3.7±1.6	2.3	
Chlorpromazine	162±43	40.5±11	4	2.3
(-)-Sulpiride	22,000±1,000	264±105	83	75
(+)-Sulpiride	28,000±17,000	15,000±1,000	1.9	-
Raclopride	100,000	379	264	13
Remoxipride	100,000	19,000	5.3	9
Azaperone	1,000±730	413±170	2.4	-
Azasilane	1,100±410	2,300±600	0.5	-
Clozapine	396±204	751± 11	0.5	65
Carbidine	9,000±2,700	1,500±320	6	20
SCH 23390	2.3±0.7	2.500±1100	0.0009	0.02

Discussion

The results of the present study in confirmation with others (4) indicate that /^{3}H/ SKF 38393 labels the sites which may be designated as the D-1 receptors. Although the regional distribution of the D-1 receptors labeled by /^{3}H / SKF 38393 very closely correlates with that of the D-2 receptors labeled by /^{3}H/ spiperone, the functional role of these sites remains unknown. Our data, however, indicate that these neuroleptics which are potent in competing for D-1 receptors are also potent in inducing catalepsy. Thus, this suggests that the interaction of neuroleptics with D-1 sites may contribute to their potency in inducing catalepsy. This proposition, nevertheless, contradicts the data that was obtained in binding studies with /^{3}H/ SKF 23390, where the most potent competitors were thioxanthenes and phenothiazines but not butyrophenones (7). However, it should be emphasized that /^{3}H/ SKF 38393 and /^{3}H/ SCH 23390 may label distinct states of the D-1 receptor. Therefore, it should not be excluded that the blockade of the D-1 high affinity state of D-1 receptors by neuroleptics may account for the development of catalepsy. On the other hand, our previous study (18) as well as many others (2,8,15) have shown that the antistereotypic potencies of neuroleptics are highly correlated with their potency to displace /^{3}H/ spiperone from the D-2 sites suggesting that the antagonism of stereotypy is mediated via the D-2 receptors. The properties of DA receptor agonists to induce yawnings do not, however, correlate with their agonistic potency at the D_1 or D_2 receptors. Recent studies have shown that the D-2 receptor can exist in two states of high and low affinity for the D-2 receptor agonists. Analysing the dose response data concerning yawning induction and stereotypy, it can be supposed that the agonistic D-2 affinity state might be involved in the mediation of yawning, whereas the agonistic low affinity state seems to mediate a stereotypic response. However, the hypothesis remains highly speculative, since at present time no direct evidence suggesting the existence of different affinity states of the DA receptors under in vivo conditions is available.

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DECREASE IN in vivo /^{3}H/SPIPERONE BINDING IN MOUSE BRAIN AFTER CHRONIC HALOPERIDOL TREATMENT

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Key words: in vivo /^{3}H/spiperone binding - chronic haloperidol

Abstract:

Administration of haloperidol (2.5 mg/kg, i.p.) for 21 days to mice resulted in disappearance of inhibition of locomotions and in increased cage climbing behaviour induced by DA receptor agonist apomorphine 0.1 and 1 mg/kg, respectively, during 2-7 days after withdrawal, suggesting the development of DA postsynoptic receptor hypersensitivity. Despite the sign of behavioural hypersensitivity in these animals, a decrease in in vivo /^{3}H/spiperone binding was observed during 2-7 after withdrawal. Reduction of /^{3}H/spiperone binding was seen in both structures studied: striatum and cortex. Similar results were also obtained after acute haloperidol treatment. On the basis of these findings, it is proposed that observed decrease in /^{3}H/spiperone binding underin vivo conditions might be caused by partial displacement of radioligand by residual haloperidol still present in the cerebral tissues.

Introduction

Repeated administration of antipsychotic drugs to laboratory animals and their subsequent withdrawal induce the

cerebral dopamine DA receptor hypersensitivity (2,4,11, 18, 19). After withdrawal from chronic treatment with typical neuroleptics, the animals displayed exaggerated stereotypic response to apomorphine and an increase in the striatal DA receptor sites (D2 sites not linked to the dopamine sensitive adenylate cyclase) identified by in vitro binding techniques using tritiated DA receptor agonists and antagonists (2,11,14,16,19). It has been proposed that such proliferaof DA receptors that was detected in in vitro binding studies is favoured by the development of behavioural supersensitivity to the DA receptor agonists in laboratory animals or dyskinetic disturbance in the patients chronically treated with neuroleptics (6,14). Although the studies using in vitro binding technique invariably show a significant increase of the D2 receptor binding sites in the striatum of the animals chronically treated with neuroleptics, the in vivo binding studies using /^{3}H/spiperone as a ligand give less consistent results.

Thus, Bishoff (1) found an increase in in vivo /^{3}H/spiperone binding in the striatal and hippocampal tissues of brain after 21 days of haloperidol treatment and 4 days of washout period. Similar results were obtained by Ferrero et al. (3). In contrast, Saelens et al. (13) reported that the chronic administration of haloperidol resulted in a decrease in in vivo /^{3}H/spiperone binding in the subcortical but not in the cortical structures of the rat brain. The authors interpreted these data in the terms of compensatory reduction of the antagonist binding sites and of a complementary increase of the agonist binding sites after chronic haloperidol treatment.

In the light of these contradictory results, it was interesting to re-examine the state of the DA receptor sensitivity in the mice chronically treated with haloperidol using both behavioural experiments and in vivo /^{3}H/spiperone binding techniques.

Methods

Male mice weighing 18-22 g were given i.p. single or repeated injections of haloperidol 2.5 mg/kg (commercial ampoules, Gedeon Richter, Hungary) for 21 consequtive days.

The corresponding control animals received saline injections. At different time after chronic haloperidol or saline treatment the animals were randomly divided into groups of 8-12 mice and taken for behavioural experiments. The animals were injected with 0.1 mg/kg of apomorphine HCl or saline and immediately placed into the individual fotocell chambers for the determination of locomotor activity. The locomotor activity was counted during 30 min after apomorphine injection . Another group of mice received 1.0 mg/kg of apomorphine HCl and the cage climbing behaviour was determined. The climbing behaviour was assessed in the cylindrical cages (12 cm dia., 15 cm high) with walls surmounted by fine wire mesh. The intensity of climbing behaviour was scored during 1 min at 10 min intervals of 30 min observation period according to the method of Wilcox et al. (21). 6-10 animals from chronic haloperidol or saline groups and the animals from acute halo - peridol or saline groups were used for the determination of in vivo /^{3}H/spiperone binding. The in vivo binding of /^{3}H/ spiperone (Amersham plc., England) was determined according to the method of Höllt at al (5). The animals were given into tail veins 5 ug/kg of /^{3}H/spiperone (spec. act. 16-19 Ci/ mmol) and 1.5 hours sacrificed by the cervical dislocation . The brains were rapidly removed, striatum and frontal and parietal cortex were dissected on the ice-cold plate and after that weighed. The radioactivity was counted in a LS-6800 liquid scintillation counter (Beckman, USA), the following combustion of the samples in a BTS tissue solubilizer (Beckman) at 50^{o}C for 2 hours. For the determination of non-specific binding the mice in the parallel experiments were given i.p. haloperidol (10 mg/kg) 30 min before the i.v. injection of radioligand.

The statistical analysis of non-parametric data has been performed by the Mann-Whitney U-test. All other behavioural and binding data were evaluated by the Student's t- test.

Results

The chronic treatment of mice with haloperidol resulted in considerable modification of the behavioural effects of the DA receptor agonist apomorphine.

Although in control animals low doses of apomorphine

(0.1 mg/kg) caused a significant reduction of looomozions, in chronic haloperidol treated animals apomorphine did not induce any inhibition of the locomotor activity within 2-6 days after withdrawal (Fig. 1). Only on the 10th day after withdrawal the inhibitory effect of apomorphine on the locomotion reappeared (Fig. 1). After chronic haloperidol treatment the mice exhibited more intense cage climbing induced by apomorphine (1.0 mg/kg) then the control animals (Fig. 2). The increased climbing behaviour was observed during 2-7 days after the withdrawal of chronic haloperidol treatment suggesting the development of the postsynaptic DA receptor hypersensitivity. Despite the behavioural signs of the DA receptor hypersensitivity in these animals, quite a marked inhibition in in vivo /^{3}H/spiperone binding in the mouse brain was found (Table 1). This effect was observed during 2-7 days after withdrawal from chronic haloperidol. On the 10th day after the withdrawal the /^{3}H/ spiperone binding tended to increase in the striatal tissues in comparison with the control animals; however, the data did not reach the level of significance (Table 1). Acute injection of haloperidol, similar to chronic treatment, also induced a decrease in in vivo /^{3}H/spiperone binding in the striatum and frontal cortex of the mouse brain (Table 1). The reduction of binding in these animals was seen, however, during a shorter period, 2-5 days after haloperidol pretreatment.

Discussion

The data of the present study, in confirmation to those reported by Saelens et al. (13), showed a reduction in the in vivo /^{3}H/spiperone binding in the mouse brain after chronic and acute haloperidol treatment. In addition, we detected a decrease in binding not only in striatum but also in frontal cortex where /^{3}H/spiperone labels preferentially serotonin S2 receptors (7,9). In our study, we were also able to detect the behavioural supersensitivity as revealed by the increased cage climbing behaviour induced by the receptor agonist apomorphine in the mice chronically treated with haloperidol. These data contradict numerous reports where the proliferation of the D2 receptors deter-

mined in vitro and the corresponding increased sensitivity to the behavioural effects of the DA receptor agonists after chronic neuroleptic treatment have been described (2,16, 19).

Keeping in view that the properties of the DA receptors are identical in respect to the antagonist affinities under in vivo and in vitro conditions (7,8), it can be concluded that the observed changes in in vivo /^{3}H/spiperone binding are due to the partial displacement of the radioligand by residual haloperidol still present in the cerebral tissues. This residual haloperidol masks the increase in /^{3}H/spiperone binding that would be expected to occur on the basis of many behavioural and binding /^{3}H/spiperone

The lack of any structural differences in the reduction of /^{3}H/spiperone binding and the decreased binding even after acute haloperidol treatment observed in our experiments indirectly support this position. The recent study of Wan et al. (20) showed that even under in vitro conditions the residual neuroleptic might induce a decrease in the affinity of the binding sites in the /^{3}H/ spiperone binding assay. Recently Öhman et al. (12) reported that after chronic and acute treatment of rats with haloperidol, the drug was still present during 2-6 days after withdrawal, in the cerebral tissues in concentrations of about 10 ng/g per tissue. This concentration of haloperidol seems to be sufficient to interfere in the /^{3}H/spiperone binding assay under in vivo conditions. These data, however, should be applied with caution when explaining our data, since considerable differences may exist in the rate of haloperidol elimination between the rat and the mouse cerebral tissues. Since /^{3}H/spiperone under in vivo conditions does not distinguish between the populations of the pre- and postsynaptic DA receptors in the striatum, it is not clear which of them might be preferentially occupied by the residual neuroleptic. Relying on the well-documented data that the inhibition of locomotor activity induced by low doses of apomorphine is related to the presynaptic DA receptors, whereas in low (presynaptic) doses haloperidol selectively antagonizes this effect of apomorphine (10,15), it may be proposed that the inability of apomorphine to induce sedation in mice after chronic haloperidol treatment, observed in

Table 1

Effect of acute or repeated (21 days) treatment with haloperidol (2.5 mg/kg/day, i.p.) on in vivo /^{3}H/spiperone binding in mouse striatum and cortex. Results are means ± SEM expressed in pmol per g tissue. N - number of animals; * - $P < 0.05$; ** - $P < 0.01$ (Student's t-test)

Treatment	N	Specific /^{3}H/spiperone binding (pmol · g^{-1})	
		Striatum	Cortex
		2nd day after withdrawal	
Control: saline	8	7.6 ± 1.0	5.2 ± 0.5
Haloperidol acute	8	2.4 ± 0.5**	2.0 ± 0.4**
chronic	10	3.0 ± 0.4**	2.4 ± 0.09*
		5th day after withdrawal	
Control: saline	8	8.7 ± 1.2	5.0 ± 0.8
Haloperidol acute	8	5.4 ± 0.6*	2.1 ± 0.7*
chronic	10	4.7 ± 0.7*	2.6 ± 0.7*
		7th day after withdrawal	
Control: saline	8	8.4 ± 1.2	4.4 ± 0.7
Haloperidol acute	6	7.4 ± 0.5	3.9 ± 0.6
chronic	8	5.1 ± 0.7*	3.2 ± 0.5
		10th day after withdrawal	
Control: saline	6	7.9 ± 0.6	4.2 ± 0.6
Haloperidol acute	10	7.8 ± 0.6	3.8 ± 0.6
chronic	6	9.1 ± 1.0	4.3 ± 0.3

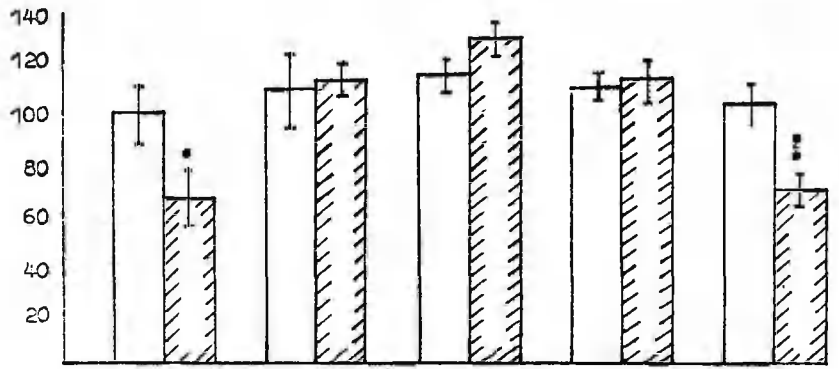

Fig. 1. The effect of apomorphine (0.1 mg/kg, s.c.) on the spontaneous locomotor activity rats withdrawn from chronic haloperidol treatment. Open columns - saline treated groups, dashed columns - apomorphine treated group. Means ± SEM of 10-12 animals per group. * - $P < 0.05$, ** - $P < 0.01$ as compared with saline injected mice.

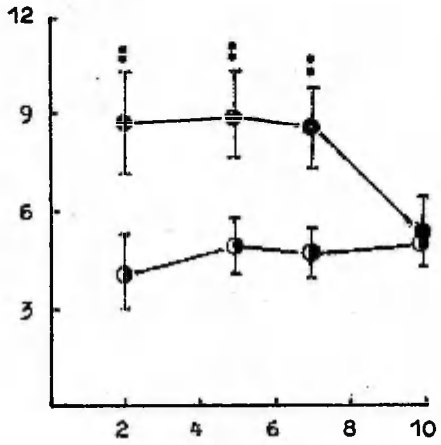

Fig. 2. The cage climbing behaviour induced by apomorphine (1.0 mg/kg) in mice after withrawal from chronic saline (- -) or haloperidol (- -) treatment. Means ± SEM of 8-10 animals per group. ** - $P < 0.01$ (Mann Whitney U-test).

the present study, is due to the interaction of residual haloperidol with apomorphine on the presynaptic receptors. However, it is not clear whether the exclusive occupation of the presynaptic DA receptors may indeed be reflected by the decrease in in vivo /^{3}H/spiperone binding, since the studies on the rats with lesions of the ascending nigrostriatal DA-ergic pathway by 6-hydroxydopamine, which eliminates the striatal presynaptic DA receptors, did not reveal any changes in the striatal in vivo /^{3}H/spiperone binding (17). It is not excluded that residual haloperidol occupies the DA postsynaptic receptors as well as the presynaptic receptors, or any other receptors (e.g., serotonin(S-2) receptors), inactivateds them and thereby prevents the sedative action of low dose of apomorphine.

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THE INFLUENCE OF MIF ON THE BEHAVIOURAL EFFECTS OF DOPAMINE RECEPTOR AGONISTS AND ANTAGONISTS

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Abstract:

In experiments on rats and mice the action of neuropeptide Pro-Leu-Gly (MIF) on the behavioural effects of dopamine (DA) receptor agonists and antagonists has been studied. It was found that MIF in dose 1 mg/kg (i.p.) enhanced the yawning behaviour that was induced by D2 receptor agonist Ly 171 555 (0.01 mg/kg, s.c.) in rats. The inhibitory effect of Ly 171 555 on the locomotions of mice was also enhanced by MIF.

In addition, MIF 0.1 mg/kg diminished the inhibitory effect of the D2 receptor antagonist sulpiride on the locomotions of mice. In contract, MIF in doses 0.1-10 mg/kg did not affect the behavioural effects of selective D1 receptor agonist and antagonist or mixed D1/D2 receptor agonists and antagonists. MIF in the doses used did not change either the level of striatal or mesolimbic DA and its metabolites HVA and DOPAC.

It is proposed that MIF excerts its modulatory effect on the DA-ergic neurotransmission allosterically interacting with the high affinity D2 binding sites which probably represent presynaptic (auto -) receptors.

Introduction

Considerable evidence indicates that the hypothalamic factor melantropin releases the inhibiting hormone (Pro-Leu-Gly-NH_2; MIF), in addition to its endocrine activity it possesses direct effects on the central nervous system independently of their interaction with the hypothalamic-pituitary axis (2,3,4). Among various central actions of MIF the best described is its effect on the dopaminergic neurotransmission. The neurochemical analysis of the mechanism of the MIF action showed that peptide exerts modulatory effect on the dopaminergic neurotransmitter system (2,3,4,5,10,14,19).

Thus, MIF selectively enhanced the binding affinity of the dopamine agonist ^{3}H-apomorphine to the dopamine receptors, augmented the turnover of dopamine, and inhibited the dopamine-sensitive adenylate cyclase activity in the caudate nucleus but did not influence the dopamine uptake, tyrosine hydroxylase or dopa-decarboxylase activity (9,18,19, 20).

Recent studies have shown that MIF antagonized the haloperidol-induced enhanced /^{3}H/spiroperidol binding in rat striatum (2,5,7,10). On the basis of these studies some authors have hypothized the existence of a unique MIF receptor site functionally coupled to the dopamine receptor through which MIF might modulate the nigrostriatal dopaminergic neurotransmission (4,5,8,9).

At present at least two different types of dopamine receptors have been described (17). These receptors were identified on the basis of their linkage to adenylate cyclase. Thus, the dopamine D1 receptor is linked positively to adenylate cyclase, and the D2 receptor is independent of or even involved in the inhibitory control of this enzyme (17, 27). In recent studies, the selective agonists and antagonists of D1 and D2 receptors have been described (6,16, 21, 28). In the light of these findings arises a question, which of these receptor subtypes are involved in the effect of

MIF on the dopaminergic neurotransmission. Therefore, the aim of the present study was to investigate the action of MIF in the behavioural effects of the specific D1 and D2 receptor agonists and antagonists.

Materials and Methods

Experiments were carried out on male rats weighing 200-300 g and male mice weighing 25-30 g. The animals were kept under standard vivarium conditions and they received food and water "ad libitum".

Behavioural Studies

Locomotor activity was measured in mice placing them into individual cylindrical activity cages (18 cm high, 30 cm diameter) each fitted with two photoelectric units located 2 cm above the floor and connected with an electromechanical counter. The number of counts was determined in a 30 min period of observation. Catalepsy was assessed in mice according to the method of Honma, Fukushima (15). Briefly, at various intervals after drug treatment the animals were placed by their forepaws onto the horizontal bar, positioned 4 cm above the floor and the time in seconds during which the animals remained in such a position was recorded. In order to assess the stereotypy and yawnings, the rats were placed into individual square plexiglass boxes (15x15x15 cm). The number of yawning episodes was recorded during a 60 minute test using a hand-held counter. The stereotypy was scored on a four-point severity scale after a 1 min observation period and at every 15 min interval for up to 60 min according to the method of Costall and Naylor (11).

Biochemical Studies

For carrying out biochemical studies the rats were decapitated, brains were rapidly removed and striata were dissected out on the ice. Dopamine (DA) and its metabolites - homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were isolated on the columns with Sephadex G-10(Farmacia, Sweden) and measured spectrofluorimetrically in a fluorescence spectrophotometer MPF-2 (Hitachi, Japan) according to the method of Early, Leonard (13).

Drugs

The following drugs were used: MIF (obtained as a gift from the Institute of Pharmacology, Academy of Medical Sciences, Moscow) was dissolved in saline and injected s.c. 20 min before the test. D1/D2 mixing agonist apomorphine · HCl (USSR) was dissolved in saline containing 0.05 % of ascorbic acid as an antioxydant and injected s.c. in the neck region of animals; the specific D2 receptor agonist Ly 171555 (qiunpirrole HCl, gift of Eli Lilly Co., Indianopolis, USA) was dissolved in saline and injected s.c.; the D2 receptor anagonist sulpiride (commercial ampoules "Eglonyl") was diluted to the appropriate volume with saline and injected i.p. in a dose 100 mg/kg; the D1 specific antagonist SCH23390 (as a gift of Schering, Corp., Bloomfeld, USA) and haloperidol (Janssen Farmaceutica, Beerse, Belgium) were dissolved in a minimal amount of acetic acid, further diluted with saline and injected i.p,

Statistics

The results were expressed as means ± SEM. For statistical calculations the 1-way analysis of variance (ANOVA) followed by Student's test or Dunnett's t-test were used.

Results

MIF in doses 0.1-10.0 mg/kg given alone failed to affect locomotions in mice within 90 min after treatment. The specific D2 receptor agonist Ly 171555 induced the inhibition of locomotions in mice. This effect of Ly 171 555 was observed even after very low doses of Ly 171 555 (0.01mg/kg). MIF (1 mg/kg) enhanced this effect of Ly 171 555 on locomotions. The effect of MIF was not dose-dependent and in a higher dose (10 mg/kg) disappeared (Fig. 1), MIF also antagonized the inhibition of the locomotions induced by sulpiride (Fig. 2). In contrast, MIF did not change the behavioural stimulation induced by SKF 38 393 or the catalepsy induced by SCH 233 390. MIF did not either change the haloperidol-induced catalepsy in mice.

Similar results were obtained in experiments on rats. Thus, the MIF potentiated by Ly 171 555-induced yawnings (Fig. 3) but did not influence either the haloperidol-ind - used catalepsy (Table 1) or the apomorphine-induced stereotypy (data not shown). MIF also did not change the levels of DA and its metabolites HVA and DOPAC in the striatal and mesolimbic tissues of the rat brain (Table 2).

Discussion

Recent studies have shown that Ly 171 555 and sulpiride selectively interacted with the D2 receptors, whereas SKF 38 393 and SCH 23390 possessed agonistic and antagonistic properties respectively at D1 receptors (6,11,16,21,27, 28). In contrast, apomorphine and haloperidol counteract with both types of receptors, D1 and D2 (6,11,16,25).
The results obtained in the present study show that MIF potentiates the behavioural effects of the selective D2 receptor agonist Ly 171 555 and abolishes the behavioural effects the specific D2 receptor agonist sulpiride and which

Table 1

Effect of MIF on the catalepsy induced by haloderidol and SCH 23390 in Mice. The data are means ± SEM in groups of 8-10 animals. Catalepsy was measured 60 min after haloperidol and 30 min after SCH 23390 treatment.

Drug (mg/kg)	Catalepsy (sec.) Haloperidol (1.0 mg/kg)	SCH 23390 (0.1 mg/kg)
Saline	229 ± 15	43 ± 6
MIF 0.1	187 ± 29	44 ± 9
1.0	178 ± 36	41 ± 7
10.0	157 ± 22	50 ± 7

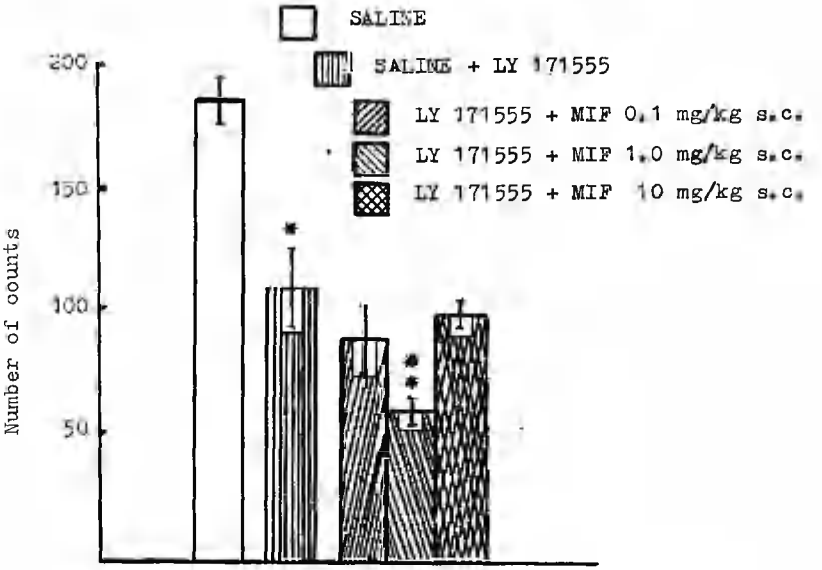

Fig. 1. Effect of MIF on the inhibition of looomotions in mice induced by Ly 171555 (0,05 mg/(0.05 mg/kg, s.c.). Locomotor activity was determined during 30 min after drug treatment. Each bar represents means ± SEM of 8-10 animals. * - $P<0.05$ v.s. saline; ** - $P<0.01$ v.s. Ly 171555 treated group.

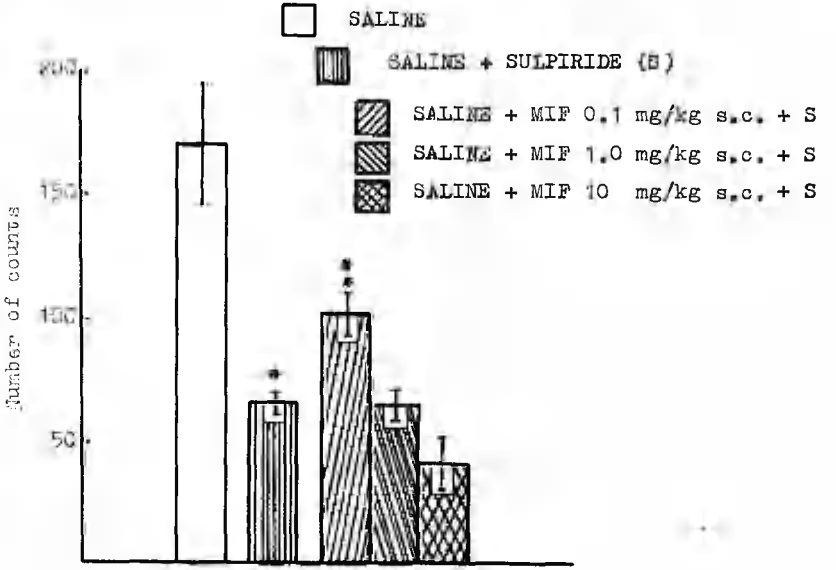

Fig. 2. Effect of MIF on the inhibition of locomotions induced by sulpiride (100 mg/kg,i.p.) Locomotor activity was determined during 60 min and 30 min after sulpiride pre-treatment. Each group consists of 10-12 animals. * - P 0.05 v.s. saline; ** - P 0.05 v.s. sulpiride treated group.

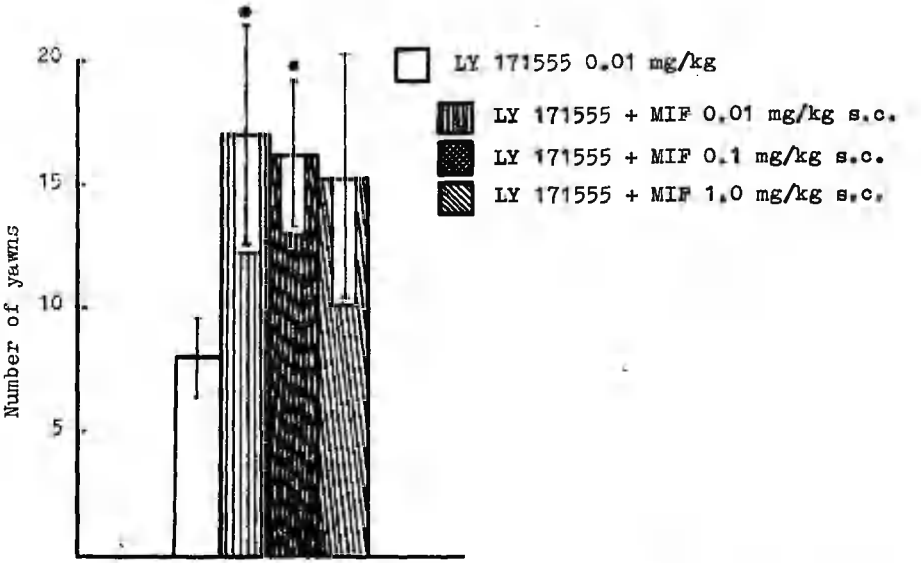

Fig. 3. Effects of MIF on the yawning behaviour induced by Ly 171555 in rats. The number of yawns was counted during 60 min after drug treatment. Each group consisted of 10-12 animals. * - P 0.05 v.s. control; ** - P 0.05 v.s. Ly 171555 treated animals.

Table 2

The effect of MIF on the concentration (μg/g tissue) of DA and its metabolites HVA and DOPAC in the rat brain. MIF was administered s.c. 30 min. before decapitation. The data are means ± SEM of four separate determinations.

Drug, dose (mg/kg)	Striatum			Limbic system		
	DA	HVA	DOPAC	DA	HVA	DOPAC
Saline	7.8 ± 0.9	0.8 ± 0.3	0.7 ± 0.07	4.2 ± 0.7	1.7 ± 0.4	1.1 ± 0.5
MIF 0.01	8.9 ± 0.7	1.3 ± 0.7	1.0 ± 0.3	3.7 ± 0.5	2.5 ± 0.6	1.0 ± 0.3
0.1	7.1 ± 0.9	0.8 ± 0.3	0.8 ± 0.1	3.0 ± 0.3	1.2 ± 0.2	0.9 ± 0.1
1.0	7.0 ± 0.6	2.2 ± 1.0	1.1 ± 0.4	3.7 ± 0.7	2.2 ± 0.4	1.2 ± 0.4

does not affect the behavioural changes induced by D1 receptor agonists and antagonists. These data suggest that MIF selectively counteracts with the D2 receptors probably by increasing their sensitivity to the D2 receptor agonists. The previous binding studies as well as the data obtained in our laboratory suggest that this modulatory effect of MIF is not dependent on the direct interaction of MIF with the D2 sites (6,8,9). Most probably, the enhancing effect of MIF on dopaminergic neurotransmission is due to the allosteric interaction of the MIF-sensitive binding sites with the D2 receptors. The previous studies have shown that the inhibition of locomotor activity or yawning behaviour induced by low doses of dopamine receptor agonists are mediated via selective activation of presynaptic (auto) receptors of the D2 type (12,22). According to some other authors, these effects are mediated via the D2 postsynaptic receptor, distinct from those which induce stereotypy or catalepsy (6, 22). Binding studies have also revealed that at least two subtypes or affinity states of the D2 receptor might exist within the CNS (27,28). The data of the present study also indicate that a novel D2 receptor agonist Ly 171 555 in the doses used selectively activates a distinct subtype (or affinity state) of the D2 receptors which is involved in the induction of yawnings and inhibition of locomotions. This proposition is further supported by the failure of MIF to change the stereotypy and catalepsy induced by apomorphine and haloperidol, respectively. On the basis of these results it might be proposed that MIF exerts its modulatory effect allosterically interacting with the high affinity subtype of the D2 receptor which represents probably a presynaptic (auto) receptor.

Finally, the modulatory effect of MIF appears only in the narrow range of doses. The higher doses of MIF failed to change the behavioural effects of Ly 171 555 and sulpiride. The ineffectiveness of high doses of MIF is difficult to explain. Previous studies have shown that the transport of a close analogue of MIF Tyr-MIF into brain may be inhibited by Tyr-MIF itself (1). Such self-inhibition may also occur in the case of MIF. Furthermore, high concentrations of MIF may inhibit the binding of peptide to its receptors resulting in the disappearance of the effects of MIF seen after lower doses.

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THE PARAMETERS OF CHOLECYSTOKININ (CCK-8) RECEPTORS IN DIFFERENT BRAIN STRUCTURES OF THE RAT

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Abstract:

The binding specificity of ^{3}H-CCK-8 was determined in rat brain structures. The analysis of the association and dissociation experiments at 23°C revealed an apparent two-site fit of the binding data in the all rat brain regions examined. The pretreatment of brain membranes at 37°C caused a complete loss of slower dissociation component of ^{3}H-CCK-8 binding. According to the Scatchard analysis the pretreatment of the membranes at 37°C did not alter the density of the CCK-8 receptors, but the affinity of the CCK-8 binding sites was decreased 1,5-2 times. The highest amount of the CCK-8 binding sites was in mesolimbic area, somewhat lower in frontal cortex and the lowest in brainstem. The calculation of the IC_{50} values for different CCK-related peptides and CCK-antagonists also revealed differences between the brain structures. The results of this study demonstrate the disparity of the CCK-8 binding sites in different brain structures. The CCK-8 binding sites differ in their affinity for different ligands and in the density in the different brain areas.

INTRODUCTION

Cholecystokinin octapeptide (CCK-8) is an important gastrointestinal hormone with well established neuronal activity. The peptide is located throughout brain in a characteristic distribution (1,5), parallelled to large extent by the distribution of its specific binding sites (8,15),

Recent electrophysiological and biochemical studies have demonstrated the existence of different population of CCK-8 receptors in the brain. CCK-8 in low concentrations inhibits the activity of pyramidal cells in hippocampus, whereas the higher concentrations are needed for exciting of these cells (2,7). In the radioligand binding studies is demonstrated the presence of central and peripheral CCK-8 receptors in the brain (3). Several authors have shown the bi-phasic dissociation of the radiolabelled CCK-8 from the binding sites in cerebral cortex (14,11). The hypothesis of this disparity of CCK-8 receptors may be supported by the fact that CCK-8 has different neurochemical interactions in the brain structures. For example, in the mesolimbic area CCK-8 has strict interaction with dopamine, while in cerebral cortex and hippocampus it is located in the same neurons with the major inhibitory neurotransmitter GABA (4,6). The main task of the present work was to characterize the CCK-8 binding sites in different brain structures for revealing the possible disparity in the properties of the CCK-8 receptors in brain structures.

MATERIALS AND METHODS

The experiments were performed on male rats, weighing 200-250 g. During the following decapitation (between 9 and 12 a.m.) the whole brain was rapidly removed. The brain structures (frontal cortex, mesolimbic area, striatum, hippocampus, brainstem) were dissected on ice and the membranes were prepared according to the method of Saito et al. (1981). The brain structures from six rats were pooled, homogenized in 10 volumes of 50 mM TrisHCl buffer (pH 7,4 at $4^{o}C$) using a motor driven teflon-glass (Potter-S) homogenizer for 10 strokes. The homogenate was centrifuged at 37000x g for 15 min, resuspended in the same volume of buffer and recentrifuged for 15 min. The pellet was then homogenized in the standard incubation buffer, consisting of the following: HEPES 10 mM, NaCl 130 mM, KCl 5 mM, $MgCl_2$ 5 mM , $EDTANa_2$ 1 mM, bovine serum albumine 0,5 mg per 1 ml (pH 6,8 at $24^{o}C$). The binding experiments were carried out in polypropylene tubes (1,5 ml), which routinely received 50 µl of ^{3}H-CCK-8 (60-80 Ci/mmole, Amersham International, U.K., final concentration 0,05-1,5 mM), 50 µl of various concentra-

tions of peptides or drugs, and 400 µl of freshly prepared brain membranes. In the majority of experiments the membranes were preincubated for 25 min at 23°C with peptides or drugs, and after that the radiolabelled CCK-8 was added into polypropylene tubes and the samples were carefully mixed. The incubation procedure was terminated by rapid centrifugation in a Beckman microfuge 12 (11000x g) for 3 min at room temperature. The supernatants were aspirated, and the pellets were washed with 1 ml of incubation buffer. The tips of tubes were cut and dropped into the scintillation vials. The radioactivity was counted by liquid scintillation spectrometry (Beckman LS 6800) in the Bray cocktail. The counting efficiency for tritium was 50-52%. The specific binding of ^{3}H-CCK-8 was defined as the difference between the degree of binding in the absence and the presence of 100 nM of caerulein sulfated. Saturation curves were analysed according to the method of Scatchard (1949). The IC50 values for drugs and peptides were obtained using the log-probit analysis.

The following drugs were used in the experiments: caerulein sulfated, caerulein desulfated (Farmitalis Carlo Erba, Italy), CCK-4 (I.C.I.,U.K.), tifluadom(Sandoz, Switzerland), proglumide (Rotta Research Labs, Italy),pentagastrin (Sanitas, USSR), haloperidol (Gedeon Richter, Hungary).

RESULTS

The binding of ^{3}H-CCK-8 to the rat brain membranes was monitored at 23°C for various time intervals. The association of radioligand reached to the equilibrium during 60 min in the mesolimbic area and hippocampus, and during 75 min in frontal cortex and striatum. The binding was stable for 4 hr. The binding of ^{3}H-CCK-8 to brain membranes was linear between 1 and 10 mg of original tissue/ assay . For routine assay 3-4 original tissue/assay was used . After reaching the state of equilibium the binding of the radiolabelled CCK-8 was reversed by adding 100 nM of caerulein sulfated. The analysis of the association experiment by ln (Beq/Beq-Bt) (Beq, the specific binding at equilibrium; Bt, the specific binding at indicated time) vs. time showed the observed association rate constant (K_{obs}). It was 0,0200 min^{-1} in frontal cortex, 0,026 min^{-1} in mesolimbic area, 0,0242 min^{-1} in striatum and 0,0220 min^{-1} in hippocampus.

The analysis of the dissociation experiment by the plot ln (Bt/Bo) (Bt, the specific binding at the indicated time; Bo, the specific binding at the start of the dissociation experiment) vs. time showed a biphasic curve. The higher dissociation rate constant (K_{-1f}) was 0,0121 min^{-1} in frontal cortex, 0,0152 min^{-1} in mesolimbic area and 0,0183 min^{-1} in striatum. The slower dissociation rate constant (k_{-1s}) was 0,0070 min^{-1} in frontal cortex, 0,0056 min^{-1} in mesolimbic area and 0,0059 min^{-1} in striatum. Using the relationship $K_{+1} = (K_{obs} - K_{-1s}):L$, in which L represents the concentration of ^{3}H-CCK, was calculated the association rate constant(table 1). The equilibrium dissociation constant (K_d) was calculated from the ratio of the dissociation and association rate constants ($K_{-1}:K_{+1}$) (table 1). The pretreatment of brain membranes at 37°C caused the complete loss of the slower dissociating component of the ^{3}H-CCK-8 binding. The results of kinetic experiments demonstrated that the affinity of the CCK-8 binding sites was higher in the mesolimbic area than in the other forebrain structures. In saturation experiments with increasing concentrations of ^{3}H-CCK-8 (0,05-1,5 nM) the specific binding was proportional to the amount of the radioligand used. The concentrations of the radiolabelled CCK-8 over 1 nM completely saturated the CCK-8 binding sites in the forebrain (figure). The Scatchard plot was linear in all brain structures studied in the present experiment (table 2). Pretreatment of brain membranes at 37°C decreased evidently the affinity of the CCK-8 binding sites to the radiolabelled CCK-8. The change was more marked in striatum (see also table 1, the difference between two kinetic constants in striatum). These results evidently demonstrate that the pretreatment of brain membranes at 37°C does not reduce the number of the CCK-8 binding sites, but obviously decreases their affinity.

In table 3 are shown the mean values of binding experiments with different brain structures. The amount of the CCK-8 binding sites in rat brain was the highest in the mesolimbic area. The number of the CCK-8 binding sites was somewhat lower in frontal cortex and striatum, whereas in hippocampus and brainstem it was significantly lower if compared to the mesolimbic area (table 3). The calculation of this IC_{50} values for different CCK-related peptides and CCK CCK antagonists also revealed some differencesbetween brain

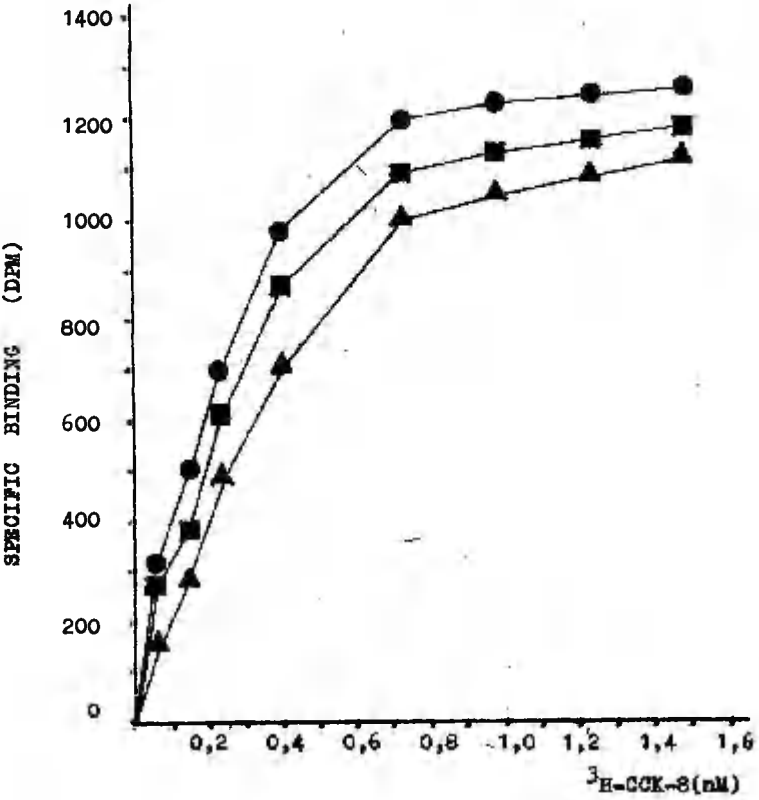

Figure. A saturation experiment showing the binding of several concentrations of ^{3}H-CCK-8 (0,08-1,5 nM) to rat brain membranes at 23°C for 120 min. The specific binding of ^{3}H-CCK-8 is presented in figure. The differences between total (in absence of 100 nM caerulein) and nonspecific (in presence 100 nM caerulein) binding is defined as specific binding. Frontal cortex —▲— ; Mesolimbic area —●— ; Striatum —■—.

Table 1

Kinetic parameters of ^{3}H-CCK-8 binding in different brain structures.

Brain structure	Association constant (k+1) ($nM^{-1} \times min^{-1}$)	Dissociation constant K, (k-1/k+1) (pM) 23°C pretreatment		37°C pretreatment	
		Fast	Slow	Fast	Slow
Frontal cortex	0,065±0,012	190	110	202	-
Mesolimbic area	0,102±0,015	150	56	130	-
Striatum	0,077±0,009	234	77	209	-

In dissociation experiments the brain membranes were preincubated during 30 min at 23°C or 37°C.

The effect of pretreatment at 37°C on ^{3}H-CCK-8 binding in different forebrain structures

Brain structure	23°C pretreatment K_d	23°C pretreatment B_{max}	37°C pretreatment K_d	37°C pretreatment B_{max}
Frontal cortex	0,35±0,04	5,80±0,25	0,65±0,03^{+}	6,00±0,24
Mesolimbic area	0,25±0,02	6,35±0,27	0,51±0,03^{++}	6,60±0,30
Striatum	0,36±0,04	5,70±0,30	0,80±0,05^{++}	6,40±0,26

Kd - dissociation constant (nM); B_{max} - density of binding sites pmoles/g tiseue. + - $p \leq 0,05$; ++ - $p \leq 0,01$ (Student's t-test).

Table 3

The binding parameters of ^{3}H-CCK-8 in different brain regiona

Brain structure	n	K_d (nM)	B_{max}(pmoles/g tiseue)
Frontal cortex	12	0,35±0,04	4,59±0,20
Mesolimbic area	12	0,32±0,05	5,21±0,22
Striatum	12	0,33±0,04	4,88±0,25
Hippocampus	6	0,35±0,05	2,25±0,25
Brainstem	3	0,30±0,05	0,59±0,08

n - number of experiments; K_d - dissociation constant B_{max} - density of binding sites.

Table 4

IC_{50} values in competitive inhibition of ^{3}H-CCK-8 specific binding to the membranes of different brain structures by CCK-related peptides and CCK-antagonists

Peptide or CCK-antagonist	n	IC_{50} values ± S.E.M. Frontal cortex	Mesolimbic area	Striatum	Hippocampus	SN+VTA
Caerulein	5	1,7±0,2 nM	1,5±0,1 nM	1,5±0,1 nM	1,2±0,1 nM	1,3±0,1 nM
CCK-4	4	395 ±22 nM	125±12 nM	147±13 nM	196±15 nM	-
Pentagastrin	4	7,0±0,5 nM	5,0±0,4 nM	4,5±0,5 nM	9,7±0,5 nM	6,5±0,3 nM
Desulfated caerulein	3	52±4 nM	54±6 nM	56±7 nM	22±5 nM	-
Proglumide	3	1,1±0,3 mM	1,2±0,3 mM	1,9±0,4 mM	0,5±0,2 mM	-
Cifluadom	3	80±10 µM	66±12 µM	67±9 µM	-	-

n - number of experiments; SN+VTA - substantia nigra + ventral tegmental area

Table 5

The IC_{50} values of caerulein and tifluadom after pretreatment at 37°C in different brain structures and pancreas

Brain structure / pancreas	n	IC_{50} ± S.E.M. 23°C caerulein(nM)	23°C tifluadom(μM)	37°C caerulein(nM)	37°C tifluadom(μM)
Frontal cortex	3	1,7±0,2	80±10	1,3±0,2	36±8+
Mesolimbic area	3	1,5±0,2	66±12	1,6±0,2	49±10
Striatum	3	1,5±0,2	67±10	2,1±0,2	42±7
Pancreas	3	1,0±0,1	3,5±0,5	-§	-

n - number of experiment; § - after pretreatment at 37°C the binding of ^{3}H-CCK-8 in pancreas was completely lost. + - p 0,05.

Table 6

The effect of long-term treatment of haloperidol (0,5 mg/kg daily, during 15 days) on ^{3}H-CCK-8 binding in different brain regions.

Brain structure	n	K_d (nM)		B_{max} (pmoles/g tissue)	
		Saline	Haloperidol	Saline	Haloperidol
Frontal cortex	6	0,35±0,03	0,34±0,04	4,93±0,18	5,13±0,24
Mesolimbic area	6	0,35±0,04	0,28±0,04	5,34±0,25	5,06±0,25
Striatum	6	0,34±0,05	0,31±0,05	5,12±0,24	4,61±0,23
Hippocampus	3	0,35±0,05	0,34±0,05	1,98±0,24	1,58±0,25
Brainstem	2	0,30±0,07	0,34±0,08	0,55±0,10	0,62±0,08

K_d - dissociation constant; B_{max} - density of binding sites

n - number of independant experiments. The studies were performed 48 hours after the last injection of haloperidol. The special studies showed that the effect of haloperidol (15 days treatment) on ^{3}H-CCK-8 binding was not different 2 hours as well as 48 hours after the last injection of haloperidol.

structures (table 4). Sulfated caerulein similarily displaced ^{3}H-CCK-8 from binding sites, whereas the action of CCK-4 was more pronounced in the mesolimbic area and striatum. The effect of pentagastrin was also the highest in the mesolombic area and striatum, but in hippocampus and frontal it had somewhat lower affinity (table 4).The CCK antagonist proglumide had the most significant effect in hippocampus, in hippocampus the action of proglumide was approximately 4 times weaker. The other CCK antagonist tifluadom displaced similarly radiolabelled CCK-8 from binding sites in all structures studied. The comparison of the effect of caerulein and tifluadom after the pretreatment of brain membranes at 37°C revealed significant changes in the action of tifluadom, although the effect of caerulein remained unchanged (table 5). The affinity of tifluadom was increased 1,5-2 times after pretreatment at 37°C. The binding sites of CCK-8 in pancreas were completely lost after the pretreatment of membranes at 37°C. It was shown in our previous experiments that the repeated administration of haloperidol caused significant changes in the behavioural effects of caerulein, an agonist of CCK-8 receptors. The inhibiting effects of caerulein became obviously weaker or the stimulating effects of caerulein became evident after repeated haloperidol treatment (12,13). The effects of long-term haloperidol treatment (0,5 mg/kg daily, during 15 days) on the ^{3}H-CCK-8 binding in different brain structures in different experiments were compared. The results of different studies are quite contradicting: the repeated haloperidol administration might increase as well as decrease the number of the CCK-8 binding sites in brain. The calculation of the mean values of 6 independent experiments has not demonstrated statistically evident differences in the ^{3}H-CCK-8 binding after haloperidol treatment (table 6). The pretreatment of brain membranes at 37°C also did not reveal the differences between the long-term administration of saline and haloperidol.

DISCUSSION

The results of the present study have supported the idea about the disparity of the CCK-8 binding sites in the brain. The dissociation experiments have revealed the biphasic nature of the ^{3}H-CCK-8 dissociation from binding sites. Pretreatment of brain membranes at 37°C results in

a complete loss of slowly (high-affinity) dissociating binding sites. This finding is in agreement with the studies of Wennogle et al. (1985) and Sekiguchi, Moroji(1986). However, the Scatchard analysis have not confirmed the viewpoint of Wennogle et al. (1985) that the pretreatment of brain membranes at 37°C significantly reduces the number of the CCK-8 binding sites. In reality, such a procedure evidently reduces the affinity of the CCK-8 binding sites, but not their density in forebrain structures. It seems possible that the interconvertible conformational states of the CCK-8 binding sites exist in the brain structure and the pretreatment of brain membranes at 37°C converts all binding sites into the low-affinity state. The ratio of high- and low-affinity binding sites for CCK-8 seems to be different in forebrain structures. Study of competition curves of different CCK-related peptides and CCK antagonists in different brain regions also supports the idea of heterogenity of the CCK-8 binding sites in brain. CCK-4 has 2,5-3 times higher affinity for subcortical CCK-8 binding sites (mesolimbic area and striatum) if compared with the binding sites in frontal cortex. Proglumide, an antagonist of the CCK-8 receptors, has very weak affinity for the CCK-8 binding sites in brain, although the disparity also exists in the action of proglumide. Proglumide has approximately 3,5 times lower affinity for the CCK-8 binding sites in striatum if compared to the binding sites in hippocampus. The differences in the action of the CCK- antagonists may be also linked to the uneven distribution of the CCK-8 binding sites in the rat brain. The density of this CCK-8 binding sites is the highest in mesolimbic area, somewhat lower in striatum and frontal cortex, whereas in hippocampus their density is approximately 2,5 times and in brainstem 10 times lower than in the mesolimbic region of rat brain.

Despite the profound changes in the action of caerulein after long-term haloperidol treatment (12,13) we fail to find any statistically significant changes in the density of the CCK-8 binding sites after 15 days haloperidol administration. It seems possible that a long-term haloperidol treatment separates the inhibiting and activating CCK-8 receptors, increasing relatively the amount of activating CCK-8 binding sites. Unfortunately in the binding studies it is impossible to separate these different binding sites.

In conclusion, the present study demonstrates the disparity of the CCK-8 binding sites in the brain. The differences in the properties of the CCK-8 binding sites are related to the uneven distribution of the CCK-8 binding sites in brain structures (mesolimbic area striatum=frontal cortex hippocampus brainstem), to the different distribution and properties of interconvertible conformational states (faster and lower dissociating component) of this CCK-8 binding sites in forebrain and to the disparity in the affinity of the CCK-8-ergic ligands to the CCK-8 binding sites in different brain regions.

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NEUROLEPTIC - CHOLECYSTOKININ INTERACTION: THE INVOLVEMENT OF Na-PUMP

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Abstract:

The experiments on male rats have shown the reduction of sodium pump activity in the different brain structures after administration of caerulein (10 ug/kg), an agonist of CCK-8 receptors, and proglumide (1 mg/kg), an antagonist of CCK-8. The simultaneous administration of caerulein and prqglumide completely reversed the decreasing effect of both compounds. The acute treatment of haloperidol (0,5 mg/kg) also decreased the activity of Na - pump, but after repeated (during 15 days) treatment the action of haloperidol was more pronounced. Acute and chronic proglumide medication antagonized the effect of repeated haloperidol treatment. In binding studies concomitant administration of haloperidol and proglumide reversed completely the effect of haloperidol on the ^{3}H-CCK-8 binding in different forebrain structures. It seems probable that the reduced activity of Na - pump and its decreased cooperativity with Na^{+} is playing an evident role in the development of depolarization inactivation of dopaminergic neurones after administration of the CCK - 8 agonists and neuroleptic drugs.

INTRODUCTION

The intravenous and iontophoretic administration of cholecystokinin octapeptide (CCK-8) were observed to activate dopamine cells to the point that they developed a state

of apparent depolarization inactivation (3). Similar effect was found after repeated administration of antipsychotic drugs. Their long-term application leads to the inactivity of the great majority of midbrain dopamine-containing cells (1,6). This inactivity has been reported to be also due to the state of tonic depolarization inactivation of neurons (2). Proglumide, an antagonist of CCK-8 receptors reverses completely the inactivation of dopamine cells induced by CCK-8 or repeated neuroleptic treatment (3,4). It seems very probable that CCK-8 is mediating the effect of a long-term neuroleptic treatment on dopamine cells activity, but the biochemical nature of this phenomenon remains still unknown. The aim of the present investigation was to study the role of Na^+/K^+ATPase in the neuroleptic-CCK-8 interaction. It is well-known that sodium pump is the key factor in the regulation of the resting potential of neurons membrane. The decreased activity of this enzyme may be the reason for the depolarization inactivation of nerve cells. Simultaneously with the changes in sodium pump activity the parameters of ^{3}H-CCK-8 binding were studied after the long-term treatment of haloperidol and proglumide.

METHODS

The experiments were performed on female albino rats, weighing 200-240 g. In acute experiments CCK-8 agonist caerulein (10 µg/kg s.c., Parmitalia Carlo Erba, Italy) was injected 15 min, proglumide (1 mg/kg i.p., Rotta Pharmaceutici, Italy) 20 min and haloperidol (0,5 mg/kg i.p., Gedeon Richter, Hungary) 60 min before the decapitation of animals. The brain was rapidly removed from the skull and the brain structures (frontal cortex, nucleus accumbens, striatum, hippocampus and substantia nigra-ventral tegmental area) were prepared on ice. The brain structures of acute experiments were used for the purifying of Na^+/K^+ATPase.

In the second part of the experiment haloperidol (0,5 mg/kg i.p. daily) and proglumide (10 mg/kg i.p. daily)were injected alone or simultaneously during 15 days. On the 15th day 1 hr after the last injection of haloperidol or 20 min after proglumide the rats were killed by the decapitation. In one group of animals, receiving only halo -

peridol, the acute injection of proglumide (1 mg/kg i..p.) was done 20 min before the decapitation of rats on the 15th day. The brains of the animals, receiving repeated haloperidol or proglumide treatments, were used for the Na^+/K^+ATPase studies as well for the measurement of ^{3}H-CCK-8 binding.

Na^+/K^+ATPase activity (pmole P_i/mg protein per min)was assayed with 5 mM ATP-Tris, 100 mM NaCl, 20 mM KCl_2 and 30 mM imidazole buffer, pH 7,4 at 37°C. The Hill's coefficient (n_H^{Na+}) were calculated from Hill plot.

^{3}H-CCK-8 binding experiments were performed in frontal cortex, nucleus accumbens and striatum. For binding studies the modified method of Praissman (5) was used. Briefly, the brain structures of 5 rats were pooled, homogenized in 10 volumes of TrisHCl buffer (50 mM, pH 7,4 at 20°C).The membranes were centrifuged twice (35000xg during 15 min). The incubation medium was the following: HepesKOH (10 mM,pH 6,8 at 20°C), 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM $EDTANa_2$ and bovine serum albumine (1 mg per 2 ml of incubation medium). The washed membranes were homogenized in incubation buffer (10 mg original tissue in ml). The homogenized membranes were preincubated during 30 min (at 23°C) in the incubation medium: 3 parallels without and 3 parallels with caerulein (100 nM). Then ^{3}H-CCK-8 (60-85 Ci/mmole, Amersham International, U.K., 0.08-1.5 nM) was added into the incubation mixture and the membranes were incubated during 120 min at 23°C. The binding was stopped by rapid centrifugation (11000xg during 3 min). The supernatant was discarded and pellet washed two times with 1 ml ice-cold incubation buffer and cut into vials. Radioactivity was counted in the Bray scintillation cocktail in a Beckman LS 6800 (counting efficacy 50-53 %). The results of binding experiments were analyzed using Scatchard analysis.

RESULTS

Acute administration of caerulein (10 ug/kg) decreased the activity of the Na-pump as well n_H for Na^+ in frontal cortex, nucleus accumbens and hippocampus (table 1).Proglumide (1 mg/kg) caused the decrease of the Na^+/K^+ATPase activity and n_H for Na^+ in all structures studied. The coadministration of proglumide and caerulein completely attenuated the changes in the Na-pump activity and in particu-

lar the changes of n_H^{Na} caused by both drugs(table 1).Acute administration of haloperidol (0,5 mg/kg) reduced the activity of Na-pump in frontal cortex and hippocampus, whereas the n_H for Na^+ was decreased in frontal cortex, nucleus accumbens and striatum (table 2). Long-term haloperidol treatment evoked the more pronounced reduction in the parameters of Na^+/K^+ATPase activity in comparison with acute treatment. The repeated treatment of proglumide (10 mg/kg) caused the significant reduction of the Na-pump activity in all structures studied. However, the acute or long-term administration of proglumide antagonized all the changes in Na^+/K^+ATPase actively caused by long-term haloperidol (table 2). A parallel study of the parameters of ^{3}H-CCK-8 binding revealed the statistically evident decrease of the ^{3}H-CCK-8 binding sites in frontal cortex after long-term treatment of haloperidol, whilein the other structures it caused an insignificant increase in their number (table 3). Long- term administration of proglumide (10 mg/kg) enhanced the affinity of the CCK-8 binding sites in nucleus accumbens and striatum, the density of the CCK-8 sites was reduced in nucleus accumbens. The coadministration of haloperidol and proglumide antagonized the haloperidol caused changes, but potentiated the effects of long-term proglumide medication (table 3).

DISCUSSION

The acute administration of caerulein, an agonist of the CCK-8 receptors, reduces obviously the activity of the Na-pump in forebrain structures. Simultaneous administration of proglumide, an antagonist of CCK-8, completely blocks the action of caerulein. A similar reduction of the Na^+/K^+ATPase activity is found after acute treatment of haloperidol, but it was more pronounced after long-term administration of haloperidol. The acute or repeated simultaneous administration of proglumide with haloperidol completely reverses the effect of haloperidol on the Na-pump activity. It seems very probable that the inhibiting action of long-term haloperidol treatment on the Na-pump activity is medicated through the CCK-8-ergic mechanisms. This opinion is supported by radioligand studies. The long-term haloperidol treatment causes the opposite changes in the density

Table 1

THE EFFECT OF ACUTE ADMINISTRATION OF CAERULEIN AND PROGLUMIDE ON Na-PUMP ACTIVITY IN DIFFERENT BRAIN STRUCTURES OF RAT

Drug/dose	Number of experiments	Frontal cortex		Nucleus accumbens		Striatum		Hippocampus		Substantia nigra+ Ventral tegmental area	
		Activity	$n_H^{Na^+}$	Activity	$n_H^{Na^+}$	Activity	$n_H^{Na^+}$	Activity	$n_H^{Na^+}$	Activity	$n_H^{Na^+}$
Saline	7	0,15± 0,02	1,41± 0,05	0,20± 0,03	1,44± 0,05	0,18± 0,02	1,42± 0,04	0,16± 0,02	1,40± 0,04	0,12± 0,01	1,35± 0,05
Caerulein 10 ug/kg	3	0,10± 0,02	1,12±* 0,04	0,07±* 0,03	1,07±* 0,03	0,15± 0,03	1,25± 0,05	0,05±* 0,02	1,0±* 0	0,11± 0,01	1,23± 0,05
Proglumide 1 mg/kg	3	0,06±* 0,03	1,17±* 0,04	0,05±* 0,03	1,03±* 0,04	0,06±* 0,03	1,03±* 0,03	0,08±* 0,03	1,2± 0,05	0,06±* 0,02	1,1±* 0,03
Caerulein,+ proglumide	3	0,11± 0,03	1,30± 0,05	0,10± 0,04	1,30± 0	0,10± 0,03	1,30± 0,05	0,11± 0,03	1,30± 0,05	0,10± 0,01	1,35± 0,05

$n_H^{Na^+}$ - Hill's coefficient for Na^+. Na-pump activity - pmole P_i/mg protein per min. + - $p<0,05$ (statistically evident difference from saline treated group).

Table 2

THE EFFECT OF ACUTE AND REPEATED TREATMENT OF HALOPERIDOL AND PROGLUMIDE ON Na-PUMP ACTIVITY IN DIFFERENT BRAIN STRUCTURES OF RAT

Drug/dose	Number of experiments	Frontal cortex		Nucleus accumbens		Striatum		Hippocampus		Substantia nigra+ Ventral tegmental area	
		Activity	$n^{Na^+}_H$	Activity	$n^{Na^+}_H$	Activity	$n^{Na^+}_H$	Activity	$n^{Na^+}_H$	Activity	$n^{Na^+}_H$
Saline (repeated)	3	0,16± 0,02	1,42± 0,05	0,19± 0,03	1,45± 0,05	0,19± 0,03	1,44± 0,04	0,17± 0,03	1,42± 0,04	0,13± 0,02	1,39± 0,04
Haloperidol 0,5 mg/kg (acute)	2	0,09±* 0,02	1,15±* 0,05	0,15± 0,03	1,05±* 0,05	0,15± 0,03	1,15±* 0,05	0,07±* 0,03	1,35± 0,05	0,09±* 0,01	1,35± 0,05
Haloperidol 0,5 mg/kg (repeated)	3	0,09±* 0,02	1,07±* 0,03	0,11±* 0,02	1,03±* 0,03	0,08±* 0,03	1,10±* 0	0,11± 0,03	1,10±* 0,05	0,08±* 0,02	1,00± 0
Haloperidol 0,5mg/kg (repeated)+proglumide 1 mg/kg (acute)	2	0,13± 0,03	1,40± 0,05	0,11±* 0,03	1,35± 0,05	0,12± 0,03	1,30± 0,05	0,12± 0,04	1,45± 0,05	0,11± 0,02	1,25± 0,05
Haloperidol 0,5mg/kg (repeated)+proglumide 10 mg/kg (repeated)	2	0,13± 0,03	1,50± 0,05	0,11± 0,03	1,40± 0,05	0,19± 0,02	1,40± 0,05	0,08±* 0,03	1,30± 0,05	0,10± 0,01	1,30± 0,05
Proglumide 10 mg/kg (repeated)	2	0,05±* 0,03	1,00±* 0	0,07±* 0,02	1,10±* 0,05	0,08±* 0,03	1,10±* 0,05	0,08±* 0,03	1,0±* 0	0,13± 0,02	1,10±* 0,05

* - $p<0,05$ (statistically evident difference from saline treated group).

Table 3

THE EFFECT OF REPEATED ADMINISTRATION OF HALOPERIDOL AND PROGLUMIDE ON ^{3}H-CCK-8 BINDING IN RAT FOREBRAIN

Drug / dose	Number of experiments	Frontal cortex		Nucleus Accumbens		Striatum	
		K_d	B_{max}	K_d	B_{max}	K_d	B_{max}
Saline	3	0,75±0,03	4,50±0,20	0,64±0,05	5,20±0,20	0,60±0,04	4,65±0,15
Haloperidol 0,5mg/kg	2	0,78±0,03	3,75±0,18^{+}	0,59±0,05	5,50±0,30	0,60±0,03	4,95±0,18
Proglumide 10mg/kg	2	0,69±0,04	4,62±0,15	0,45±0,03^{+}	4,30±0,30^{+}	0,46±0,04^{+}	4,50±0,20
Haloperidol + proglumide	2	0,63±0,05	6,00±0,30^{+}	0,25±0,02^{++}	2,75±0,15^{++}	0,47±0,03^{+}	3,85±0,22^{+}

+ - $p<0,05$; ++ - $p<0,01$ (statistically evident difference from saline treated group)

K_d - constant of dissociation (nM); B_{max} - number of binding sites (pmoles/g wet weight tissue).

of the ^{3}H-CCK-8 binding sites in frontal cortex and subcortical structures, whereas coadministration of proglumide with haloperidol completely attenuates the effect of haloperidol on ^{3}H-CCK-8 binding. The results of the present investigation are consistent with the studies of Bunney et al. (3,4), revealing the similar antagonism of proglumide against the depolarization inactivation of dopamine-containing neurons by CCK-8 and longterm haloperidol medication. There is existing possibility that haloperidol by blocking dopamine$_2$ receptors increases the release of CCK-8 from neurons. Increased stimulation of the CCK-8 receptors leads to the decrease of the Na-pump activity in different brain structures. The reduced activity and cooperativity with Na^+ of this enzyme seems to play an evident role in the development of depolarization inactivation of dopaminergic neurones induced by the CCK-8 agonists and neuroleptic drugs.

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AN ELEVATED PLUS-MAZE: A POSSIBLE TOOL FOR DETECTING STATE AND TRAIT ANXIETY IN RODENTS

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ABSTRACT:

The effect of anxiolytic and anxiogenic drugs (diazepam and DMCM respectively) on the exploratory activity of mice in two models was compared. It was shown that an elevated plus-maze model had significant advantage in respect to simple open field model. Mice and rats selected according to their exploratory activity in elevated plus-maze as anxious and non-anxious animals had dissimilar neurochemical characteristics. Namely, anxious animals had significantly lower number of central benzodiazepine binding sites in frontal cortex, but not in cerebellum. It is concluded that animal selection procedures may represent a possibility of creation of models for analysing the predisposition toward feeling anxious in some individuals.

INTRODUCTION

Pharmacotherapy of pathological mental states depends upon our understanding of the neurobiological mechanisms underlying such conditions. It has been shown that for the study of anxiety mechanisms anxiogenic compounds besides anxiolytics are also useful (32). A wide variety of animal models for anxiety have been pursued and several exploratory behaviour paradigms are most widely exploited (9). The simplest system for measuring exploratory activity is placement of the animal in an unfamiliar open field. However, the sedative effect of anxiolytics will produce much noise in this trivial test (9). Recently a new apparatus - an elevated plus-maze - was described and validated for detecting

the effects of anxiolytic and anxiogenic drugs in rat(22, 24). We compared the influence of a classical anxiolytic diazepam and a potent anxiogenic compound DMCM (methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) on the behaviour of mice in a combined open field/hole board apparatus and in an elevated plus-maze.

Psychologists distinguish between "state" and "trait" anxiety. State anxiety refers to the anxiety felt at a particular moment; trait anxiety implies the predisposition toward feeling anxious as a personality trait.Persons with high trait anxiety are more likely to feel anxious at any instant because they have a lowered threshold for the induction of fear and tension (17). The efforts of experimental psychobiology are mainly directed to the elucidation of the substrate of anxiety in state anxiety models(exposition of subjects to acute stressful events)whereas the trait anxiety paradigms have received a quite poor attention. However, it is suggested that the anxiety, experienced by animals, is not too dissimilar from such human psychic condition (14). It is possible to select experimental animals according to their individual behavioral peculiarities related to anxiety and to find some neurochemical correlates (21,27).

The compounds, acting at central benzodiazepine receptors, have clear relations to anxiety states(2,30). Therefore, it is logical to seek for the differences in benzodiazepine receptor characteristics in the CNS of animals, selected according to their more or less "anxious" behaviour in novel environment.

MATERIALS AND METHODS

Male white laboratory mice and rats weighing 22-25 g and 200-220 g respectively were used in this study.The animals were housed 40-45 (mice) or 15-20 (rats) per cage.The exploratory activity of mice was measured in open field and in plus-maze model. Mice were placed singly into the open field/hole board apparatus (30x30x18 cm,16 equal sectors, 16 holes with diameter 1,4 cm) and observed during the first 2 min. The number of crossed sectors, head-dips and rears was counted. The plus-maze consisted of two open arms (22x5 cm) and two enclosed arms (22x5x15 cm) with an

open roof. The maze was elevated to the height of 25 cm. To determine the exploratory activity in the open part of the maze the pair of open arms was divided into 7 equal sectors. During a 4 min test session the latency period of the first open part entry, the number of sectors crossed in open arms, and the total time spent in open arms were measured. To begin the experiment mice were placed at the centre of the plus-maze, facing one of the enclosed arms. Locomotor activity was registered in a multicage photocell motor activity meter. Each cage of the actometer was a cylinder with the height of 20 cm and diameter of 30 cm. Locomotor activity of mice was measured for a period of 30 min. DMCM (Schering AG, West Berlin) and diazepam(Seduxen, Gedeon Richter, Hungary) were administered intraperitoneally 15 or 30 min before the experiment respectively.

In the plus-maze selection experiments, mice and rats first were observed in a plus-maze for 4 min. The apparatus for rats was in principle similar to the one for mice except that the size was different (50x10 cm and 50x10x40 cm for the open and enclosed arms respectively). The animals were killed by decapitation immediately after the behavioural test. Dissected and homogenized brain regions were washed twice in TRIS-HCl buffer by centrifugation (48 000 g for 20 min) and resuspension. The binding was carried out in the presence of 0,125-8 nM of ^{3}H- flunitrazepam (spec. act. 81 Ci/mmol, Amersham Radiochemicals) using a total incubation volume of 500 µl. To determine nnnspecific binding unlabelled flunitrazepam (10 uM, Hoffmann - La Roche, Basel) was added. After 60 min incubation at 0°C the reaction was stopped by rapid filtration over the GF/B (Whatman, England) filters. Specific binding was calculated by subtracting the non-specific from total binding at each given radioactivity concentration.

The analysis of variance and Student's t-test for paired observations were used to determine statistical significance.

RESULTS AND DISCUSSION

Anxiogenic β-carbolines can aggravate the behavioral responses to stressful events or induce fear by itself (5, 12,23,25). As shown in Table 1, administration of a potent

Table 1

The effect of DMCM and diazepam on the exploratory activity of mice in a combined open field/hole board apparatus and on the locomotor activity in a multicage photocell motor activity meter. Results are expressed as a mean ± SEM.

Treatment (mg/kg)	Crossed sectors	N of rears	N of head-dips	Actometer counts
Vehicle	19,8±2,8	4,5±1,1	13,5±1,9	160±15
DMCM 0.5	20.2±3.0	4.3±0.7	17.0±2.5	151±25
1.0	16.7±3.9	2.8±0.7	13.4±1.9	154±17
1.5	10.1±3.5	1.8±0.4^{x}	12.1±2.1	130±20
2.0	5.3±2.5^{xx}	0.8±0.2^{xx}	5.3±1.4^{xx}	100±19^{x}
Diazepam 0.5	24.1±2.1	6.5±1.2	17.7±1.6	181±16
1.0	21.0±2.6	8.7±1.9	13.9±2.2	160±17
1.5	15.2±2.9	3.1±0.8	13.1±2.3	120±17
2.0	10.4±2.1^{x}	1.2±0.7^{x}	9.5±1.7^{xx}	95±15^{x}
DMCM 0.5 + diazepam 0.75	23.3±4.3	4.8±0.4	13.4±2.0	155±20
DMCM 1.0 + diazepam 0.75	27.8±2.0^{x+}	10.1±2.0^{x++}	19.2±1.9	152±18

x - $P < 0.05$ as compared to vehicle treated animals
xx - $P < 0.01$ as compared to vehicle treated animals
+ - $P < 0.05$ as compared to DMCM 1.0 treated animals
++ - $P < 0.01$ as compared to DMCM 1.0 treated animals

anxiogenic drug DMCM decreased all parameters of exploratory behaviour observed in open field only in quite high doses. The number of rears seems to be the most sensitive behavioral pattern to DMCM. The doses of DMCM which significantly attenuated the number of crossed sectors and head - dips in mice also lowered the basal locomotor activity.

We were not able to demonstrate the anxiolytic effect of diazepam in our open field experiments. The activating effect of little doses of diazepam did not reach signi - ficance and in doses 1.5 mg/kg and more diazepam had a sedative effect. This discrepancy with the results, obtained by several authors, may be related to a different strain of the mice used or to a high baseline level of the response (8,28). In any case, such results are not unexpected,as the data from open field experiments are quite inconsistent(13). It has been pointed out that the qualities of the testarena are also important in this paradigm.

Using the borderline doses of DMCM and diazepam together we found that this combination can lead to a significant increase in exploratory behaviour.(Table 1). Benzodiazepine inverse agonists can support information processing (33). Possibly a combination of anxiolysis and intensified information processing gives rise to such enhanced exploration. It is apparent that, if this is the case, distinct primary targets may be involved for the action of benzodiazepine agonists and inverse agonists.

Animals display fear not with regard to novelty per se but when they are prevented from responsing normally to the new conditions (20).There is no unique relationship between locomotor activity and fear (1), and rodents will walk around a test arena to explore it or to escape from if or both(11). Therefore, an apparatus for detecting anxiety state has to be somewhat more artificial and complicated than a simple open field. An elevated plus-maze has recently been validated for testing anxiety in rats (22,24). In our experiments with mice (Table 2) administration of DMCM had clear dose dependent attenuating effect on all the parameters registered. The conflict in the behaviour of animals between the exploration drive and "agarophobia" was obvious, but requires a detailed ethological analysis. Nevertheless, the parameters used as the latency period of the first open part entry, the number of crossed sectors in open arms, and to-

The effect of DMCM and diazepam on the exploratory activity of mice in an elevated plus-maze. Results are expressed as a mean ± SEM.

Table 2

Treatment (mg/kg)	Latency of first open part entry	N of open arm sectors crossed	Total time spent in open arms
Vehicle	28± 6	20.6±2.4	54± 7
DMCM 0.5	39± 7	14.9±4.2	39± 4
1.0	55±13	8.4±3.5^{x}	29± 5^{x}
1.5	89±21^{x}	5.3±2.8^{xx}	17± 6^{xx}
2.0	95±20^{x}	4.2±2.0^{xx}	15± 5^{xx}
Diazepam 0.5	25± 6	22.2±2.7	54± 9
1.0	21± 4	24.7±3.1	48± 8
1.5	16± 3	33.5±3.4^{x}	94± 9^{xx}
2.0	27± 9	25.3±2.6	70±10
DMCM 1.5 + diazepam 0.75	33± 8^{+}	18.3±3.6^{+}	46± 8^{+}

x - $P < 0.05$ as compared to vehicle treated animals
xx - $P < 0.01$ as compared to vehicle treated animals
+ - $P < 0.05$ as compared to DMCM 1.5 treated animals

Table 3

Selection experiment according to the exploratory activity of mice in an elevated plus-maze. Results are expressed as a mean ± SEM.

Group	N of animals	Latency of first open part entry	N of open arm sectors crossed	Total time spent in open arms
Total	84	58 ± 12	18.3 ± 1.8	38 ± 6
Non-anxious subgroup	12	13 ± 2^{xx}	31.3 ± 4.1^{x}	78 ± 13^{x}
Anxious subgroup	12	209 ± 18^{xx+}	8.4 ± 2.4^{x+}	15 ± 3^{xx+}

x - $P < 0.05$ as compared to total group

xx - $P < 0.01$ as compared to total group

+ - $P < 0.01$ as compared to non-anxious subgroup

Table 4

Selection experiment according to the exploratory activity of rats in an elevated plus-maze. Results are expressed as a mean ± SEM.

Group	N of animals	Latency of first open part entry	N of open arm sectors crossed	Total time spent in open arms
Total	39	36 ± 10	9.4 ± 0.6	50 ± 4
Non-anxious subgroup	5	7 ± 1^{x}	14.6 ± 0.4^{xx}	79 ± 7^{xx}
Anxious subgroup	5	102 ± 23^{x+}	3.2 ± 1.0^{xx+}	13 ± 5^{xx+}

x - $P < 0.05$ as compared to total group

xx - $P < 0.01$ as compared to total group

+ - $P < 0.01$ as compared to non-anxious subgroup

Table 5

Scathard analysis of saturation data of ^{3}H-flunitrazepam binding in mice selected according to their exploratory activity in an elevated plus-maze. Each value is the mean ±SEM of three separate experiments.

Animal group	^{3}H-flunitrazepam binding B_{max} (fmol/mg protein)	K_D (nM)
	C E R E B R A L C O R T E X	
Non-anxious	1410 ± 90	1.41 ± 0.12
Anxious	1070 ± 70[x]	1.46 ± 0.18
	C E R E B E L L U M	
Non-anxious	980 ± 60	1.51 ± 0.28
Anxious	890 ± 90	1.43 ± 0.24

x - $P < 0.05$ as compared to non-anxious subgroup

Table 6

Scatchard analysis of saturation data of ^{3}H-flunitrazepam binding in rats selected according to their exploratory activity in an elevated plus-maze. Each value is the mean ±SEM of three separate experiments.

Animal group	^{3}H-flunitrazepam binding B_{max} (fmol/mg protein)	K_D (nM)
	C E R E B R A L C O R T E X	
Non-anxious	1290 ± 40	1.55 ± 0.09
Anxious	1100 ± 30[x]	1.54 ± 0.11
	CEREBELLUM	
Non-anxious	920 ± 40	1.27 ± 0.19
Anxious	870 ± 20	1.54 ± 0.21

x - $P < 0.05$ as compared to non-anxious subgroup

tal time spent in open arms, are also acceptable for indication of anxious behaviour, if using non-sedative doses of drug (see Table 1, actometer counts). It seems that this model is more reliable for measuring the action of anxiogenics, because the basal activity of rodents was usually high. However, the positive effect of diazepam on exploratory activity was also observable, confirming the results of Pellow et al, obtained on rats (22).

The anxiogenic effect of DMCM on the exploratory behaviour of mice in the plus-maze was completely abolished by the administration of diazepam in the dose (0.75 mg / kg) without any measurable action by itself. It seems that DMCM and diazepam exert their effect on the exploratory activity in this model through a common site of action, probably central benzodiazepine receptors.

In several works carried out with rodents, animals have been selected according to their distinct spontaneous(3,26, 31) or drug-induced (7,21,27) behaviour. Such selection experiments have given some worthful preliminary information about the neurochemical basis of diversity in animal behaviour (21,26,27,31). It has been suggested that the animals' general level of fear, determined by its past history, will also interact with exploratory behaviour (16). As the variability in the exploratory activity of mice and rats in an elevated plus-maze was quite high we decided to separate the most extremistic individuals into subgroups for the following binding studies. As shown in Tables 3 and 4, it was possible to select group-housed mice and rats according to their more or less "anxious" spontaneous exploratory behaviour in this test. We termed these selected subgroups as non-anxious or anxious, because their spontaneous behavioural characteristics resembled the effects of anxiolytics and anxiogenics respectively.

In vitro ^{3}H-flunitrazepam binding in the cerebral cortex and cerebellum of non-anxious rodents demonstrated similar differences both in mice and rats. Namely, the number of benzodiazepine binding sites was significantly lower in cerebral cortex but not in the cerebellum of anxious animals (Tables 5 and 6). No reliable differences in the binding affinity were found between subgroups.

The present finding that the rodents which display

re anxious behaviour in plus-maze have lower benzodiazepine receptor density in cerebral cortex might be interpreted in two ways. First, if the exposition to stressful events would decrease ^{3}H-flunitrazepam binding as proposed by Medina et al (18), decreased exploratory activity and lower benzodiazepine receptor density in cerebral cortex have to be regarded as the reflection of the reaction to an acute environmental change. However, there is also evidence that acute stress situation may cause rapid increase in brain benzodiazepine receptor binding (19) and enhance the activity of chloride ion channel function (15,29). Therefore,we prefer another possible interpretation. It seems that the neurochemical correlates of behavioural differences in the anxiety - measuring model used reflect the individual trait to have a lowered threshold for the induction of fear. Such an individual trait might be either formed in social interactions or determined genetically or is,most probably,influenced by both named factors. There is no sufficient data available to make decisions about the genetic aspects of the observed problem yet. At the same time, several works indicate that durable lifetime stressors cause naurochemical alterations. Chronic administration of anxiogenic β-carbolines induced long-lasting proconflict effect in rats(6), and decreased the density of low-affinity GABA receptors (probably coupled with benzodiazepine receptors) in different rat brain areas (4). Furthermore, chronic psychogenic stress suppressed the exploratory activity of rats in open field and decreased the density of benzodiazepine receptors in cerebral cortex (10). We have recently demonstrated that the mice, more sensitive to the sedative action of baclofen, a $GABA_B$ receptor agonist, were also more sensitive to the sedative action of diazepam and had a lower density of benzodiazepine receptors in the forebrain (27). Baclofen-responders displayed more anxious behaviour in an elevated plus-maze than baclofen-nonresponders (our unpublished data). It seems that social interactions in the animal populations were responsible for these behavioural and neurochemical differences, because separation of baclofen-responders and baclofen-nonresponders into distinct cages abolished all differences between subgroups. However, the real origin of distinct exploratory behaviour in the plus - maze model remains to be clarified in further experiments, so as

the possibility to use animal selection in plus-maze for detecting trait anxiety.

In some conditions stress situations do not influence basal ^{3}H-flunitrazepam binding but the potency of chloride ions to enhance it (15). As our binding experiments were performed in TRIS-HCl buffer there is a possibility that our results do reflect not the differences in the benzodiazepine receptor characteristics of behaviourally selected animals but the differences in the chloride channel function. For elucidation of the role of the GABA/benzodiazepine/Cl^- ionophore receptor complex in the anxiety trait more complete studies are required.

CONCLUSIONS

1. It is possible to use an elevated plus-maze as an exploratory behaviour model of anxiety in mice. This model is more reliable than a trivial open field test.

2. Mice and rats, divided according to their exploratory activity into anxious and non-anxious subgroups, have distinct benzodiazepine receptor binding properties in cerebral cortex.

3. The possibility of creation of trait anxiety models using animal selection procedures in exploratory behaviour paradigms is suggested.

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THE ADAPTATION PROBLEM IN PHARMACOLOGY

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Abstract:

As every drug affects normal (or pathological) homeostasis certain adaptive-compensatory reactions of varying intensity develop practically to any drug administered. The adaptive-compensatory reactions can be observed on molecular-cellular and on systematic levels. These reactions play a certain role in formation of drug action, in development of adverse reactions and abstinence or withdrawal syndrome. In consequence, the drug action is a complex of immediate functional changes developed as a result of drug-receptor interaction and of adaptive-compensatory reactions aimed at neutralizing them.

The investigation of these reactions is of great importance in assay of new drugs. These reactions must also be considered in the course of determination of rational drugs, therapeutic regimens and tactics.

Adaptation to changing external and internal factors is an important property of living matter. It is a factor determining the persistence and evolution of Life.

In the course of phylogenesis all species have developed most effective machanisms, protecting them against various factors affecting homeostasis. Drugs (xenobiotics) are potent homeostasis affecting agents. A drug (remedium or poison) is defined as a substance, which, if introduced, changes the functions of a living organism, i.e. affects the physiologic (or pathologic) homeostasis. Several defence mechanisms are activated in order to guarantee the etabili-

ty of internal medium. These mechanisms are pointed at the delivery of the organism from drug and at the recovery of the affected functions.

The adaptive reactions of the organism to foreign substances. The reactions aimed at the elimination of foreign substances are as follows:

- Biotransformation: transformation of a lipid soluble substance into water-soluble matabolites, which can be easily eliminated. This transformation is carried out by specific or nonspecific enzyme systems, such as microsomal monooxygenase system containing cytochrome P_{450}, several transferases (glucuronyl transferase, acetyltransferase)etc. (14,18).

- Immunological defence: specific binding of the xenobiotic to antibodies or to complementary binding sites. The foreign substance itself seldom acts as an antigene, usually the drug complex or its metabolite with plasma or tissue proteins act as antigenes. The formed antigene-antibody complexes poorly penetrate into tissues, they do not react with receptors and are finally transformed and excreted (11, 12). As the formation of antibodies needs some time, this compensatory mechanism works especially in case of repeated exposure to drugs.

- The excretion of a foreign substance or its water soluble metabolites via kidneys, gut or other ways.

The reactions aimed at the recovery of the affected functions are carried out by the common homeostasis maintaining mechanisms, functioning on the molecular, cellular, organic or systemic levels. On the molecular level the most widely investigated reactions are the receptor sensitivity and/or the endogenic ligand turnover changes as the adaptation to receptor agonists or antagonists. So, the receptor stimulation by an agonist is followed by an adaptive inhibition of ligand turnover and a decrease of receptor sensitivity (cholinomimetics, dopaminomimetics) (1, 9, 15). The receptor blockade by an antagonist is followed by the enhancement of ligand turnover and the hypersensitivity of the receptors (neuroleptics, cholinergic, adrenergic, histaminergic, etc. blocking agents (5,6,7,10, 15,16)). The enhancement of ligand release (indirect acting adrenomimetics) lowers the receptor sensitivity, the decrease of ligand re-

lease (antiadrenergic drugs) is followed by the receptor hypersensitivity (1,3). These reactions are realized mainly by physiologic feed-back mechanisms.

On systemic level, the homeostasis is maintained by the common reciprocal regulatory mechanisms. A majority of the functions are regulated by at least two contrary acting mechanisms. Such regulating systems as sympathetic and parasympathetic nervous systems, sleep-alertness, nociceptive and antinociceptive, pressor and depressor, coagulating and anti - coagulating systems, the hypothalamic liberins and statins , enzyme activators and inhibitors, etc. are well known. In case a drug causes a functional shift in one or another direction, immediately the opposite mechanisms are activated . The drug action can be neutralized also by feedback mechanisms working on different levels. The adaptive-compensatory reactions are well demonstrated in the endocrine system - a prolonged administration of any hormone or its synthetic analogue depresses the secretion of the endocrine gland (corticosteroids, thyroxine, sexual hormones, etc.). It is based on the inhibition of hypophysar tropines or on the hypothalamic liberins' release. A long-term administration of anticoagulants is finally followed by an increase in blood coagulation, and vice versa. Several drugs which are metabolized by microsomal enzymes, cause the enzyme induction (14). This enhances the metabolism of the drug used as well as that of many other drugs. The use of hypotensive drugs having different mechanisms of action can be followed by the compensatory activation of pressor mechanisms, as e.g., the use of hypertensive drugs with their opposites. The changes in body fluid volume or osmotic pressure cause compensatory changes in the activity of the renin-angiotensine system or vasopressin.

The role of adaptive-compensatory reactions in formation of drug action and adverse reactions. The adaptive-compen - satory reactions developing practically to any drug administred, modify their action. It can be postulated that drug action is a complex of the immediate functional changes developed as a result of the drug-receptor interaction and of the adaptive-compensatory reactions aimed at neutralizing them. The role of these components can be different depend - ing on a drug, its dose, duration of administration and on the peculiarities of the organism. Usually, the effect of

the adaptive-compensatory reactions is not of great importance, but in some cases it is decisive. For instance, the osmotic diuretics changing the plasma osmotic parameters caused a compensatory increase in the diuresis. The adaptive-compensatory reactions can be the factors terminating the drug action as in the cases of ethanol (2) and barbiturates (17). It is shown that the recovery after these drugs occurs at much higher drug blood levels than at the onset of the actions. The adaptive reactions play an important part in the development of tolerance to many different drugs (neuroleptics, tranquillizers, barbiturates, opiates, etc.) (2, 4, 17). The tolerance does not diminish the drug effectiveness, but also complies to the correct dosage and drug schedule. Adaptation to drug action can be the main mechanism of adverse reactions (the tardive dyscinesia during neuroleptic therapy) (13). The adaptive-compensatory reactions are of determining importance in the development of the abstinence-syndrome in drug addicts and in the withdrawal symptoms in the case of an abrupt termination of long-lasting cures. It is well known that the abstinence syndrome developed in alcohol, morphine, cocaine, barbiturate, amphetamine addicts is generally opposite to the main actions of the drugs used (2, 19), it points significantly at its compensatory character. The interruption or termination of a long-lasting drug cure can be followed by certain withdrawal reactions. In this case activation of the primarily depressed symptoms or the occurrence of the opposite effects can be observed. Such syndroms are, for instance, as follows: the adrenal cortex insufficiency and the activation of inflammation after an abrupt termination of glycocorticoid therapy; hypertension after the administration of hypotensive drugs, stenocardial attacks after use of nitrites; the increase of coagulation and the occurrence of thrombosis after anticoagulant therapy; the activation of psychotic symptomatics after having neuroleptics; increased anxiety after the effect of tranquillizers, nightmares after using barbiturates; increased appetite after administration of anorexigenes; hypovitaminosis after massive doses of vitamins (especially ascorbic acid).

The adaptive-compensatory reactions and the pharmaco-therapeutical tactics. Any pathologic process can be described as a complex consisting of a pathogenetic factor induced

changes in homeostasis (i.e., the pathogenesis) and adaptive compensatory reactions against them (i.e., sanogenesis). The therapy can be pointed either at the depression of the patogenesis or at the enhancement of sanogenesis. In most cases the therapy is aimed at the depression lof the patho - genesis (the contraria contrariis curantur principle), although by itself, the therapy can be ethiotropic, symptomatic or pathogenetic. The drug action is the opposite to the influence of the pathogenetic factor, and thus supporting sanogenesis. The adaptive-compensatory reactions to the drug action in this case can cause therapeutical complications (1, 5,13) producing several untoward effects.

There can also be a principally different approach to therapy: the drag effect is in some way similar to the action of the pathogenetic factor (the similia similibus curantur principle). The common effect of the pathogenetic factor and of the drug potentiates the adaptive mechanism's activity (i.e., the sanogenesis): in this case the untoward effects are not of such a great importance. There are but few examples of this approach: the analgetic effect of local irritant drugs, the immunostimulant effects of "biogenic-stimulants", enzyme induction by phenobarbital in treatment of the icterus of the newborn, etc. can be explained this way. It is supposed that the therapeutic effectiveness of antidepressant drugs in depression treatment (3,13), also that of neuroleptics and apomorphine in schizophrenia treatment are based on analogous mechanisms (6).

To sum it up, the adaptive-compensatory reactions of various intensity develop practically to any drug. These reactions can be of importance in formation of drug action and in adverse effects occurring during or after drug treatment (tolerance, eide effects, abstinence or withdrawal syndrome). The investigation of these reactions is of great significance in the assay of new drugs. The adaptive-compensatory reactions must also be considered in the course of rational drug choice and determination of therapeutic regimen and tactics.

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CHRONOPHARMACOLOGICAL ASPECTS OF PHENIBUT

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Abstract:

It was shown that there are time-dependent daily variations of pharmacological (behavioural and biochemical) effects of phenibut. The deprimative effects of phenibut on spontaneous locomotor activity, threshold of agression, coordination of movements on rota-rod are notable at 12.00. The nootropic, antihypoxic and anticonvulsant effects are strong at midnight, the antiamnestic effects by the low level of amnestic functions. The efficiency of phenibut is minimal at 18.00 when toxicity is the highest. It can be concluded that the variations of nootropic activity depend on the daily variations of biochemical effects (ratio of DA striatum, concentration of c AMP in blood) of phenibut.

Introduction

Nootropic drugs, such as phenibut, are considered to act on the integrative functions of the central nervous system, learning and memory, to ameliorate the ageing brain processes and organic brain syndromes (1). An elevation of the ATP-turnover in the brain and some other processes suggested a possible stimulation of synaptic transmission by the compound. Based on this assumption, experiments were made on phenibut, a centrally acting derivative, employed clinically for about 20 years as a brain activating drug for the treatment of acute and chronic impairment of brain functions (7,8)

Phenibut has certain nootropic (antihypoxic, antitoxic

and antiamnestic) properties. Myorelaxing sedative properties characteristic to tranquillizers have been stated in experiments on animals. Marked tranquillizing effects could be observed in asthenic and alcohol abstinence patients. Phenibut is an agonist of $GABA_B$ receptors giving the increase of dopamine (DA) and DA metabolites in animal brain.

Numerous investigators have demonstrated the presence of diural rhythmic changes in catecholamine and GABA content in the mice brain (2,5,9,10). Rhythmical variation in neurotransmitter contents produce the rhythmical variations of pharmacological effects of phenibut.

The present report makes an attempt to summarize the results of the recent experiments performed in our laboratory to demonstrate the presence of time-dependent daily rhythms in the behavioural and biochemical effects of phenibut.

Material and Methods

Male and female white mice averaging 25 $\pm$ 2 g in weight were used. Four weeks prior to each series of studies the animals were kept in a cage at a nearly constant temperature (20°C). The mice were given food and water ad libidum and they were exposed to 12:12 hr regimen of alterating darkness and artificial light. The light phase ran daily from 07.00 to 19.00. To determine circadian rhythms, the experiments were carried out on groups of mice consisting of 8-10 animals at 06.00, 12.00, 18.00 and 24.00 respectively in winter months. Phenibut (50 and 100 mg/kg) was administered intraperitoneally. The animals were put into experiments 60 min after injection.

For behavioural studies were used: the determination of spontaneous motility was tested by means of a photoelectric actometer, emotionality was determined using the electric-pain test, coordinating activity - using rota-rod and the influence of the drug by rectale temperature (6).

In order to determine the special nootropic activity of phenibut, three possible test procedures were carried out: swimming test at 5° C; antihypoxic effect (the model of hypoxic hypoxia) test; active avoidance responses' test in a classical shuttlebox of lighted and dark sections (4). In se-

parate experiments, the coadministration of phenibut and corazol was used for determining the interactions. The chronotoxicity of phenibut was also studied.

As to the biochemical studies, the concentration of dopamine (DA) and its main metabolite DOPAC were determined spectrofluorimetrically by the method described by Early and Leonard. Finally, a circadian-phase-dependency in cyclic AMP content in the blood of mice was found.

The statistical significance of the differences between the mean values was calculated by the Student's unpaired t-test.

Results and Discussion

In our experiments at least three aspects of the diurnal action of phenibut were studied: the action of intraperitoneal, its administration in case of behavioural effects, specific nootropic effects and biochemical effects on the dopamine content in brain and cyclic AMP content in blood.

In the area of behavioural testing, the spontaneous circadian cycle in untreated mice was compared with the behavioural response to 50 and 100 mg/kg of phenibut I.P. as determined at 6-hour intervals over a 24-hour period under normal lighting conditions, using separate groups of mice at each point (Table 1).

Distinct, apparently circadian rhythms were detected. During control studies, in which saline was injected, the mice showed almost continuous active locomotion during the dark period, but displayed a relatively low and interrupted activity during the light period. Phenibut injection induced the sedation of mice, which depended on the time of administration. The ratio of locomotion depression between each time period was 4:1. At 12.00 the effect exceeded 5 times that of the diurnal mean, being minimal at 18.00.

Table 2 indicates a similar decrease of the effects of rectal temperature of mice under the phenibut administration. Despite the decreased spontaneous locomotion, the phenibut-treated mice showed increased coordination and strength on the rota-rod and swimming test by 5° C.

The rate of appearance of small anxielytic, tranquilizer effect of phenibut at different time moments was not

significant. However, the increased pain sensitivity was correlated with the increased aggressiveness of mice (Table 1).

Table 2 shows the difference in the duration of life of mice by corazol-induced convulsions during a 24-h period. After injection, especially at 00.00 and during the whole dark period, the effect of phenibut remains high. At 00.00 all the mice remained alive.

Clinical feedback caused us to examine the diurnal variation of protective effects on animal brain. Hypoxic hypoxia model was used. As expected, the antihypoxic effect was parallel to the lowering of rectal temperature.

In both cases the effect was maximal after injection at 00.00 during the dark period.

An avoidance-learned response was recorded when the mouse moved to the other side of the shuttle-box during the 5-sec warning period preceding the shock.

During the initial learning phase, the animals were submitted daily to one session in the shuttle-box in morning at 08.00 and another session in the afternoon at 19.00. In the case of nontreated mice, the memory processes were better at 19.00 and worse at 08.00. Phenibut seemed to be active when memory processes were impaired at 08.00. The differences between treatment were significant in the 100mg/kg phenibut-treated groups at 08.00. The duration of being in dark section was significantly lower. This may depend on the higher pain sensitivity of animals.

After a few preliminary trials we decided to take a phenibut dose of 850 mg/kg i.p. for the determination of toxicity. Lethality was the highest at 18.00 (Table 2). The chronotoxicity (or chronopathology) defined as the rhythmic variations in the toxic effects and lethality of animals depends on phase differences in rhythms of 24 hours, e.g., in metabolic pathways and processes at various hierarchies of biological organization. Relatively high motility, heart frequence and diuresis, the activity of certain hepatic drug-metabolizing enzymes occur during the dark period. It is possible that these circumstances play a certain rôle in the low toxicity of phenibut during the night time. The data in the table reveal that the anticonvulsant effects of phenibut after corazol administration are higher during the dark period at about 00.00.

Table 1

Time Course of Phenibut (50 mg/kg) Effects (mean±SEM group of 10 mice) 60 min after i.p. Administration. Data are shown for daylight in 7 am - 7 pm.

x $P \leq 0.05$

Hour	Dosis mg/kg	Spontaneous activity during 30 min	Number of arising (investigation)	Threshold of agression (volts)	Rota-rod (sec)	Continuance of swimming at 5°C (sec)
1	2	3	4	5	6	7
06.00	C	401.3 ± 47.4	14.6 ± 3.4	32.5 ± 1.4	247 ± 53	108.4 ± 18.1
	50	227.0 ± 43.1^{x}	14.0 ± 1.9	41.2 ± 1.3^{x}	268 ± 32	131.0 ± 13.5
12.00	C	402.3 ± 76.3	16.4 ± 2.7	35.0 ± 0	251 ± 49	74.0 ± 15.1
	50	73.0 ± 23.5^{x}	0.4 ± 0.1^{x}	23.7 ± 1.2	132 ± 68	154.0 ± 7.4^{x}
18.00	C	328.9 ± 68.3	8.0 ± 1.7	30.0 ± 0	167 ± 62	111.1 ± 7.8
	50	319.7 ± 64.0	7.0 ± 2.2	32.5 ± 1.4	300 ± 0^{x}	89.1 ± 4.2
24.00	C	248.3 ± 33.4	24.0 ± 1.8	25.0 ± 2.9	246 ± 54	136.4 ± 19.5
	50	147.3 ± 27.2	9.5 ± 1.4^{x}	22.5 ± 1.4	160 ± 57	172.0 ± 24.0
Diurnal		345.0 ± 56.4	15.8 ± 2.4	30.6 ± 2.1	227 ± 54	107 ± 15
Mean		197.8 ± 39.5^{x}	7.8 ± 1.4^{x}	30.0 ± 4.4	215 ± 39	157 ± 12

Table 2

Time course of phenibut (50,100 mg/kg) effects (mean±SEM group of 10 mice) 60 min after i.p. administration. x $P \leq 0.05$.

Hour	Dose mg/kg	Rectal temperature	Duration of life: in the model of hypoxic hypoxia	Duration of life: by coramine-induced convulsions	cAMP mol/ml of blood	Lethality % of group n=15 after 850 mg/kg
0600	C	33.4±0.3x	471±51	217± 72	75.4± 8.0	10
	50	27.8±0.7x			93.6±14.0	
	100		775±22x	1134±162	106.0± 4.6	
1200	C	33.0±0.5	450±33	600±191	95.0±12.0	
	50	30.3±0.5			77.9± 8.5	0
	100		824±73	1000±325	119.2±11.1	
1800	C	31.2±0.4	369±32	484±218	87.6± 8.3	
	50	29.0±0.4			74.0± 5.2	
	100		726±98x	1126±328	90.1± 6.1	85
2400	C	31.6±0.3	426±28	600±170	72.0± 7.1	
	50	28.7±0.4x			66.5± 9.2	25
	100		903±116x	all alivex	68.0±4.6	
Diurnal Mean	C	32.3±0.3	429±36	475±162	85.3±8.8	
	50	28.9±0.5x			78.0±9.4	22.5
	100		807±62		93.3±6.6	

Table 3

Time course of phenibut effects 60 min after i.p. administration. x $P \leq 0.05$

Hour	Dose mg/kg	An avoidance-learned response: Latence of the 1st entering the dark side	An avoidance-learned response: Total time while away in the dark side (sec)	DA g in striatal slice	DOPAC g
08.00	C	91±16	129±13	5.66±0.1	0.6±0.07
	50	135±15	84±7	9.57±0.8x	0.7±0.08
	100	239±30xx	41±2		
19.00	C	144±20	69±1	4.32±0.6	0.5±0.09
	50	193±17	45±3x	11.4 ±0.9x	0.4±0.04

Previous research concerning the pharmacological profile of phenibut has demonstrated that in the case of mice acute administration enables to increase the brain levels of dopamine and of its major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) which both are reliable indicators of intraneuronal dopamine synthesis (7).

A marked phenibut-induced rhythm could be observed in the levels of dopamine and DOPAC rodent striatal contents within a 24-hour period. The mean values indicate that the content is higher in the mornings and lower at daytime. A comparative phasing of their rhythms and those of typical motor activity of rodents reveals that the maximum phenibut induced DA levels correspond to the animals' activity, high pain sensitivity and aggressive behaviour. The striatal content of DA seems to be inversely proportional to the phenibut (850 mg/kg) induced lethality of mice. During daytime, the lethality was significantly lower than the diurnal mean.

Previous studies have demonstrated the increase of the AMP content under some psychoterapic agents. The increase of the cyclic AMP content could be induced by the blockade of GABA receptors or by the inhibition of GABA synthesis (3).

On the other hand, phenibut was capable to modify the cyclic adenosine monophosphate (cAMP) content in blood. In line with these data, behavioural studies have evidenced that over compound phenibut increase motor activity during morning period when the content of cAMP is high.

Conclusion

It may be said in conclusion that the central behavioural and some physiological effects of phenibut can be explained by its action upon the daily varying concentration and the turnover of neurotransmitters (including DA) and it may likewise be related to its action on biochemical effects in blood. During morning hours when the synthesis of DA and GABA has increased, may occur the increase of the activating effects of phenibut on the memory processes and the effects of depressive agents may diminish.

At the end of the light period at 18.00 the inhibiting effects of phenibut prevail. The phenibut-induced toxicity

was maximal and antihypoxic effects were minimal.

Any drug, e.g. phenibut, is capable of producing different effects depending on its biological timing.

Several circadian metabolic rhythms at cellular level are synchronized by the circadian pattern of neurotransmitters' secretion functioning as a pacemaker. It is clear that those numerous pharmacokinetic, pharmacodynamic and endogenous neurobiological changes have contributed to the circadian alteration in the effects of phenibut. Daily changes in the phenibut action may have certain clinical implication.

Attempts have already been made to chrono-optimize the use of pharmacologic agents. For example, if patients, like rats, are most susceptible to the effects of phenibut (beneficial or toxic) when asleep this pattern suggests daily dosing in the mornings rather than at bedtime, as is a common clinical practice now. Such timing may be additionally beneficial if coupled with smaller doses of nootropics than normally used, provided that they are clinically effective.

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ПЕРИФЕРИЧЕСКИЕ МЕСТА СВЯЗЫВАНИЯ БЕНЗОДИАЗЕПИНОВ: ФУНКЦИОНАЛЬНЫЕ РЕЦЕПТОРЫ ИЛИ АКЦЕПТОРЫ

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Р е з ю м е

Связывающее место бензодиазепинов (БД) центрального типа, часто называемое также бензодиазепиновым рецепторов, является интегральным звеном комплекса рецептора $ГАМК_А$-хлорного канала в мозге млекопитающих, через которое бензодиазепины и небензодиазепиновые вещества позитивно или негативно аллостерически модулируют функции хлорного канала со всеми последующими характерными фармакологическими эффектами. Пока БД рецепторы не найдены вне ЦНС. В противоположность центральным БД рецепторам места связывания БД периферического типа широко распространены во многих органах и тканях, а также в ЦНС. Несмотря на то, что периферические места связывания БД известны уже десять лет, главное внимание исследователей было сосредоточено, в основном, на центральных местах связывания БД. Однако в последние годы достигнут определенный успех в выяснении множества функций и свойств периферических мест связывания БД. В обзоре описано распределение, развитие, физиологическая регуляция и функции, а также проблема существования возможных эндогенных и экзогенных лигандов периферических мест связывания БД. В свете этих данных обсуждается принадлежность этих мест связывания к функциональным рецепторам.

БИОХИМИЧЕСКАЯ И ПОВЕДЕНЧЕСКАЯ ХАРАКТЕРИСТИКА ИНТАКТНЫХ И КОНТАКТНЫХ КРЫС

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Р е з ю м е

Хроническое стрессирование животных (т.е. контактные крысы) проводилось путем привыкания их к манипуляциям в условиях, предшествующих декапитации в течение 10-12 дней. Интактных крыс держали при стабильных условиях вивария до дня опыта. Хроническое стрессирование вызывало уменьшение плотности мест связывания ^3H-флунитразепама во фронтальной коре,

почках и надпочечниках, уменьшение количества $ГАМК_A$-рецепторов в коре мозга и гиппокампе. Уменьшалась также плотность мест связывания ^3H-спиперона во фронтальной коре и плотность мест связывания ^3H-спиперона и ^3H-дипренорфина в мезолимбических структурах. Изменения наблюдались также в концентрации диеновых коньюгатов во фронтальной коре и соматотропного гормона в крови. В модели поднятого крестообразного лабиринта контактные крысы оказывались более тревожными по параметрам исследовательской активности и дефекации. Таким образом, наблюдаемые различия в поведении и биохимических параметрах между контактными и интактными животными указывают на более выраженное стрессовое состояние контактных крыс.

ИЗМЕНЕНИЯ В РЕЦЕПТОРАХ ГАМК И БЕНЗОДИАЗЕПИНОВ ПОСЛЕ ЭЛЕКТРОБОЛЕВОГО РАЗДРАЖЕНИЯ У КРЫС - ВЛИЯНИЕ ДИАЗЕПАМА

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Р е з ю м е

В качестве однократного стрессового воздействия использовалась модель электроболевого раздражения в течение 3 мин непосредственно перед декапитацией животных. На основании опытов связывания выяснилось, что электроболевое раздражение вызывало уменьшение плотности мест связывания ^3H-флунитразепама и ^3H-мусцимола в коре головного мозга и гиппокампе. Уменьшалась и плотность периферических мест связывания бензодиазепинов в почках и надпочечниках. При этом одинаковое уменьшение наблюдалось у интактных крыс и у крыс, хронически привыкающих к манипуляциям в условиях, предшествующих декапитации. Таким образом, хроническое стрессирование крыс не влияло на действие однократного стрессового воздействия на рецепторном уровне. Предварительное введение диазепама (2,5 мг/кг) в наших опытах не устраняло эффект электроболевого раздражения на рецепторы ГАМК и бензодиазепинов.

ФУНКЦИОНАЛЬНАЯ РОЛЬ ДОФАМИНОВЫХ Д1 РЕЦЕПТОРОВ В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ

К.Черешка, А.Жарковский

Р е з ю м е

В опытах радиолигандного связывания с мембранами мозга крыс изучалось связывание избирательного агониста Д1-рецепторов ^{3}H-СКФ 38393. Установлено, что связывание ^{3}H-СКФ 38393 зависит от состава инкубационной среды и температуры инкубации. Наибольшее отношение специфического связывания к неспецифическому было достигнуто в фосфатном буфере, если инкубация проводилась при 0^{o}C. Анализ связывания в координатах Скэтчарда показал, что ^{3}H-СКФ 38393 связывается с гомогенной популяцией рецепторов с плотностью 384 $\pm$ 43 фмоль/мг и $K_{Д}$=5,8 $\pm$ I,0 нМ. Регинональное распределение в переднем мозге крыс Д1-рецепторов, меченых ^{3}H-СКФ 38393, соответствовало распределению Д2-рецепторов, меченых ^{3}H-спипероном. Способность нейролептиков угнетать связывание ^{3}H-СКФ 38393 и ^{3}H-спиперона сочиталась с их более выраженной способностью вызывать каталепсию. Возможно, каталептогенное действие нейролептиков связано с блокадой обоих типов дофаминовых рецепторов Д1 и Д2.

УМЕНЬШЕНИЕ СВЯЗЫВАНИЯ ^{3}H-СПИПЕРОНА IN VIVO В МОЗГЕ МЫШЕЙ ПОСЛЕ ХРОНИЧЕСКОГО ПРИМЕНЕНИЯ ГАЛОПЕРИДОЛА

А.Жарковский, К.Черешка

Р е з ю м е

В опытах на мышах введение галоперидола в дозе 2,5 мг/кг в/бр приводило к ослаблению угнетающего действия стимулятора дофаминовых рецепторов апоморфина (0,I мг/кг) на локомоцию в течение 2-7 дней после отмены галоперидола. Напротив, синдром вертикализации, вызываемый апоморфином в дозе I мг/кг, был усилен, что свидетельствовало о повышении чувствительности постсинаптических дофаминовых рецепторов после отмены галоперидола. Несмотря на признаки гиперчувствительности постсинаптических дофаминовых рецепторов, наблюдаемых в поведенческих опытах, у мышей после отмены галоперидола наблюдалось уменьшение связывания ^{3}H-спиперона в стриатуме и мезолимбиче-

ских структурах в условиях in vivo. Снижение связывания было обнаружено и после однократного введения галоперидола. На основании этих данных предполагается, что в стадии отмены галоперидол в низких концентрациях избирательно блокирует пресинаптические дофаминовые рецепторы, с чем, по-видимому, связано ослабление пресинаптических эффектов апоморфина и уменьшение связывания ^{3}H-спиперона

ВЛИЯНИЕ МИФ НА ПОВЕДЕНЧЕСКИЕ ЭФФЕКТЫ АГОНИСТОВ И АНТАГОНИСТОВ ДОФАМИНОВЫХ РЕЦЕПТОРОВ

К.Черешка, Г.Цеберс, Т.Ээпик, А.Жарковский

Р е з ю м е

В опытах на крысах изучалось влияние трипептида (пролей-гли, МИФ) на поведенческие и биохимические эффекты агонистов и антагонистов дофаминовых рецепторов. МИФ в дозе I мг/кг (в/бр) усиливал угнетающее влияние избирательного агониста Д2-рецепторов Ly 171555 (0,01 мг/кг, п.к.) на двигательную активность мышей и провокацию зевательных движений у крыс. Напротив, МИФ не влиял на поведенческие эффекты агониста Д1-рецепторов СКФ 38393. Он не влиял также на поведенческие эффекты галоперидола, однако антагонизировал действие сульпирида на двигательную активность. На основании этих данных предполагается, что МИФ избирательно повышает чувствительность пресинаптических дофаминовых рецепторов Д2 типа.

ПАРАМЕТРЫ ХОЛЕЦИСТОКИНИНОВЫХ (ХЦК-8) РЕЦЕПТОРОВ В РАЗЛИЧНЫХ СТРУКТУРАХ МОЗГА КРЫС

А.Соосаар, Э.Васар, А.Ланг

Р е з ю м е

Для изучения показателей холецистокининовых (ХЦК-8) рецепторов были проведены опыты связывания с ^{3}H-ХЦК-8 в различных структурах мозга крыс. В опытах ассоциации связывание ^{3}H-ХЦК-8 достигало равновесия в течение 60-75 мин при 23°С и было стабильным в течение 4 часов. Диссоциирование ^{3}H-ХЦК-8 в переднем мозге носило двухфазный характер. Преинкубация мембран мозга при 37°С ликвидировала более аффинные места связы-

вания. Важно отметить, что преинкубация мембран при 37°С не изменяла плотность ХЦК-8 рецепторов, а понижала в 1,5-2 раза их аффинность. Число мест связывания ХЦК-8 было наивысшим в мезолимбических структурах, несколько меньше в стриатуме и во фронтальной коре и самым низким - в стволе мозга. Константы полуингибирования разных лигандов ХЦК-8 рецепторов были весьма одинаковыми в различных структурах мозга, различаясь только для ХЦК-4 и проглумида, антагониста ХЦК-8 рецепторов. При них константы полуингибирования различались 3-4 раза. Преинкубация мембран при 37°С не изменяла аффинность ХЦК-8 рецепторов для церулеина, аналога ХЦК-8, однако повышала аффинность для тифлуадома, антагониста ХЦК-8 рецепторов, в 1,5-2 раза. Длительное введение галоперидола (0,5 мг/кг в день, в течение 15 дней) подопытным животным не привело к статистически достоверным изменениям в параметрах ХЦК-8 рецепторов. Проведенный анализ свидетельствует о том, что параметры ХЦК-8 рецепторов в различных структурах мозга весьма разные. Эти места связывания отличаются как по аффинности к разным лигандам ХЦК-8 рецепторов, так и по плотности в различных структурах мозга.

ВЗАИМОДЕЙСТВИЕ МЕЖДУ НЕЙРОЛЕПТИКОМ И ХОЛЕЦИСТОКИНИНОМ: УЧАСТИЕ НАТРИЕВОГО НАСОСА

Э.Васар, М.Цильмер, А.Соосаар

Р е з ю м е

В опытах на белых крысах-самцах было установлено, что церулеин (10 мкг/кг), агонист ХЦК-8 рецепторов, и проглумид (1 мг/кг), антагонист ХЦК-8, уменьшают активность натриевого насоса в ралзичных структурах мозга. Одновременно применение церулеина и проглумида полностью подавляло действие обоих веществ. Острое введение галоперидола (0,5 мг/кг) уменьшало активность натриевого насоса, однако после длительного введения (в течение 15 дней) наблюдалось заметное усиление подавляющего действия галоперидола. Острое или длительное введение проглумида полностью антагонизировало действие многократных введений галоперидола. В опытах радиолигандного связывания совместное длительное введение галоперидола и проглумида устраняло влияние галоперидола на связывание ^{3}Н-ХЦК-8 в различных структурах переднего мозга. По всей вероятности,

пониженная активность натриевого насоса, а также уменьшение кооперативности ионов натрия к натриевому насосу находятся в основе развития деполяризационной блокады дофаминовых нейронов при введении агонистов ХЦК-8 и нейролептических веществ.

ПОДНЯТЫЙ КРЕСТООБРАЗНЫЙ ЛАБИРИНТ КАК ИНСТРУМЕНТ ДЛЯ ИССЛЕДОВАНИЯ СОСТОЯНИЯ ТРЕВОГИ И СТРАХА НА ГРЫЗУНАХ

Я.Харро, Р.-А.Кийвет, М.Пыльд, Л.Ряго

Р е з ю м е

ДМСМ дозозависимо уменьшал поведенческую активность мышей в поднятом крестообразном лабиринте. Диазепам обладал противоположным действием. В модели открытого поля оба вещества были эффективны только в дозах, нарушающих базальную двигательную активность.

На основании спонтанной более или менее тревожной исследовательской активности в поднятом крестообразном лабиринте животные были разделены на две группы. Показано, что у мышей и крыс с более тревожным поведением связывание ^{3}H-флунитразепама с бензодиазепиновыми рецепторами в коре больших полушарий было более низким, чем у животных с менее тревожным поведением. Различий в связывании ^{3}H-флунитразепама в мозжечке обеих групп обнаружено не было. Обсуждается вопрос о возможности использования экспериментов селектирования грызунов в моделях исследовательской активности для выявления животных с тревожным состоянием и изучения нейрохимических основ этого явления.

ПРОБЛЕМА АДАПТАЦИИ В ФАРМАКОЛОГИИ

Л.Нурманд

Р е з ю м е

Показано, что адаптивно-компенсаторные реакции возникают на разных уровнях регуляции практически к любому лекарственному веществу.

На молекулярном-клеточном уровне из подобных реакций наиболее изучены изменения чувствительности рецепторов и/или

скорости оборота лиганда в ответ на воздействие агонистами или антагонистами. На системном уровне к этим реакциям можно отнести индукцию ферментов, компенсаторную активацию противоположно действующих систем, подавление секреции гормонов в ответ на введение экзогенного гормона и т.д.

Все эти реакции нередко имеют решающую роль в проявлении и продолжительности действия, а также в возникновении побочных реакции и синдрома абстиненции или отмены. Проявление действия любого лекарственного вещества складывается из непосредственных функциональных сдвигов, возникающих в результате взаимодействия вещества с рецептором, и адаптивно-компенсаторных реакции организма на эти сдвиги.

Изучение этих реакций особенно важно при оценке действия новых лекарственных веществ. Учитывание этих реакций имеет также большое значение в формировании мышления врача, при выборе рациональных лекарств, тактики и схемы лечения.

ХРОНОФАРМАКОЛОГИЧЕСКИЕ АСПЕКТЫ ДЕЙСТВИЯ ФЕНИБУТА

М. Оттер

Р е з ю м е

В опытах на белых мышах, проводимых 4 раза в день (06.00, 12.00, 18.00 и 24.00) было выяснено, что в фармакологических, биохимических и поведенческих эффектах фенибута появляются суточные вариации. В течение суток депримирующие эффекты фенибута на определенные параметры (исследовательская активность, порог агрессивности, координация движений) сильнее выражены в полдень (12.00). Что касается ноотропных эффектов, то антигипоксический и противосудорожный эффекты сильнее в полночь (24.00), а антиамнестический эффект - при низком исходном уровне мнестических способностей. Отрицательные стороны действия фенибута, уменьшение продолжительности плавания в воде при 5°С и летальный эффект самые выраженные в 18.00. Определенную роль в изменениях эффектов фенибута играют суточные колебания содержания ДА в стриатуме и цАМФ в крови.

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