DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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NEUROPHARMACOLOGY OF ATYPICAL ANTIPSYCHOTICS AND AN ANIMAL MODEL OF PSYCHOSIS

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LIST OF ORIGINAL PUBLICATIONS

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I **Rudissaar R**, Pruus K, Skrebuhhova T, Allikmets L, Matto V (1999) Modulatory role of 5-HT_3 receptors in mediation of apomorphine-induced aggressive behaviour in male rats. Behav Brain Res 106:91–96

II Pruus K, Skrebuhhova-Malmros T, **Rudissaar R**, Matto V, Allikmets L (2000) 5-HT_{1A} receptor agonists buspirone and gepirone attenuate apomorphine-induced aggressive behaviour in adult male Wistar rats. J Physiol Pharmacol 51:833–846

III Skrebuhhova-Malmros T, Pruus K, **Rudissaar R**, Allikmets L, Matto V (2000) The serotonin 5-HT_{2A} receptor subtype does not mediate apomorphine-induced aggressive behaviour in male Wistar rats. Pharmacol Biochem Behav 67:339–343

IV Matto V, Vaarmann A, **Rudissaar R**, Pruus K, Skrebuhhova-Malmros T, Allikmets L (2000) Apomorphine-induced aggressive behaviour and post-mortem monoamine content in male Wistar rats. Neurosci Lett 289:131–134

V Pruus K, **Rudissaar R**, Skrebuhhova-Malmros T, Allikmets L, Matto V (2000) Development of apomorphine-induced aggressive behaviour: comparison of adult male and female Wistar rats. Meth Find Exp Clin Pharmacol 22:47–50

VI **Rudissaar R**, Pruus K, Vaarmann A, Pannel P, Skrebuhhova-Malmros T, Allikmets L, Matto V (2001) Acute trazodone and quipazine treatment attenuates apomorphine-induced aggressive behaviour in male rats without major impact on emotional behaviour or monoamine content post mortem. Pharmacol Res 43:349–358

VII **Rudissaar R**, Pruus K, Allikmets L, Harro J (2006) The role of NMDA and 5-HT_{2A} receptors in the effects of second generation antipsychotics: interactions with MK-801 and DOI. Pharmacol Res (submitted)

Author's contribution

Paper I: Performed around half of the experiments. Participated in study design and writing of the manuscript.

Paper II: Performed around half of the experiments. Participated in study design and writing of the manuscript.

Paper III: Performed around half of the experiments. Participated in study design and writing of the manuscript.

Paper IV: Performed around half of the experiments. Participated in study design and writing of the manuscript.

Paper V: Performed around half of the experiments. Participated in study design and writing of the manuscript.

Paper VI: Performed around half of the experiments. Participated in study design and writing of the manuscript.

Paper VII: Main person responsible for writing. Performed around half of the experimental work and all calculations.

ABBREVIATIONS

[^{35}S]GTPγS	guanosine-5'-(γ-thio)-triphosphate
1-PBG	1-phenylbiguanide
5-HIAA	5-hydroxyindole-3-acetic acid
5-HT	5-hydroxytryptamine; serotonin
5-HTP	5-hydroxytryptamine
8-OH-DPAT	(±)-8-hydroxy-2-dipropylaminotetralin hydrobromide
ANOVA	analysis of variance
CNS	central nervous system
DOI	[±]-2,5-dimethoxy-4-iodoamphetamine
DOPAC	3,4-dihydroxyphenylacetic acid
DSP-4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride
GABA	γ-aminobutyric acid
GDP	guanosine diphosphate sodium
HPLC-ECD	high perfomance liquid chromatography with electrochemical dedection
HVA	homovanillic acid
i.p.	intraperitoneal(ly)
ICS-205930	3-tropanyl-tropanyl-indole-3-carboxylate HCl; tropisetron
MAO	monoamine oxidase
mCPBG	1-(m-chlorophenyl)-biguanide
MDL-72222	3-tropanyl-3,5-dichlorobenzoate
MK-801	(5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5–10-imine maleate; dizolcipine
NMDA	N-methyl-D-aspartatic acid
PCP	phencyclidine
s.c.	subcutaneous(ly)
SR 57227A	4-amino-(6-chloro-2-pyridyl)-1-piperidine hydrochloride
SSRI	selective serotonin reuptake inhibitor
TWEEN-85®	polyoxyethylene-(20)-sorbitan oleate
WAY-100635	[N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexane-carboxamide maleate

INTRODUCTION

Compounds having antipsychotic activity belong to a chemically hetereogenous group of drugs and can be divided into two generations — first generation (classical neuroleptics) and second generation (atypical antipsychotics). The blockade of dopamine D_2 receptors is one of the main features of mechanism of action (Allikmets *et al.,* 1984; Assie *et al.,* 2005). But it is clear now that most antipsychotic drugs have significant affinity also for serotonin (5-HT) receptors (Roth *et al.,* 2003), often greater than their affinity to dopamine receptors (Arnt and Skarsfeldt, 1998; Goldstein, 2000). There is growing evidence about anatomical and functional interaction between serotonergic, dopaminergic and glutamatergic systems. For example, 5-HT modulates striatal dopamine outflow in conditions in which dopamine synthesis and/or release are activated or inhibited (Schmidt *et al.,* 1992; Ichikawa *et al.,* 1995). The serotonergic system inhibits dopamine function at the level of the midbrain by reducing the firing of dopamine cells in the substantia nigra, inhibiting the synaptic release of dopamine in striatum and cortex (Kapur and Remington, 1996). Glutamate is also known to contribute to the control of dopamine release in various brain structures (Whitton *et al.,* 1994).

The present study was aimed to extend our understanding of the mechanism of action of atypical antipsychotic drugs. In particular, some of atypical antipsychotics are used as antiaggressive drugs, though with controversial results, but the mechanism of the antiaggressive effects of atypical antipsycotics is unknown.

The main objectives of our study were to characterize the apomorphine aggressiveness model with regard to its behavioural specificity and also underlying neurochemistry. Also we have tried to reveal the role of 5-HT and glutamate receptors in the behavioural effects of atypical antipsychotics.

The serotonergic system is linked to psychotic behaviour and regulation of aggressive behaviour in animals and humans. It has been found that drugs with 5-HT_{1A} agonistic or 5-HT_{2A} antagonistic properties elicit antiaggressive effects in some tests of aggressive behaviour in animals. However, there are controversies in this issue as well, as only a very limited number of studies have been devoted to investigation of the effect of atypical antipsychotics on aggressiveness. In this context the objective of our studies was to investigate effects of various ligands of 5-HT receptors and atypical antipsychotics (quetiapine, ziprasidone, olanzapine, risperidone, sertindole, melperone) on apomorphine-induced aggressiveness. As it is known that classical neuroleptics are very strong in inhibiting apomorphine-elicited aggressive behaviour in rats, we compared the behavioural and biochemical effects of atypical antipsychotics with haloperidol, the etalon compound from the first generation antipsychotics.

REVIEW OF LITERATURE

Mechanism of action of atypical antipsychotics

Antipsychotic drugs have been classified into first generation or typical (classical) and second generation or atypical agents based on their affinities for D_1, D_2 and 5-HT_2 receptors (Meltzer *et al.,* 1989) and their different neurochemical, pharmacological and clinical properties.

Much effort has been made to reveal the neurochemical basis of antipsychotic action, but it can be claimed that the precise mechanism of action that accounts for the effects of antipsychotic medications is still unknown. There are certain mechanisms that undoubtedly contribute to this action. The dopamine hypothesis is the predominate theory used to explain the action of these drugs. There are two core components to the dopamine theory: (1) psychosis is induced by increased levels of dopamine activity and (2) most antipsychotic drugs block postsynaptic dopamine receptors (Kontkanen, 2002). Early pharmacological studies, which classified dopamine receptors into D_1 and D_2 receptors, suggested that the D_2 receptor was most closely associated with the antipsychotic activity and that this action correlated with dopamine receptor occupancy. Typical antipsychotic drugs have a propensity to cause various side effects. In long-term use high striatal D_2 occupancy by haloperidol, as well as by other typical antipsychotic drugs, may lead to the appearance of extrapyramidal symptoms, such as rigidity, parkinsonism and tardive dyskinesia. Other side effects of typical antipsychotic drugs are related to their affinities to various other neurotransmitter receptors. For example, sedation is caused by blockade of histamine (H_1) receptors, hypotension through α_1-adrenoreceptors, and dry mouth, constipation and blurred vision result from the blockade of muscarinic cholinergic receptors (Stahl, 1996; Tamminga, 1999; Nestler *et al.,* 2001). The side effects and other unwanted properties of typical antipsychotic agents have prompted a search for better-tolerated and more effective antipsychotic drugs.

5-HT-receptor-based mechanisms have been postulated to play a critical role in the action of the new generation of antipsychotic drugs that is usually referred to as atypical antipsychotics because of their ability to achieve an antipsychotic effect with lower rates of extrapyramidal symptoms compared to the first generation antipsychotics such as haloperidol (Meltzer *et al*., 2003). These agents have possibly greater efficacy in reducing negative symptoms and, as a group, they also have a superior effect on cognitive function and greater ability than typical antipsychotic drugs to treat mood symptoms in patients with either schizophrenia or affective disorders.

Meltzer *et al*. (1989) proposed that the potent 5-HT_{2A} receptor antagonism together with weak D_2 receptor antagonism are the principal pharmacologic features that differentiate atypical antipsychotics from typical antipsychotics. 5-HT_{1A} receptor agonism has also been suggested to contribute to the atypical

antipsychotic profile (VanderMaelen and Braselton, 1990; Wadenberg and Ahlenius, 1991). The atypical antipsychotic agents vary in their affinities for other types of serotonin as well as dopamine, muscarinic, glutamatergic, adrenergic, and histaminic receptors, some, or all of which may contribute to their differences in efficacy and side effect profiles (Meltzer, 1999).

Currently available antipychotic drugs alter glutamatergic activity in multiple ways: by enhancing release of glutamate in the striatum, directly interacting with NMDA receptors, altering glutamate receptor density, and changing the subunit composition of glutamate receptors. Many of these effects are regionally selective and vary among the antipsychotics, with important differences emerging between atypical and conventional drugs (Goff and Coyle, 2001).

All clinically effective antipsychotic drugs produce their effects slowly, as a rule after weeks of continuous administration, whereas dopamine and serotonin receptor antagonism is immediate. Long-term antipsychotic drug administration produces altered neuronal and synaptic morphology in animal models (Zharkovsky and Belyakov, 1983; Harrison, 1999; Konradi and Heckers, 2001).

Serotonergic systems

Serotonin synthesis, metabolism and serotonergic pathways in the brain. 5-HT was chemically identified by Rapport *et al.* (1948) as one of the major vasoconstricting substances. Serotonin in CNS forms only 1–2% of its total amount in the body (Bradely, 1989), but the indoleamine can not cross the blood-brain barrier. 5-HT is formed by a two-step process involving the hydroxylation of the essential amino acid L-tryptophan to 5-hydroxytryptophan (5-HTP), which is then decarboxylated to 5-HT (5-hydroxytryptamine). 5-HT can be synthesized in both the cell bodies (raphe nuclei) and the terminals, although the latter site is probably more important for the short-term regulation of serotonin synthesis. 5-HT, which is formed in the cell body, is transported to the terminals and stored in vesicles. Release of 5-HT is a Ca^{2+} dependent process (Kuhn *et al.,* 1986).

Following its release, the effect of 5-HT is terminated principally by re-uptake into serotonergic nerve terminals using a Na^{+}/K^{+}-ATPase-dependent transporter (Shaskan and Snyder, 1970). Once back inside the serotonergic neurone the transmitter is either re-stored in the vesicles (Slotkin *et al*., 1978) or metabolized by monoamine oxidase (MAO). Not only does MAO metabolize serotonin, it deaminates many amines with the general formula $R\text{-}CH_2\text{-}NH_2$, where R is a substituted aryl or alkyl group. The amines that fall in this category include serotonin, dopamine, noradrenaline, adrenaline, tyramine and tryptamine (Marsden, 1996).

Serotonergic neurons originate in the dorsal and median raphe nuclei of the brain stem and project to virtually every region of the brain with primary targets including the substantia nigra, hypothalamus, thalamus, amygdaloid-hippocampal area, caudate putamen and nucleus accumbens and cerebral cortical

areas including the frontal, occipital, insular, parietal, temporal and cerebellar cortices (Azmitia and Whitaker-Azmitia, 1991).

The role of serotonin and the serotonin receptor family. 5-HT is involved in a large number of CNS processes, including the regulation of aggression, mood, pain, anxiety, feeding behaviour etc (Bradley *et al.,* 1986; Roth, 1994). To mediate these functions there is a family of receptors divided into 7 main classes and designed 5-HT_1 to 5-HT_7 comprising at least 16 molecular by distinct receptor subtypes (Bovento and MacKenzie, 1997). Except of the 5-HT_3 receptors, which are ligand–gated ion channels, all 5-HT receptors interact with G-proteins. Based on the homology of their structure and coupling to second messengers, these receptors can be divided into families. The 5-HT_1 family contains receptors that are negatively coupled to adenylate cyclase: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}. The 5-HT_2 family includes receptors that stimulate pospholipase C: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. The adenylate cyclase stimulatory receptors are a heterogeneous group including the 5-HT_4, 5-HT_6 and 5-HT_7 receptors. The effector systems of 5-HT_{5A} and 5-HT_{5B} receptors still remain to be unknown (Stark *et al.*, 1998). The localization of 5-HT receptors and their putative functions are summarized in Table 1.

Table 1. Localisation and function of some serotonin receptors, which are implicated in atypical antipsychotics effects.

Receptor	**Regional localization**	**Subcellular localization**	**Functions**
5-HT_{1A}	High in limbic brain areas, hippocampus, lateral septum, cortex and dorsal and median raphe nuclei in the mesencephalon	Postsynaptically on the 5-HT neurons (in forebrain regions), and on the 5-HT neuron soma and dendrites (in mesencephalic and medullary raphe nuclei)	In the midbrain raphe nuclei act as auto-receptors that control negatively 5-HT firing and synthesis/ release of 5-HT; adaptive responses to aversion, ingestive behaviours, neuroendocrine control
5-HT_{1D}		Unknown	Unknown
5-HT_{2A}	In cortex of forebrain regions: especially neocortex, entorhinal and pyriform cortex, claustrum; nucleus caudatus, nucleus accumbens, olfactory tubercle and hippocampus	Postsynaptic membranes	Control of noradrenaline release, hallucinogenic-induced behaviours, sleep, aversion

Receptor	Regional localization	Subcellular localization	Functions
5-HT$_{2C}$	In the choroid plexus, areas of cortex (olfactory nucleus, pyriform cortex); nucleus accumbens, hippocampus, amygdala; caudate nucleus, substantia nigra	Postsynaptic	Aversive behaviours, hypoactivity, ingestive behaviours, anticonvulsive effects
5-HT$_3$	Within the dorsal vagal complex in the brainstem. The region comprises the nucleus tractus solitarius, area postrema and dorsal motor nucleus of the vomiting reflex. Receptor expression in the forebrain is low, but relatively higher levels are expressed in the hippocampus, amygdala and superficial layers of the cerebral cortex	Postsynaptic membranes	Sensory function, emesis, control of neurotransmitter release, anxiety?, cognition?, psychosis?
5-HT$_6$	Cortex, accumbens, caudate, hippocampus	Unknown	Unknown motor function?, affective behaviour?
5-HT$_7$	Hippocampus, hypothalamus, raphe nuclei	Unknown	Unknown, similar to 5-HT$_6$?

[Data compiled from Pazos *et al.,* 1985; 1987; Pratt *et al.*, 1990; Radja *et al.,* 1993; Bufton *et al.,* 1993; Hoyer *et al.,* 1994; Lucas and Hen, 1995; Parker *et al.,* 1996; Ereshefsky, 1996; Kulikov *et al.,* 1997; Lopez-Gimenez *et al.,* 1997; Barnes and Sharp, 1999].

Regulation of serotonergic system. Absolutely specific agonists do not exist for any of the 5-HT receptors, although moderately selective agonists are available for some. For a few of the receptors moderately selective antagonists exist, although most antagonists have variable affinities for a number of 5-HT receptor subtypes (Hoyer *et al.*, 1994).

Serotonin — dopamine interactions. It has been suggested that abnormalities in the interaction between monoaminergic systems, in general, and between serotonergic and dopaminergic systems in particular (Meltzer, 1989), rather than abnormalities in any system alone are important in schizophrenia patho-

physiology. It is particularly difficult to discuss dopamine without mentioning its interactions with 5-HT. Both neurotransmitter systems are highly intertwined, anatomically and functionally, with 5-HT having an inhibitory modulation on dopamine function (Kahn and Davidson, 1993).

It has been known for long that the central serotonergic system modulates the activity of the nigrostriatal dopaminergic pathway (Dray, 1981; Soubrié *et al.,* 1984; Spoont, 1992). Most recent studies have focused on the involvement of 5-HT_2 receptors in this interaction, and suggested a potential significance for this mechanism in the treatment of neuropsychiatric disorders related to central dopamine dysfunction, such as schizophrenia (Meltzer and Nash 1991; Kapur and Remington, 1996).

5-HT modulates striatal dopamine outflow in conditions in which dopamine synthesis and/or release are activated or inhibited (Schmidt *et al.,* 1992; Ichikawa *et al.,* 1995). The 5-HT system inhibits dopamine function at the level of the midbrain by reducing the firing of dopamine cells of the substantia nigra, inhibiting the synaptic release of dopamine in striatum and cortex (Kapur and Remington, 1996). Thus, according to the suggestion that negative symptoms of schizophrenia are connected with a hypodopaminergic function in the prefrontal cortex (Weinberger and Berman, 1988), the blockade of serotonin activity should result in disinhibition of dopamine activity in these areas, resulting in fewer negative behavioural and extrapyramidal symptoms.

Different subtypes of 5-HT receptors are involved in these serotonergic control mechanisms over dopaminergic activity. The population of 5-HT_{2C} receptors inhibitory to the dopaminergic neurons projecting to frontal cortex is likely to be localized in the ventral tegmental area itself — presumably on GABAergic interneurons (Pompeiano *et al.,* 1994).

5-HT_{2A} receptors may actually potentiate frontocortical dopaminergic and noradrenergic transmission (Millan *et al.,* 2000). 5-HT_{2A} receptors are found on interneurons in caudate nucleus and in certain cortical areas (Garlow *et al.,* 1993).

It has been suggested that excitatory 5-HT_3 receptors on the dopaminergic nerve terminals in the frontal cortex enhance the release of dopamine, but equivalent actions of 5-HT_3 receptors have been documented more convincingly for subcortical dopaminergic projections (Chen *et al.,* 1992; Tanda *et al.*, 1995; Iyer and Bradberry, 1996; De Deurwaedere *et al*., 1998).

Serotonin — glutamate interactions. Historically, research into the neurochemistry of schizophrenia and its treatment has predominantly focused on the dopaminergic system. The possible dysfunction of glutamatergic system in schizophrenia is not in conflict with the dopamine hypothesis since reciprocal connections within the glutamatergic and dopaminergic systems are well established in the forebrain. Projection neurons from the prefrontal cortex use glutamate as their neurotransmitter to innervate striatum. Striatal dopaminergic projection neurons innervate the prefrontal cortex through the mesocortico-

limbic dopaminergic system (Bantick *et al.,* 2001) and are likely to affect cortical glutamatergic neurotransmission.

Dall`Olio *et al*. (1999) have shown that unlike competitive N-methyl-D-aspartate (NMDA) receptor antagonists, the non-competitive antagonists enhanced the expression of serotonergic stimulation, and suggested that a glutamate deficiency could contribute to the pathogenesis of schizophrenia, not only through dopaminergic, but also through serotonergic hyperactivity.

As a major proportion of neocortical 5-HT_{1A} receptors appear to be located on pyramidal, putatively glutamatergic, cells (Burnet *et al.,* 1995; Azmitia *et al.,* 1996), the increased receptor numbers may have implications for pyramidal cell function in the illness. Interestingly, cell density and glutamatergic elements have been reported to be elevated in the prefrontal cortex in schizophrenia (reviewed in Deakin and Simpson, 1997; Bantick *et al.,* 2001).

Stimulation of 5-HT_{2A} receptors generally leads to activation of serotonergic neurones by multiple mechanisms, including a direct or indirect mechanism to inhibit GABAergic interneurons, and a direct effect to excite glutamatergic and other neurons (Matsuyama *et al.,* 1997; Celada *et al.,* 2001).

Apomorphine-induced aggressiveness

Aggressiveness in psychotic patients is an important medical and social problem (Mann, 1995). Mechanisms of aggressive behaviour and also the experimental models of aggression have been studied extensively (Allikmets, 1996). Aggressiveness can be induced in laboratory animals (mice, rats, primates, cats, etc) by limiting of territorial area, by pain or the other sensory irritations, removal of positive reinforcement, electrical or chemical stimulation or lesioning of certain brain structures, and administration of certain drugs or by drug withdrawal (Allikmets, 1975). In animal studies the apomorphine–induced aggressiveness test has been proposed to be an equivalent to human pathological aggressive behaviour (Ueda *et al.,* 1999) or even a homological model of schizophrenia or psychosis (Lang *et al.,* 1994; 1995). Apomorphine-induced aggressiveness is a robust method to study defensive aggression, being clearly expressed at least in the majority of adult male rats.

Apomorphine is a direct but unselective dopamine receptor agonist equally potent at D_1 and D_2 receptor subtypes (Creese *et al.,* 1983). Therefore, the administration of apomorphine produces a number of behavioural effects. Administration of apomorphine or indirect dopamine agonists such as amphetamine and cocaine to laboratory animals increases locomotor activity and induces stereotyped behaviour (fixed posture, stereotyped body movements, stereotyped sniffing, yawning, licking and other stereotyped mouth muscle movements).

In humans, repeated use of direct or indirect dopamine agonists can often cause severe and sometimes long-lasting adverse effects, such as amphetamine

psychosis. Repeated administration of various dopamine agonists (apomorphine, amphetamine, cocaine) to laboratory animals induces sensitisation of the dopaminergic system and thereby increases locomotor activity and irritable aggression consisting of defensive upright postures, vocalization and biting attacks in pairs of responsive rats (Lang *et al.*, 1995; Uijke, 2001). It is a widespread opinion that the neurobiological mechanisms underlying these behavioural effects of repeated administration of dopaminergic agents in laboratory animals and in humans are similar (Mattingly *et al.,* 1991).

Aggressive behaviour of animals can be influenced by a variety of chemicals acting upon different neurobiological systems. So far, there is no specific antiaggressive drug, and still the sedative neuroleptics are drugs of choice for clinical use (Mann, 1995).

The apomorphine-induced aggressive behaviour can be effectively antagonized by clinically used typical neuroleptics and other D_2 receptor blockers, morphine and NMDA receptor antagonists (Lang *et al.,* 1992; 1994). These drugs have similar effects on human psychosis, which further confirms the general validity of apomorphine-induced aggressive behaviour paradigm.

Although the phenomenon of the apomorphine-induced aggressive behaviour in rats is known over two decades, its neurobiology is still unclear. Furthermore, it is an enigma why the aggressiveness does not develop in all animals subjected to the same apomorphine-treatment regimen.

There are several data indicating that the serotonergic system is linked to aggressive behaviour (Molina *et al.,* 1987; White *et al.,* 1991; Olivier and Mos, 1992; Muehlenkamp *et al*., 1995). Apomorphine is known as a potent dopamine agonist, deprived of direct effect on the metabolism of 5-HT and neurons (Lee and Geyer, 1984; Trulson and Crisp, 1984).

The action of serotonergic compounds has been studied in various animal models of aggressive behaviour (Sanchez and Hyttel, 1994). Sanchez and collaborators have repeatedly shown that the serotonergic compounds, even if they share a common mechanism of action (for example, the SSRIs), may have different antiaggressive profile (Sanchez *et al.,* 1993; Sanchez and Hyttel, 1994).

Involvement of the 5-HT_{1A} receptors in the mediation of aggressive behaviour has been reported earlier, while the special emphasis has been put on the 5-HT_{1A} and 5-HT_{1B} receptors. Sanchez and co-workers have found that the 5-HT_{1A} receptors are involved in the neurobiology of isolation-induced aggressiveness of male mice (Sanchez *et al*., 1993; Sanchez and Hyttel, 1994). Buspirone, a 5-HT_{1A} receptor partial agonist and anxiolytic, suppresses the dominant behaviour in rats (Tornatzky and Miczek, 1995) and the territorial aggression in single-housed mice (Olivier *et al.,* 1989). The specific serotonergic 5-HT_{1A} agonist 8-OH-DPAT exerts an antiaggressive effect in the dominance and maternal aggression paradigms in rats (Mos *et al.,* 1992), but is ineffective on defensive aggressive behaviour (Muehlenkamp *et al.,* 1995). Today only busipirone is clinically used as an antiaggressive drug (Pabis and

Stanislav, 1996). It has been reported that 5-HT_{1A} receptor expression in forebrain regions of aggressive house mice is enhanced (Mechiel *et al.,* 1996). Enhanced aggressiveness has been found in animals lacking 5-HT_{1B} receptors (Saudou *et al.,* 1994) while *vice versa*, aggressiveness can be attenuated by the 5-HT_{1B} receptor agonist (Fish *et al.,* 1999).

The stimulation of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 receptors reduces offensive aggression, whereas defensive aggression is only decreased by 5-HT_2 stimulation (Muehlenkamp *et al.,* 1995).

It has been demonstrated (Olivier and Mos, 1992; Sanchez *et al.,* 1993; Sanchez and Hyttel, 1994; Miczek *et al.,* 1995; Olivier *et al.,* 1995; Mechiel *et al.,* 1996; Matto *et al.,* 1999) that at least some elements of the aggressive behaviour are mediated via the 5-HT_{2A} receptors, but the exact role of HT_{2A} receptors in the CNS is not clear. There are conflicting data available, for example 5-HT_2 and 5-HT_{1C} agonist DOI increased aggression (Sakaue *et al.,* 2002) or had an antiaggressive effect (Olivier and Mos, 1992; Sanchez *et al.,* 1993) on isolation-induced aggressive behaviour. The 5-$HT_{2A/2C}$ antagonist ritanserin was ineffective on isolation-induced aggression in mice (Sanchez *et al.,* 1993; Muehlenkamp *et al.,* 1995) or decreased it (White *et al.,* 1991, Sakaue *et al.,* 2002). Ketanserin (5-HT_2 receptor and α_1-adrenoceptor antagonist) had antiaggressive effect in isolation-induced aggressive behaviour in mice (Sanchez *et al.,* 1993). Pirenperone antagonist of 5-HT_2 receptors selectively decreased the intensity of apomorphine aggressiveness (Vasar *et al.,* 1984). The 5-HT_{2A} antagonists have been introduced as antipsychotic drugs in humans (Staley *et al.,* 1998).

The role of 5-HT_3 receptors in the mechanism of aggressive behaviour is not known. It has been evidenced that 5-HT_3 heteroreceptors mediate dopamine release in mammalian CNS (Benloucif *et al.,* 1993) and therefore possible apomorphine-5-HT_3 receptor interaction deserves special attention. The 5-HT_3 antagonist ondansetron is ineffective in isolation-induced aggression (Sanchez *et al.,* 1993; Young *et al.,* 1993; Muehlenkamp *et al.,* 1995), but reduced intruder-induced aggression in transgenic mice (McKenzie-Quirk *et al.,* 2005). Ricci *et al,* (2004; 2005) demonstrated that tropisetron alone reduced aggressiveness dose-dependently, but mCPBG was ineffective on cocaine-induced aggression. mCPBG prior to tropisetron blocked cocaine-induced aggression in hamsters in higher dosages. SR 57227A, a selective 5-HT_3 receptor agonist, reduced isolation-induced aggressivity in mice by 50 to 85% (Poncelet *et al.,* 1995). 5-HT_3 agonists may cause dopamine overflow in mammalian brain thereby activating the postsynaptic dopamine receptors and causing cataleptic-like delay by the onset of the first attack (Benloucif *et al.,* 1993).

Serotonergic neurotransmission has profound effects on dopamine-mediated behaviours. Serotonin can modulate the effects of dopamine in mammalian forebrain, but the interactions are complex and not fully understood. Findings of both enhanced and decreased dopamine release associated with increased

availability of serotonin have been reported (Ennis *et al.,* 1981; Benloucif and Galloway, 1991; Baldessarini and Marsh, 1992).

From the neurochemical point of view, Rowlett *et al.* (1991) found that after repeated apomorphine treatment the basal dopamine synthesis is enhanced. On the other hand, these changes in the dopaminergic neurotransmission are insufficient to clarify the intimate mechanism of aggressiveness elicited by apomorphine because most of the drugs that attenuate the apomorphine-induced aggressiveness do not interact directly with the dopamine receptors or transporter (Lang *et al.,* 1995). The behavioural experiments by Võikar *et al.* (1999) demonstrated that the apomorphine-induced behavioural sensitisation is a very individual phenomen and is not subject to normal distribution.

AIMS OF THE STUDY

The aim of present study was to clarify the following questions:

1. **To analyze and further characterize the apomorphine aggressiveness model with regard to its behavioural specificity and underlying neurochemistry.**

2. **To reveal the role of 5-HT and glutamate receptors in the behavioural effects of atypical antipsychotics and in the apomorphine aggressiveness paradigm.** The serotonergic system is linked to aggressive behaviour in animals and humans. It has been found that drugs with 5-HT_{1A} agonistic or 5-HT_{2A} antagonistic properties elicit antiaggressive effects in some tests of aggressive behaviour in animals. However, there are some controversies in this issue as only a very limited number of studies have been devoted to investigation of the effect of atypical antipsychotics on aggressiveness.

3. **To compare the behavioural and biochemical effects of atypical antipsychotics — olanzapine, melperone, quetiapine, sertindole and ziprasidone — with the classical neuroleptic haloperidol.**

MATERIALS AND METHODS

Animals and laboratory conditions

Male and female Wistar rats, mostly from Kuopio National Animal Center (Kuopio, Finland), but also from Grindex Breeding Center (Riga, Latvia), weighing 200–300 g (350–400 g in the apomorphine-induced aggressiveness experiments) were used in all studies. The animals were housed four or five per cage (or one per cage for the apomorphine-induced aggressiveness experiments) under standard laboratory conditions; water and food were available *ad libitum*. The animal room had controlled temperature (20ºC±2ºC) and a light/dark cycle (light on from 8.00 a.m. to 8.00 p.m.). One hour before an experiment the animals were moved in their home cages from animal room into the behavioural testing room, unless stated otherwise. The experimental protocols were approved by The Ethics Committee of University of Tartu.

Drugs and chemicals

In the behavioural experiments, the following drugs were used:

1) **Antipsychotics**: sertindole, melperone (both from H. Lundbeck, Denmark), quetiapine (from AstraZeneca, U.K.), ziprasidone (from Pfizer, U.S.A.), olanzapine (from Eli Lilly, U.S.A.), risperidone (from RBI Chemicals, U.S.A.) and haloperidol (from Gedeon Richter Rt., Hungary)
2) **Serotonergic ligands:**
   5-HT$_{1A}$ receptor agonists: 8-OH-DPAT (from Tocris, U.K.); buspirone, gepirone (both from Bristol-Myers-Squibb, U.K.)
   5-HT$_{1A}$ receptor antagonists: WAY-100635 (from RBI Chemicals, U.S.A.)
   5-HT$_{2A/2C}$ receptor agonists: DOI (from Sigma RBI, U.S.A), quipazine (from RBI Chemicals, U.S.A.)
   5-HT$_{2A}$ antagonists: ketanserin, ritanserin, trazodone (all from RBI Chemicals, U.S.A.)
   5-HT$_3$ agonists: mCPBG, 1-PBG (both from RBI Chemicals, U.S.A.)
   5-HT$_3$ antagonists: MDL-72222, tropisetron (both from RBI Chemicals, U.S.A.), ondansetron (from GlaxoGroup Ltd., U.K.)
3) **Other drugs**: apomorphine (from Reakhim, Russia), MK-801 (from Tocris, U.K.), DSP-4 (from RBI Chemicals, U.S.A.), citalopram (from H. Lundbeck, Denmark).

For the neurochemical experiments all standards of monoamines and their metabolites, and monobasic sodium phosphate were obtained from Sigma RBI (St. Louis, MO, U.S.A.). Perchloric acid and sodium disulfite were purchased from

Ridel-deHaën AG (Seelze, Germany); octanesulfonic acid sodium salt was from Fluka Chemie (Buchs, Switzerland) and HPLC grade methanol from Rathburn Chemicals Ltd. (Walkerburn, Scotland). The microdialysis disposables were obtained from Agn Tho's AB, (Lidingö, Sweden). The chromatography columns were obtained from ESA, Inc. (Bedford, MA, U.S.A.) and Polypro filters from Gelman Laboratory (Ann Arbor, MI, U.S.A.).

Guanosine-5'-(γ-thio)-triphosphate ([^{35}S]GTPγS) was purchased from Perkin Elmer Life Sciences (U.S.A.), guanosine diphosphate sodium salt (GDP), (+)-butaclamol hydrochloride and 3-hydroxytyramine hydrochloride (dopamine) were from Sigma-Aldrich Fine Chemicals (U.S.A.). The scintillation cocktail OptiPhase HiSafe®3 was obtained from Wallac Perkin Elmer Life Sciences (U.S.A.).

Behavioural experiments

Apomorphine-induced aggressiveness test. The measurement of aggressive behaviour was performed in specially designed cages (35×35×55 cm, length x width x height, with transparent plastic side walls and stainless steel floor, covered with wood shavings). Immediately after the apomorphine injection (1 mg/kg, s.c.), the animals were placed pairwise into the test cage and observed for (1) the time of latency (time before the first attack or the first aggressive posture) and (2) the intensity of aggressive behaviour. The animals were observed for 15 min and the intensity of aggressive behaviour was scored on the 0–3 point scale (modified after Allikmets *et al*., 1979):
0 — no aggressive manifestations
0.5
1 — infrequent aggressive postures or attack of the other rat, no vocalisations
1.5
2 — frequent upright aggressive postures or intensive attacks or boxing with the other rat, vocalisations but no biting or continuous fighting
2.5
3 — continuous fighting or attempts to bite the opponent, loud vocalisations.
In case the highest score of aggressive behaviour was given, the test was interrupted immediately to avoid injuries.

The same apomorphine-pretreated animals were used repeatedly, but for no more than five independent experiments. The interval between the independent experiments was not less than three days during which the apomorphine treatment (0.5–1 mg/kg, s.c., once or twice a daily) was continued. Apomorphine pretreated rats were included in the acute drug treatment experiments when their score of aggressiveness was higher than 1.5. The apomorphine-pretreated animals were semirandomly divided to apomorphine plus vehicle-treated and apomorphine plus drug-treated group, but the same animal pairs were always

used and both rats in a pair received similar treatment. Apomorphine pretreatment lasted for two weeks (at the same time no behavioural experiments were performed). On the test day apomorphine was injected immediately before the measuring of aggressiveness.

All the drugs in the acute experiments were injected intraperitoneally (i.p.) 30 min before the treatment with apomorphine.

Apomorphine-induced stereotypy. Apomorphine-induced stereotypy was measured as described previously (Allikmets and Vasar, 1982; Võikar *et al.* 1999). Rats were placed into transparent cages (35×35×55 cm, length x width x height) and observed for 15 min. The latency and intensity of stereotyped behaviour were recorded. The scoring system used for estimation of the intensity of stereotyped behaviour was the following: 0, asleep or still; 1, discontinuous sniffing and locomotor activity; 2, continuous sniffing and small head movements, periodic locomotor activity; 3, constant stereotyped activity such as sniffing, rearing, or head bobbing and discontinuous biting or chewing, brief periods of locomotor activity; 4, constant stereotyped activity with continuous licking and/or gnawing and biting of cage grids.

The scoring of stereotyped behaviour was started immediately after an injection of apomorphine (1 mg/kg s.c.), which was administrated 48 h after repeated antipsychotic treatment. In experiments with acute treatment, drugs were injected i.p. 30 min before apomorphine.

Open field test. In the experiments with acute MK-801, 8-OH-DPAT, DOI and repeated apomorphine treatment a metal quadrate arena 50×100 cm with 40 cm sidewalls was used. The floor was divided into eight squares. In the other experiments a wooden, grey painted arena 100×100 cm with 40 cm sidewalls was used, its floor was divided into sixteen squares of equal size. On the test day, one hour before the experiment the animals were moved into the testing room. After drug treatment (30 min before test) the animals were returned to the home cage. For the test, the animal was placed into the centre of the arena and was observed for four min for (1) horizontal (number of line crossing on the floor) and (2) vertical (number of rears) activity. The horizontal activity was counted only if the animal crossed the line with four paws. Vertical activity was counted whenever the animal removed the forepaws from the ground and stretched itself. All the drugs in the acute experiments were injected i.p. 30 min before the start of experiments.

Quipazine-induced wet-dog shake test. Head twitches were induced by quipazine, an agonist of 5-HT receptors (Vetulani *et al.,* 1980). Quipazine-induced wet-dog shakes were observed in individual polycarbonate cages (20×14×20 cm, length x width x height, the floor covered with wood shavings). Immediately after the administration of quipazine (2.5 mg/kg i.p.), the animals were placed into the individual test cages and observed for (1) time of latency to

the first shake and (2) the number of body shakes. The animals were observed for 40 or 60 min. The drugs were injected i.p. 30 min before treatment with quipazine.

Neurochemical methods

Measurement of monoamine neurotransmitters and their metabolites in tissue samples

Brain dissection and collection of brain samples for HPLC-ECD analysis. Randomly selected animals from each drug treatment group were killed by decapitation either in a separate room in the animal facilities or after having been moved to the biochemical laboratory located in the same building. The skulls were opened and the brains were quickly removed and prepared on an ice-cold plate. This procedure took no longer than five minutes. The brain samples were stored in polypropylene tubes at –80° C until assayed.

Measurement of post-mortem monoamine content. Monoamine content was measured as described previously (Pruus *et al.,* 2002). HPLC-ECD analysis was performed with a Coulochem Electrode Array System (CEAS, Model 5600) equipped with two Model 582 pumps and a Model 540 autoinjector. Two coulometric array cell modules, each containing four electrochemical detector cells, were used. The analytical column (150×3 mm i.d.) used was a stainless-steel column packed with 3 µm particles of silica-based C_{18} materials (MD-150/RP-C_{18}). The column and detectors were housed in a thermal chamber maintained at 30°C. The system was controlled and the data were acquired and processed using the CoulArray software on a Pentium-based computer.

The mobile phase was made of 10% (v/v) methanol in 0.1 M monobasic sodium phosphate, 0.55 mM octanesulfonic acid with pH 3.10. The buffer solution was filtered through 0.2 µm GHP Polypro filters and degassed under vacuum for 10 min. The flow-rate was 0.5 ml/min and the cell potentials (*versus* palladium reference) constituted an increasing array: 0 mV at electrode 1, 50 mV at electrode 2, with increments of 100 mV at each subsequent electrode until a value of 650 mV.

The frozen brain samples were weighed and then sonicated for 30 s in 300–1000 µl of ice-cold 0.12 M perchloric acid ($HClO_4$) containing 0.1% sodium disulfite ($Na_2S_2O_5$) and 5 ng/ml 3,4-dihydroxy-benzylamine (DHBA) as an internal standard. After centrifugation (20 min at 4°C, 13,400 X*g*) 30 µl of supernatant was injected into the HPLC system. Dissolving 10–20 mg of the component in 25 ml 0.12 M perchloric acid made the primary stock standard solutions. These concentrated solutions were stored in 1 ml portions at -20°C and thawed when necessary at 4°C. Secondary standard solutions were made by

dilution to give a concentration of 2–4 μM. Working standards in nM range were made freshly every day.

Correct identification of the peak was obtained from the retention time (±4 %) and the relative ratio (at least 0.75) of the peak height measured with two or three electrodes at different voltages. Quantization of the compound was based on the peak area obtained for an external standard.

Microdialysis

Procedure. Under chloral hydrate anaesthesia (350 mg/kg, diluted in distilled water that served as vehicle, injected intraperitoneally) the guide cannula of the microdialysis probe (Agn Tho`s AB, Lindigö, Sweden) was implanted unilaterally into the left frontal cortex according to the coordinates taken from the Paxinos and Watson (1986) brain atlas of rat: AP +3.4 mm, DV –6.0 mm, L –2.5 mm relative to bregma, and secured using 3 screws and dental base material. The standard surgical technique was used. After surgery, the animals were accommodated in individual cages for 5–7 days.

Before the experiment day the all system was perfused overnight for 12 h (1 μl/min) with destilled water. On the morning of the experiment, rats were moved into the testing room (room temperature about 25°C) and same time was perfused the all system with 95% ethanol and furher with Ringer solution for 2 h before starting the experiments. The microdialysis probe (Agn Tho`s; exposed tip: 4 mm) was inserted into the guide cannula and connected via the polyethylene tubing to a 1 ml microsyringe and modified Ringer solution (NaCl 147.0, KCl 2.7, $CaCl_2$ 1.2, $MgCl_2$ 1.0, ascorbic acid 0.02 mM) was infused through the microdialysis probe with a microinjector pump (2 μl/min). After two and a half hours stabilization period the samples were collected every 15 min. The first 4–6 samples were considered as a baseline. The average value was taken as 100 per cent.

Measurement of monoamine content in microdialysis samples. The same equipment and method as described above for the experiments in tissue level were used with the following changes. A model 5014B microdialysis cell and the first CoulArray detector cell were set in series. The mobile phase consisted of 50 mM monobasic sodium phosphate, 0.50 mM sodium acetate, 0.42 mM octanesulphonic acid and 10% (v/v) of methanol, pH was adjusted to 4.10. The flow-rate was 0.5 ml/min and the cell potentials (*versus* palladium reference) constituted an increasing array: –100 mV at electrode 1, 375 mV at electrode 2, 400 mV at electrode 3 and 500 mV at electrode 4.

Verification of the location of microdialysis probes. After completion of the experiment, the animals were killed under chloral hydrate anaesthesia by neck dislocation and the brains were removed from the skulls. The frozen brains were dissected using blades and the localization of every microdialysis probe was

verified *in situ* without staining. Animals with instable baseline of monoamine levels or wrong probe location were excluded from the analysis. The number of animals per control groups (vehicle and 8-OH-DPAT) included and subjected to statistical analysis was 8–9 and per drug combination group was 3–6.

[^{35}S]GTPγS binding assay. Rat striatal membranes were prepared as described previously (Lepiku *et al.,* 1996). Brain tissue samples were homogenized in 100 vol (ww/v) of ice-cold homogenization buffer (HB, 50 mM Tris-HCl, pH 7.4) by Bandelin Sonoplus sonificator (2 passes, á 10 sec). The membranes were collected by centrifugation at 25,000 Xg for 20 min at 4°C and washed by homogenization in HB and centrifugation two more times. The final pellets were homogenized in 90 vol (ww/v) of the incubation buffer (IB, 20 mM K-Hepes, 7 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4) and were used directly for binding experiments. Binding of [^{35}S]GTPγS was carried out as described earlier (Rinken *et al.,* 1999) with slight modifications. In brief, the membranes (500 μg per tube) in IB were incubated with 0.2 nM [^{35}S]GTPγS and different concentrations of GDP (3 mM–1 μM) in the presence of 1 mM dopamine or 10 μM butaclamol for 90 minutes at 30^{o}C, and the reactions were terminated by rapid filtration through GF/B filters using a Brandel cell harvester with three washings of 5 ml of ice-cold washing buffer (20 mM NaKphosphate buffer, 100 mM NaCl, pH 7.4). The radioactivity content of the filters was counted in 5 ml of scintillation cocktail OptiPhase HiSafe®3 (Wallac Perkin Elmer Life Sciences, U.S.A.) by Beckman LS 1800 scintillation counter.

Data analysis and statistics

The statistical analysis of the behavioural data (plus-maze, open field, forced swimming, quipazine-induced wet-dog shakes) was carried out by one-way analysis of variance (ANOVA) followed by Fisher's LSD test or Scheffe's test.

The data from repeated experiments (locomotor activity and stereotyped behaviour) were analysed using repeated measures ANOVA (between factor: drug treatment; within factors: day). Whenever an interaction effect was found, the data were further analyzed by one-way ANOVA followed by Fisher's LSD test or Scheffe's test.

Data from the *in vivo* microdialysis experiments were subjected to repeated measures analysis of variance (repeated measures ANOVA), (factors: drug treatment and time between 15 and 195 min). Whenever a significant drug treatment effect or drug treatment x time interaction was found, the data were further analysed by separate time points using ANOVA followed by Fisher's LSD test (factor: drug treatment).

The data from apomorphine-induced aggressiveness experiments were analysed by Kruskal-Wallis' one-way ANOVA or Mann-Whitney U test follo-

wed by Kolmogorov-Smirnov two sample test. Alternatively, results were subjected to ANOVA, for post-hoc data comparison Scheffe's test was used. The data from acute drug treatment of apomorphine aggressiveness experiments were subjected to Student's *t*- test.

The data obtained from the monoamine content measurements were subjected to one-way ANOVA, and where appropriate, Fisher's LSD test or Scheffe's test was used for post-hoc group comparison.

All binding data were analysed by nonlinear least-squares regression analysis using Graph Pad PRISM 4.02 (GraphPad Software, San Diego, U.S.A.).

All data are expressed as means ± SEM. The probability levels $P<0.05$ were considered statistically significant.

RESULTS

Development of apomorphine aggressiveness (I–VII, previously unpublished results)

The well-documented set of behavioural effects caused by acute apomorphine treatment, is characterized by repeated sniffing, licking and gnawing, were observed following the first injection (1 mg/kg s.c.). Repeated administration of apomorphine made animals irritable, as observed on the third day of treatment: rats presented sudden bursts of locomotor activity in response to noise or the approach of another rat. Also, some rats displayed the upright threatening posture, sham boxing and vocalization. Increasingly intensive tail-vibration and short bursts of locomotion always preceded this behavioural syndrome of aggressive behaviour. The syndrome of apomorphine-induced aggressive behaviour was very stable. Once induced, any subsequent injection of apomorphine resulted in a similar behaviour.

The repeated treatment with the low doses of apomorphine (0.5 or 1 mg/kg s.c., twice or once daily, respectively) during 10–14 days induced in the majority (over 80%) of male animals spontaneous and gradually increasing aggressiveness as evidenced by the day-by-day shortened time of latency before the first attack toward the opponent and increasing (intensified score) intensity of aggressive postures (II, III, VI; Fig. 1). In the female animals, a similar, but weaker tendency was found (V; Fig. 1). Nevertheless, in most of female animals, treatment did not lead to motor hyperactivity and aggressive attempts. On all test days, the nonaggressive females elicited strong stereotyped behaviour (cage licking, repeated stereotyped movements, and other behavioural phenomena remote from normal). Although not specifically quantified in our experiments, female rats displayed even stronger stereotyped behaviour than males, indicating the effectiveness of apomorphine treatment.

In the experiment comparing the effect of chronic apomorphine treatment only in home-cage *vs.* in association with repeated aggressive contacts, the development of aggressive behavior was measured on the third, sixth, ninth and twelfth days in two groups: vehicle and apomorphine treatment. In the other apomorphine treatment group, aggressive behaviour was measured only once at the twelfth day. Development of aggressive behaviour was present in these animals, which had fighting experience, but was completely absent in rats, which had received apomorphine in their home cage (Fig. 1).

We have also studied monoamine contents in four brain regions, but no consistent changes caused by apomorphine treatment or correlation with the development of aggressive behaviour was found (IV; Table 1). However, repeated apomorphine administration in adult male aggressive Wistar rats induced a moderate increase of contents of dopamine metabolites in some brain regions with a concomitant decrease of dopamine content. The magnitude of this effect was higher immediately after the last apomorphine injection than 24 h later (Matto *et al.,* 2000; Table 1).

Pre-treatment with DSP-4, a toxin, which destroys the noradrenergic nerve terminals, significantly accelerated the development of aggressive behaviour (I; Fig. 1). After the ninth day of the experiment, the animals of both groups were aggressive, but a moderate difference still remained. Thus, the intensity of aggressiveness in the control animals varied from two to three points, while the DSP-4 pre-treated animals always received the maximal score.

Co-administration of serotoninergic antidepressant trazodone (3 mg/kg), but not quipazine (1 mg/kg), was able to suppress the development of apomorphine–induced aggressive behaviour. This effect of tradozone was statistically significant from day nine (VI; Fig. 2).

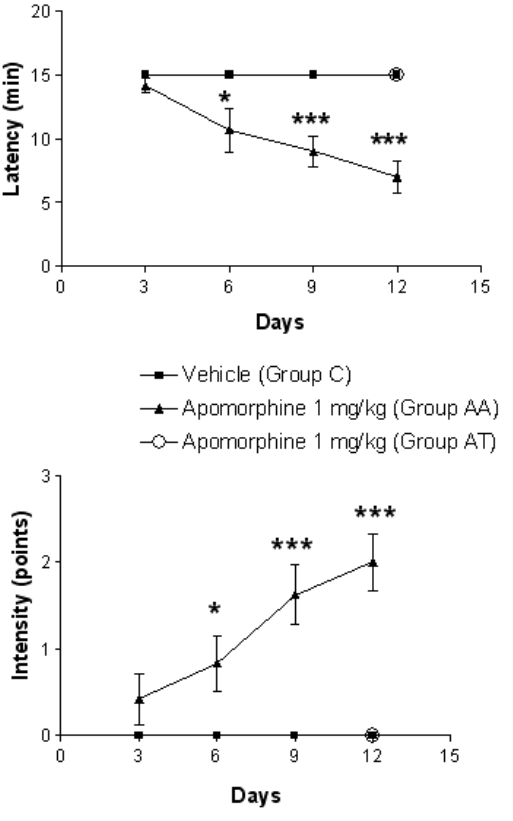

Figure 1. Effect of repeated apomorphine administration on time of latency and intensity of aggressiveness durning 12 consecutive days. The animals (n=33) were divided in to three groups: C — control animals which were not subjected to any treatment (n=11; ■), AT — apomorphine treated animals which were in apomorphine-aggressiveness test only once in the last day (n=10; ▲), AA — apomorphine aggressive animals which were in the apomorphine-aggressiveness test every 3rd day (n=12; ○). Data expressed as means±S.E.M. *p<0.05, ***p<0.001 as compared with vehicle (Fisher's LSD test).

Repeated treatment with apomorphine had an effect on GDP binding affinity to striatal membranes. Thus, two weeks of apomorphine administration in the home cage increased GDP binding affinity, but this affect was not present in apomorphine-treated rats, which had developed aggressive behaviour (Fig. 2). Similar differences in GDP affinity were detected in the presence of dopamine (10 μM) or butaclamol (1 μM) (data not shown). The effect of dopamine receptor activation on the GDP affinity was similar in all groups. In the presence of 100 μM dopamine and 40 μM GDP, lower level of [^{35}S]GTPγS binding was found in striatal membranes of apomorphine-treated rats that did not develop aggressive behaviour (Fig. 3).

Repeated administration of apomorphine, irrespective of whether aggressiveness developed or not, did not affect behaviours in forced swimming test neither open field activity (data not shown), and none of the apomorphine treatment groups differed from the vehicle group in wet-dog shakes test (Fig. 4).

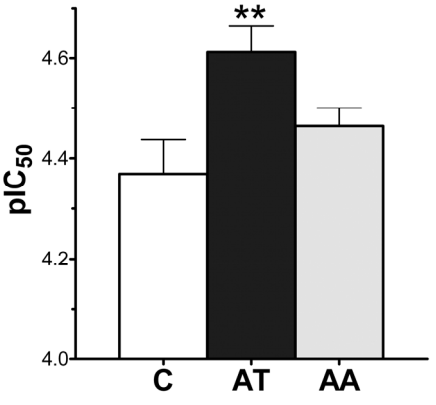

Figure 2. Effect of chronic apomorphine treatment on the affinity of GDP measured by its ability to inhibit [^{35}S]GTPγS (0.2 nM) binding to rat striatal membranes in the presence of dopamine (10μM). C — control animals which were not subjected to any treatment (n=5); AT — apomorphine treated animals which were in apomorphine-aggressiveness test only once in the last day (n=4); AA — apomorphine aggressive animals which were in the apomorphine-aggressiveness test every 3rd day (n=4). Data expressed as means±S.E.M. **p<0.01 vs. control (Student's *t*-test).

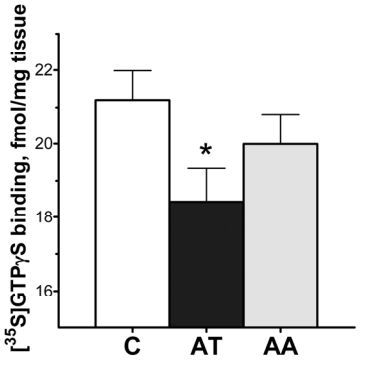

Figure 3. Effect of chronic apomorphine treatment on the dopamine dependent activation of [^{35}S]GTPγS binding in the presence of 100 μM dopamine and 40 μM GDP. C — control animals which were not subjected to any treatment (n=5); AT — apomorphine treated animals which were in apomorphine-aggressiveness test only once in the last day (n=4); AA — apomorphine aggressive animals which were in the apomorphine-aggressiveness test every 3rd day (n=4). Data expressed as means±S.E.M. *p<0.05 vs. control (Student`s *t*-test)

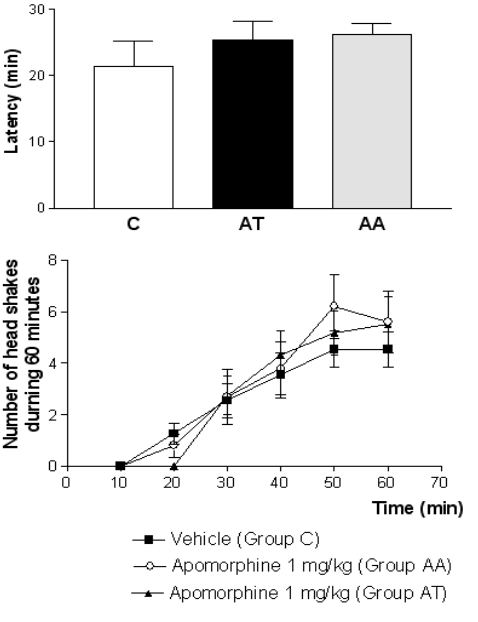

Figure 4. Effect of repeated apomorphine treatment on time of latency and intensity of quipazine-induced wet-dog shake test counted by 10 minute intervals. C — control animals which were not subjected to any treatment (n=11; ■), AT — apomorphine treated animals which were in apomorphine-aggressiveness test only once in the last day (n=10; ▲), AA — apomorphine aggressive animals which were in the apomorphine-aggressiveness test every 3rd day (n=12; ○). Data expressed as means±S.E.M.

Effects of atypical antipsychotics and selective 5-HT receptor ligands on expression of apomorphine-induced aggressiveness (I–VII, previously unpublished results)

The acute effect of atypical antipsychotic drugs on apomorphine-induced aggressive behaviour. In the experiment comparing the effect of different doses of atypical antipsychotics on apomorphine-induced aggressiveness test, the development of aggressive behaviour was measured on the first, fourth, seventh and ninth days. Melperone, sertindole, ziprasidone and quetiapine all elicited a similar anti-aggressive effect, while the dose-response curves for sertindole, ziprasidone, and quetiapine were almost identical (Fig. 5). Melperone treatment attenuated the apomorphine-induced aggressiveness from the dose of 3 mg/kg, and from 10 mg/kg blocked the aggressiveness almost completely. Other drugs had a significant anti-aggressive effect from the dose of 10 mg/kg on latency to the first aggressive encounter, and at doses 30 mg/kg and higher on the aggressiveness score. Nevertheless, the attenuation of the aggressiveness was incomplete even at the highest doses of quetiapine, sertindole and ziprasidone that were tested.

Effect of chronic antipsychotic treatment on apomorphine stereotypy. None of the studied atypical or classical antipsychotics had any statistically significant effect in apomorphine stereotypy test (Fig. 6).

Effect of concomitant serotonergic drug administration of apomorphine-induced aggressiveness. After completion of the repeated concomitant vehicle or drug (trazodone, quipazine) plus apomorphine treatment experiments, the animals were re-tested on the 3^{th} and 13^{th} day using apomorphine challenge without concomitant drug treatment. No significant effects of serotonergic drugs could be detected with repeated measures ANOVA (data not shown).

The effects of 5-HT_{1A} agonists (busiprone, gepirone and 8-OH-DPAT) and antagonist (WAY-100635) on apomorphine-induced aggressive behaviour. Buspirone (2.5 and 5 mg/kg) reduced the intensity of aggressiveness and prolonged the latency to attack (II; Table 1). However, gepirone treatment influenced similarly the latency parameter only at higher doses (10 mg/kg) and failed to have significant effect on the intensity of aggressiveness even at this dose, although a strong trend toward it was found. Administration of 8-OH-DPAT had no effect either on the intensity of aggressiveness or on the latency before the first aggressive posture. Effects of buspirone (2.5 mg/kg) and gepirone (10 mg/kg) were not antagonized by WAY-100635, a selective antagonist of 5-HT_{1A} receptors.

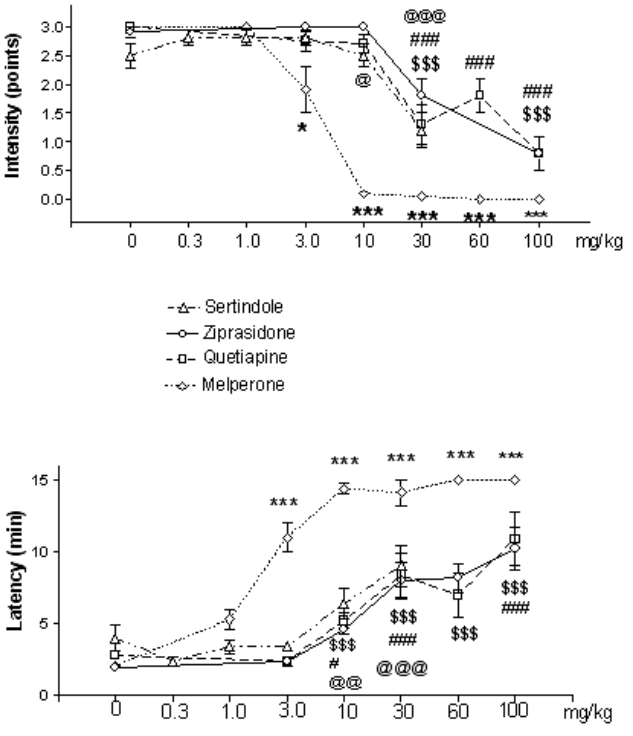

Figure 5. Effect of acute treatment with sertindole (▲), ziprasidone (○), quetiapine (□), and melperone (◊) on the apomorphine induced aggressive behaviour in rats measured in intensity of aggressiveness (upper panel) and in time of latency before first aggressive posture or attack toward the opponent rat (lower panel). 4–5 animal pairs per group, were compared, and the behavioural criteria were observed separately for each animal. Data expressed as means ± S.E.M. Significance of the difference, determined by Fisher's LSD test, of respective dose of drug in comparison with its vehicle group are presented as *p<0.05, ***p<0.001 for melperone $^{\$\$}$p<0.01, $^{\$\$\$}$p<0.001 for ziprasidone, $^{\#}$p<0.05, $^{\#\#\#}$p<0.001 for quetiapine, $^{@@@}$p<0.001 for sertindole.

Effect of 5-HT$_{1A}$ agonists (buspirone, gepirone and 8-OH-DPAT) on stereotyped behaviour and locomotor activity of apomorphine-sensitized aggressive rats. Buspirone (2.5 mg/kg) and gepirone (10 mg/kg) had no effect on the stereotyped behaviour in the apomorphine sensitized animals (II; Fig 2). In the open field, 5-HT$_{1A}$ receptor agonists tested (buspirone, gepirone and 8-OH-DPAT) dose-dependently decreased the number of line crossings and number of rearings of the experimentally naïve rats (II; Table III), while buspirone (2.5 mg/kg) and gepirone (10 mg/kg) had no effect on locomotor activity in apomorphine presensitized rats (II; Fig. 2).

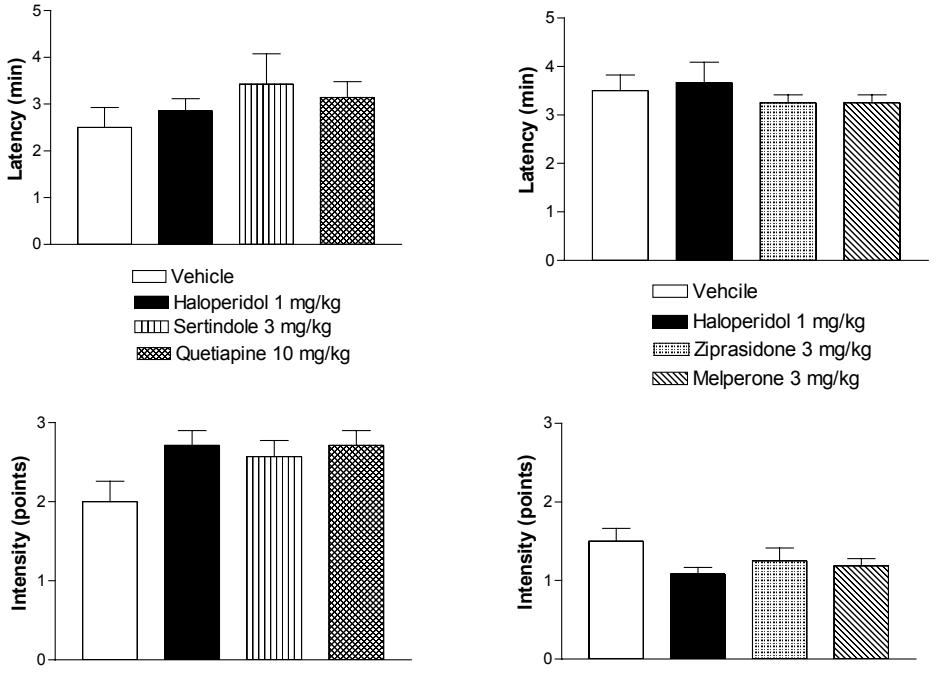

Figure 6. The effect of chronic administration (3 weeks) of atypical antipsychotics sertindole and quetiapine, melperone and ziprasidone in comparison with haloperidol on apomorphine stereotypy test, 48 hrs after last injection in rats. Data expressed as means±S.E.M.

Effect of 5-HT$_2$ receptor agonists (DOI and quipazine), antagonists (trazodone, ketanserine and ritanserine) and antipsychotics (risperidone and haloperidol) on apomorphine-induced aggressive behaviour. An apomorphine-sensitized rats, all doses of trazodone and quipazine attenuated aggressive behaviour. However, the doses used were not sufficient to block aggressive behaviour completely (VII; Table 1). Ketanserine and ritanserine (0.5 and 5 mg/kg) had no effect on the latency and intensity of apomorphine-induced aggressive behaviour (III; Table 1). In contrast, risperidone in doses 0.5 and 1 mg/kg inhibited aggressive behaviour (III; Table 2), and DOI (3 mg/kg) itself had no effect on apomorphine-induced aggressiveness (Skrebuhhova-Malmros *et al.,* 1999; Fig. 2). Risperidone (0.5 mg/kg) and DOI (0.3 and 3 mg/kg) co-administration elicited a decrease of aggressiveness (IV; Table 2). In the case of risperidone (0.5 mg/kg) and haloperidol (0.03 and 0.3 mg/kg) co-administration, already the minimal effective dose of haloperidol potentiated the effect of risperidone and aggressive behaviour was blocked completely.

Effect of co-administration of trazodone and quipazine on the effect of apomorphine sensitization on brain monoamine content. Neither repeated trazodone (3 mg/kg) nor quipazine (1 mg/kg) treatment had a major impact on the tissue levels of monoamines in apomorphine-treated animals (VII; Table 6). However, as compared with the non-treated and non-tested control animals, apomorphine aggressiveness was associated with a decreased dopamine content in the striatum and its metabolite contents increased. These changes in the striatum were more pronounced in rats given quipazine also, and were observed to a smaller degree in a few other brain regions.

Effect of 5-HT_3 receptor agonists (mCPBG and 1-PBG) and antagonists (MDL-72222, tropisetron and ondansetron) on apomorphine-induced aggressive behaviour. The 5-HT_3 receptor agonist mCPBG (1 and 10 mg/kg) failed to change apomorphine-induced aggressive behaviour in normal as well as DSP-4 pretreated rats (I; Table 1, Skrebuhhova-Malmros *et al.,* 1999; Fig. 2). 1-PBG (3 and 30 mg/kg) on the other hand, attenuated the intensity of aggressiveness and prolonged the time of latency. However, in the higher dose, in contrast, the drug shortened the time of latency in DSP-4 pretreated rats after citalopram challenge. The 5-HT_3 receptor antagonist MDL-72222 (0.4 and 4 mg/kg) and, to lesser extent, tropisetron (0.3 mg/kg) reduced the intensity and increased latency of apomorphine-induced aggressiveness. In the DSP-4 pretreated rats, MDL-72222 and tropisetron (0.03 and 0.3 mg/kg) were without any effect. Tropisetron (0.3 mg/kg) reduced aggressiveness only after citalopram (10 mg/kg) challenge. The 5-HT_3 receptor antagonist ondansetron (1 and 4 mg/kg) did not have any major effect on the apomorphine-induced aggressive behaviour (Skrebuhhova-Malmros *et al.,* 1999; Fig. 2).

Effects of atypical antipsychotics in conditions of serotonin and glutamate receptor modulation
(VII, previously unpublished results)

Effect of chronic antipsychotic treatment on open field activity. Chronic treatment with haloperidol (1 mg/kg) or sertindole, melperone and ziprasidone (all 3 mg/kg) had no effect on locomotor activity in the open field (Fig. 7). Chronic administration of quetiapine (10 mg/kg) increased locomotor activity significantly compared with vehicle and haloperidol treated groups.

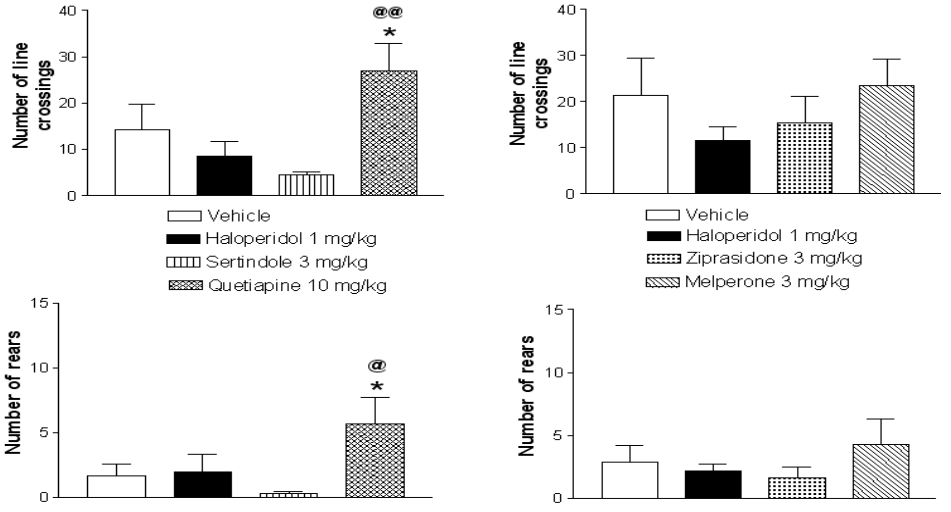

Figure 7. The effect of chronic administration (3 weeks) of sertindole and quetiapine, melperone and ziprasidone in comparison with haloperidol on open field test, 48 hrs after last injection in rats. n/control group=6–8, n/drug group=7–8. *$p<0.05$ as compared with vehicle group; $^{@}p<0.05$, $^{@@}p<0.01$ as compared with haloperidol treated group (Fisher's LSD test).

Effect of acute and repeated antipsychotic treatment on brain monoamine content. Acute treatment with sertindole (3–30 mg/kg), ziprasidone (10–100 mg/kg) or quetiapine (10–100 mg/kg) had no major effect on noradrenaline, dopamine or 5-HT as well as on 5-HIAA content compared with the vehicle-treated group in any of the three brain regions studied (Table 2). In striatum, sertindole (30 mg/kg), quetiapine (100 mg/kg) and ziprasidone (10, 30 and 100 mg/kg) increased the levels of DOPAC. Smaller doses of sertindole (3 and 10 mg/kg) and quetiapine (10 mg/kg) decreased the levels of HVA, but ziprasidone increased the levels of HVA in all doses.

In the frontal cortex, repeated melperone (3 mg/kg), ziprasidone (3 mg/kg) and haloperidol (1 mg/kg) treatment reduced noradrenaline and 5-HT levels (Table 3). However, this effect of haloperidol was not observed in the other experiment. Melperone increased both DOPAC and dopamine levels. Ziprasidone and haloperidol decreased HVA levels. In the striatum, sertindole (3 mg/kg) and haloperidol decreased the levels of HVA, and sertindole also reduced DOPAC and 5-HIAA levels. Haloperidol increased dopamine levels in one of the two experiments. None of the treatments affected monoamine levels in the hypothalamus.

Table 2. The effect of acute administration of atypical antipsychotics on monoamine content in the rat brain

Brain structure and treatment	**Noradrenaline**	**DOPAC**	**HVA**	**Dopamine**	**5-HIAA**	**Serotonin**
Frontal cortex						
Vehicle	265 ± 14	152 ± 52	59 ± 24	67 ± 18	359 ± 15	328± 15
Sertindole 3 mg/kg	267 ± 40	205 ± 54	39 ± 15	73 ± 13	364 ± 27	296± 27
Sertindole 10 mg/kg	318 ± 36	84 ± 51	35 ± 11.	119 ± 31	421 ± 16*	330 ± 20
Sertindole 30 mg/kg	226 ± 24	110 ± 56	39 ± 10	56 ± 14	380 ± 27	298 ± 16
Ziprasidone 10 mg/kg	278 ± 23	94 ± 35	67 ± 16	61 ± 12	368 ± 23	332 ± 15
Ziprasidone 30 mg/kg	247 ± 35	90 ± 27	91 ± 28	75 ± 16	418 ± 30	535 ± 79**
Ziprasidone 100 mg/kg	272 ± 26	224 ± 72	79 ± 17	63 ± 17	404 ± 19	398 ± 27
Quetiapine 10 mg/kg	323 ± 31	21 ± 17	20 ± 3	52 ± 10	326 ± 28	347 ± 41
Quetiapine 30 mg/kg	314 ± 37	162 ± 60	52 ± 11	65 ± 10	331 ± 35	416 ± 37
Quetiapine 100 mg/kg	293 ± 36	161 ± 64	41 ± 11	50 ± 9	317 ± 11	372 ± 13
Striatum						
Vehicle	355 ± 56	1981 ± 264	659 ± 140	6109 ± 461	472 ± 32	356 ± 16
Sertindole 3 mg/kg	286 ± 29	2195 ± 211	372 ± 58**	6711 ± 441	406 ± 35	318 ± 56
Sertindole 10 mg/kg	381 ± 55	2680 ± 302	386 ± 49**	7850 ± 709	511 ± 57	382 ± 51
Sertindole 30 mg/kg	291 ± 48	3299 ± 227**	628 ± 68	5935 ± 427	518± 28	316 ± 22
Ziprasidone 10 mg/kg	236 ± 15	4240 ± 488***	1331 ± 53***	7927 ± 225	491 ± 19	351 ± 19
Ziprasidone 30 mg/kg	273 ± 31	3717 ± 323***	1203 ± 67***	6446 ± 561	513 ± 29	367 ± 20
Ziprasidone 100 mg/kg	255 ± 34	3491 ± 309***	1120 ± 36***	5981 ± 541	502 ± 27	360 ± 22
Quetiapine 10 mg/kg	320 ± 66	2200 ± 295	440 ± 59*	7156 ± 872	446 ± 40	317 ± 16
Quetiapine 30 mg/kg	231 ± 36	2482 ± 170	576 ± 41	6739 ± 499	423 ± 51	331 ± 32
Quetiapine 100 mg/kg	233 ± 34	2903 ± 311*	620 ± 38	6581 ± 566	422 ± 19	312 ± 25

Brain structure and treatment	Noradrenaline	DOPAC	HVA	Dopamine	5-HIAA	Serotonin
Hypothalamus						
Vehicle	1161 ± 253	405 ± 118	33 ± 11	679 ± 226	561 ± 88	530 ± 91
Sertindole 3 mg/kg	1222 ± 185	317 ± 107	34 ± 12	291 ± 76	528 ± 60	508 ± 74
Sertindole 10 mg/kg	1256 ± 60	291 ± 48	25 ± 7	445 ± 143	537 ± 25	489 ± 35
Sertindole 30 mg/kg	1407 ± 214	298 ± 44	45 ± 12	572 ± 235	675 ± 85	543 ± 109
Ziprasidone 10 mg/kg	929 ± 77	285 ± 48	41 ± 10	368 ± 117	703 ± 86	431 ± 78
Ziprasidone 30 mg/kg	1073 ± 238	647 ± 234	27 ± 17	616 ± 219	677 ± 116	462 ± 106
Ziprasidone 100 mg/kg	1017 ± 186	410 ± 153	29 ± 6	925 ± 308	721 ± 120	444 ± 85
Quetiapine 10 mg/kg	1094 ± 123	384 ± 114	42 ± 14	402 ± 138	598 ± 96	387 ± 63
Quetiapine 30 mg/kg	1207 ± 167	470 ± 149	38 ± 9	286 ± 49	627 ± 73	469 ± 67
Quetiapine 100 mg/kg	1041 ± 174	610 ± 139	47 ± 13	747 ± 176	628 ± 89	509 ± 77

Effect of increased dosages of atypical antipsychotics sertindole, ziprasidone and quetiapine on monoamine content in the rat brain in 60 min after acute i.p. administration. All data are expressed as ng/g wet weight tissue, (means±SEM; n/control group=8–9, n/drug group=6), *p< 0.05, ** p<0.01, ***p<0.001 as compared with vehicle-treated group (difference from control, Fisher`s LSD test after significant ANOVA).

Table 3. The effect of chronic administration of atypical antipsychotics in comparison with classical neuroleptic on monoamine turnover in rat brain

Brain structure and treatment		Noradrenaline	DOPAC	HVA	Dopamine	5-HIAA	Serotonin
Frontal cortex	***Exp.1***						
Vehicle		240 ± 22	81 ± 14	36 ± 7	30 ± 7	451 ± 25	501 ± 13
Sertindole 3 mg/kg		236 ± 14	62 ± 10	26 ± 3	34 ± 8	453 ± 30	553 ±16
Quetiapine 10 mg/kg		276 ± 27	94 ± 26	51 ± 10	30 ± 9	455 ± 41	496 ±28
Haloperidol 1 mg/kg		223 ± 42	66 ± 28	30 ± 5	28 ± 9	547 ± 24	489 ±16
	Exp.2						
Vehicle		566 ± 65	62 ± 34	35 ± 7	31 ± 8	334 ± 37	349 ± 26
Melperone 3 mg/kg		414 ± 33*	152 ± 37*	30 ± 4	50 ± 7*	296 ± 9	299 ± 11*
Ziprasidone 3 mg/kg		407 ± 38*	28 ± 20	16 ± 2**	27 ± 5	345 ± 20	278 ± 10**
Haloperidol 1 mg/kg[1]		378 ± 30**	77 ± 6	13 ± 3**	28 ± 3	327 ± 18	243 ± 19***
Striatum	***Exp.1***						
Vehicle		206 ± 81	2531.4 ± 345.4	898 ± 113	8242 ± 927	577 ± 36	387 ± 20
Sertindole 3 mg/kg		456 ± 16	1553.6 ± 157.0**	514 ± 57**	6538 ± 497	429 ± 25**	342 ± 26
Quetiapine 10 mg/kg		476 ± 171	2408.4 ± 190.6	741 ± 57	8379 ± 1353	609 ± 35	411 ± 46
Haloperidol 1 mg/kg		364 ±132	2511.8 ± 226.0	479 ± 41**	7804 ± 794	570 ± 20	350 ± 4
	Exp.2						
Vehicle		345 ± 47	2860 ± 444	410 ± 58	4837 ± 396	404 ± 34	226 ± 31
Melperone 3 mg/kg		351 ± 33	2941 ± 191	494 ± 34	5708 ± 277	405 ± 14	272 ± 12
Ziprasidone 3 mg/kg		365 ± 27	2932 ± 196	389 ± 27	5359 ± 403	405 ± 25	255 ± 24
Haloperidol 1 mg/kg		398 ± 68	2488 ± 150	324 ± 16	6845 ± 577**	401 ± 17	276 ± 18

Brain structure and treatment	Noradrenaline	DOPAC	HVA	Dopamine	5-HIAA	Serotonin
Hypothalamus ***Exp.1***						
Vehicle	2425 ± 80	150 ± 21	36 ± 6	291 ± 37	723 ± 17	773 ± 36
Sertindole 3 mg/kg	1945 ± 200	136 ± 8	25 ± 2	318.2 ± 11	734± 32	780 ± 33
Quetiapine 10 mg/kg	1967 ± 167	112 ± 17	59 ± 22	590 ± 169	743 ± 12	667 ± 36
Haloperidol 1 mg/kg	1870 ± 251	161 ± 36	32 ± 7	318 ± 46	815 ± 52	721 ± 19
Exp.2						
Vehicle	1435 ± 62	222 ± 36	21 ± 5	214 ± 17	462 ± 25	388 ± 32
Melperone 3 mg/kg	1454 ± 119	184 ± 17	18 ± 3	217 ± 12	486 ± 28	377 ± 7
Ziprasidone 3 mg/kg	1258 ± 107	244 ± 42	26 ± 6	281 ± 49	447 ± 37	399 ± 27
Haloperidol 1 mg/kg	1490 ± 124	245 ± 48	24 ± 6	215 ± 32	407 ± 23	420 ± 18

Effect of chronic (28 & 30 days) administration of atypical antipsychotics sertindole, quetiapine, melperone and ziprasidone in comparison with neuroleptic haloperidol on monoamine turnover in the rat brain in 48 h after last injection. All data are expressed as ng/g wet weight tissue (means±SEM; n/drug group = 7–8, n/control group = 8–9). *$p<0.05$, ** $p<0.01$, ***$p<0.001$ as compared with the corresponding vehicle group (Fisher`s LSD test after significant ANOVA).

The effect of 5-HT_{1A} agonist (8-OH-DPAT) and antagonist (WAY-100635) on the effects of atypical antipsychotics in open field test. The 5-HT_{1A} agonist 8-OH-DPAT (0.03–0.5 mg/kg i.p.) had statistical significant effect on line crossings [$F(3,28)$ 3.73; $p<0.05$ and $F(2,25)$ 11.9; $p<0.001$] and on rearings [$F(2,25)$ 18.9; $p<0.001$] in open field test measured in two different experiments (Fig. 8). Depending on base activity of rats, 8-OH-DPAT produced either increase or decrease of spontaneous locomotor activity. The 5-HT_{1A} antagonist WAY-100635 does not change locomotor activity dose-dependently significantly.

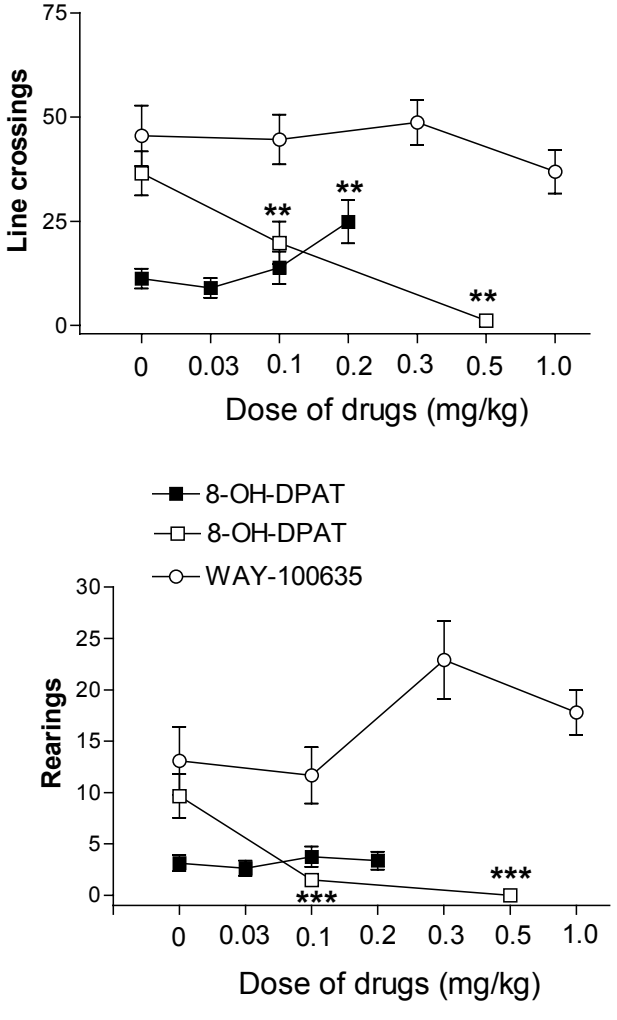

Figure 8. Dose-dependent effect of 5-HT_{1A} agonist and antagonist. All data are expressed means±S.E.M. **$p<0.01$, ***$p<0.001$ as compared with own vehicle (Fisher's LSD test).

Atypical antipsychotic sertindole (1 and 3 mg/kg) had no effect on rearing, but according to post-hoc tests after significant ANOVA [F(3,20) 4.09; p<0.05] together with 8-OH-DPAT (0.03 mg/kg) the rearing activity was strongly reduced by sertindole (3 mg/kg) (Fig. 9). The 5-HT_{1A} receptor antagonist WAY-100635 (0.3 mg/kg) potentiated the inhibitory action of sertindole (3 mg/kg) [F(5,50) 5.21; p<0.001]. In contrast, sertindole (1 mg/kg) in combination with WAY-100635 enhanced locomotor activity [F(5,50) 8.47; p<0.001].

The effect of ziprasidone (10 mg/kg) in open field test depends also on base activity of rats. In case of low base activity, ziprasidone enhanced locomotor activity on line crossings [F(3,20) 5.63 and 7.01; p<0.01 on rearings, respectively] and with high base activity it decreased [F(4,59) 5.07 and 7.75; p<0.001, respectively] (Fig. 10). Also, 8-OH-DPAT (0.03 and 0.1 mg/kg) acted the same way. A tendency of potentiation by 8-OH-DPAT (0.1 mg/kg) was observed with co-administration with ziprasidone, which reduced line crossings and rearings on their own. WAY-100635 (0.3 mg/kg) in combination with ziprasidone antagonized suppression of locomotor activity.

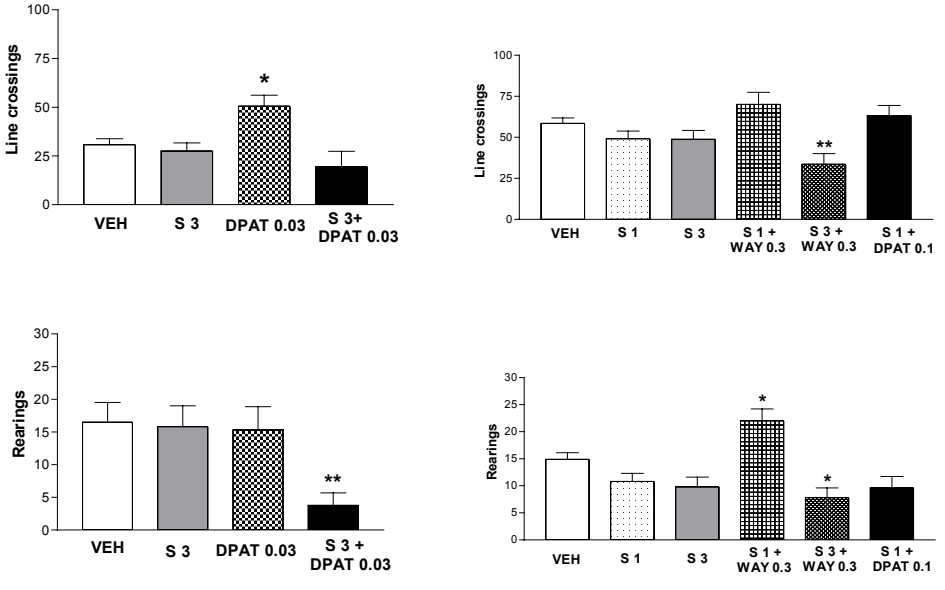

Figure 9. Effects of 5-HT_{1A} receptor agonist 8-OH-DPAT and antagonist WAY-100635 on the effect of sertindole in open field test**.** VEH = vehicle, S1 and S3 = sertindole 1 and 3 mg/kg, DPAT 0.03 and 0.1 = 8-OH-DPAT 0.03 and 0.1 mg/kg, S3 + DPAT 0.03 or S1 + DPAT 0.1 = sertindole 3 mg/kg + 8-OH-DPAT 0.03 or sertindole 1 mg/kg + 8-OH-DPAT 0.1 mg/kg, S1 + WAY 0.3 = sertindole 1 mg/kg + WAY 0.3 mg/kg. All data are expressed means±S.E.M. *p<0.05, **p<0.01 (Fisher's LSD test).

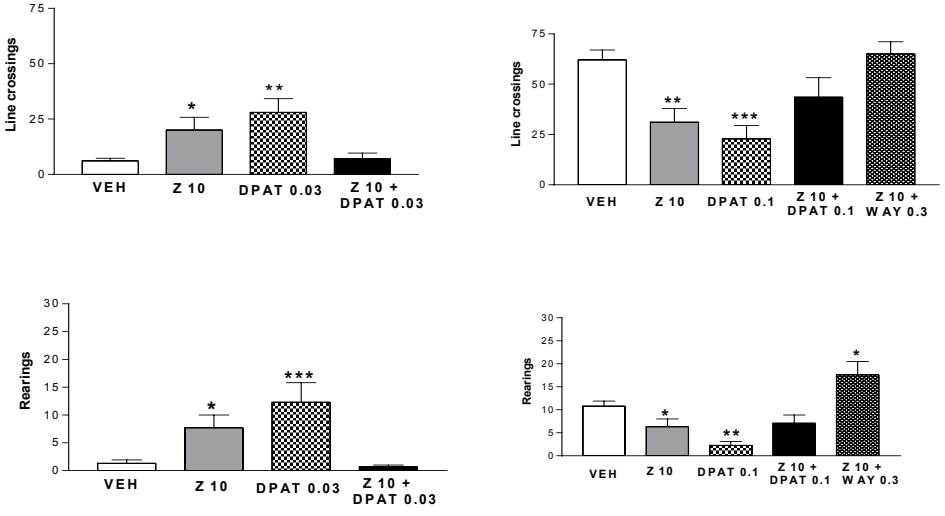

Figure 10. Effects of 5-HT_{1A} receptor agonist 8-OH-DPAT and antagonist WAY-100635 on the effect of ziprasidone. VEH = vehicle, Z10 = ziprasidone 10 mg/kg, DPAT 0.03 and 0.1 = 8-OH-DPAT 0.03 and 0.1 mg/kg, Z10 + DPAT 0.03 or 0.1 = Ziprasidone 10 mg/kg + 8-OH-DPAT 0.03 or 0.1 mg/kg, Z10 + WAY 0.3 = ziprasidone 10 mg/kg + WAY 0.3 mg/kg. All data are expressed means±S.E.M. *p<0.05, **p<0.01, ***p<0.001 (Fisher`s LSD test).

The effect of 5-HT_{1A} agonist (8-OH-DPAT) on the effects of second generation antipsychotics monoamine content in the studies of tissue level. In frontal cortex, acute treatment with melperone (3 mg/kg) and ziprasidone (30 mg/kg) had no effect on noradrenaline, dopamine or 5-HT as well as its metabolites content as compared with the vehicle-treated groups (Table 4). 8-OH-DPAT (0.1 mg/kg) decreased 5-HIAA in frontal cortex and in hypothalamus increased noradrenaline and decreased 5-HIAA content. Both sertindole (10 mg/kg) as single treatment and in combination with 8-OH-DPAT decreased significantly the content of 5-HT [F(3,28) 7.80; p<0.001]. Quetiapine (30 mg/kg) as single treatment significantly increased content of dopamine [F(3,24) 2.95; p<0.05], but in combination with 8-OH-DPAT decreased content of 5-HIAA [F(3,23) 23.4; p<0.001].

In striatum, melperone as single treatment increased dopamine, DOPAC, HVA levels [F(5,32) 7.02, 8.23, 10.6; p<0.001, respectively] and in co-administration with 8-OH-DPAT it decreased noradrenaline content [F(5,30) 12.7; p<0.001]. Ziprasidone increased the content of HVA [F(5,32) 10.6; p<0.001] and decreased noradrenaline level [F(5,30] 12.7; p<0.001]. Ziprazidone, in combination with 8-OH-DPAT, decreased contents of noradrenaline, dopamine and 5-HIAA significantly (Tables 4–6).

In hypothalamus, quetiapine, as a single treatment and also in combination with 8-OH-DPAT, decreased the content of 5-HIAA [F(3,24) 21.3; p<0.001]. Sertindole

as single treatement increased level of noradrenaline [F(3,27) 5.81; p<0.01], but in combination with 8-OH-DPAT decreased significantly the content of 5-HIAA [F(3,28) 4.53; p<0.01] in hypothalamus and also increased dopamine and 5-HT contents [F(3,28) 3.93; p<005, and 9.57; p<0.001, respectively] in hypothalamus. Ziprasidone, in combination with 8-OH-DPAT, increased the content of DOPAC [F(5,32) 2.64; p<0.05] and HVA [F(5,31) 4.41; p<0.001].

The effect of 5-HT_{1A} agonist (8-OH-DPAT) on the effects of atypical antipsychotics in microdialysis studies. Repeated measures ANOVA did not reveal any significant change in the 5-HIAA output after 8-OH-DPAT (0.05 mg/kg i.p.) and selected antipsychotics (Fig. 11).

One-way ANOVA for repeated measures revealed a significant effect of time [F(8,48) 4.24; p<0.001] on extracellular DOPAC levels. Further post-hoc analysis showed no effect of 8-OH-DPAT, but an increase of DOPAC after administration of of sertindole (3 mg/kg) and quetiapine (30 mg/kg). This increase continued to be present at approximately the same level in both experiments after 8-OH-DPAT treatment. Quetiapine (10 mg/kg) as a single treatment had no effect on serotonin and dopamine metabolites. Administration of 8-OH-DPAT after quetiapine (10 mg/kg) treatment did not change the levels of extracellular monoamines. Ziprasidone (10 mg/kg) had no effect on DOPAC levels.

ANOVA revealed a significant effect of treatment [F(2,5) 21.5; p<0.05], time [F(13,26) 42.1; p<0.001] and their interaction [F(65,26) 7.68; p<0.001] on HVA concentrations. 8-OH-DPAT as a single treatment increased the levels of HVA. Increased levels of HVA were observed after injection of quetiapine (30 mg/kg) and ziprasidone (10 mg/kg), and this effect was also continued after administration of 8-OH-DPAT. Sertindole (3 mg/kg) had no effect on the levels of HVA.

The effect of 5-HT_{2A} agonist (DOI) on second generation antipsychotics. Sertindole (3 mg/kg) had no significant effect on locomotor activity. Quetiapine and ziprasidone both at 30 mg/kg reduced the number of rears. Haloperidol (1 mg/kg), sertindole at 3 mg/kg, and quetiapine and ziprasidone both at 30 mg/kg reduced locomotion in the open field after 5-HT_{2A} receptor stimulation by DOI (1 mg/kg) (VII; Fig. 2–6).

Effect of acute and chronic treatment with antipsychotics on quipazine-induced wet-dog shakes. Acute administration of haloperidol (1 mg/kg), melperone (3 mg/kg), quetiapine (10 mg/kg), ziprasidone (3 mg/kg) and sertindole (3 mg/kg) significantly increased the latency to quipazine–elicited wet-dog shakes and reduced the number of shakes (Fig. 12). With the exception of quetiapine (10 mg/kg), the effect of quipazine was completely blocked.

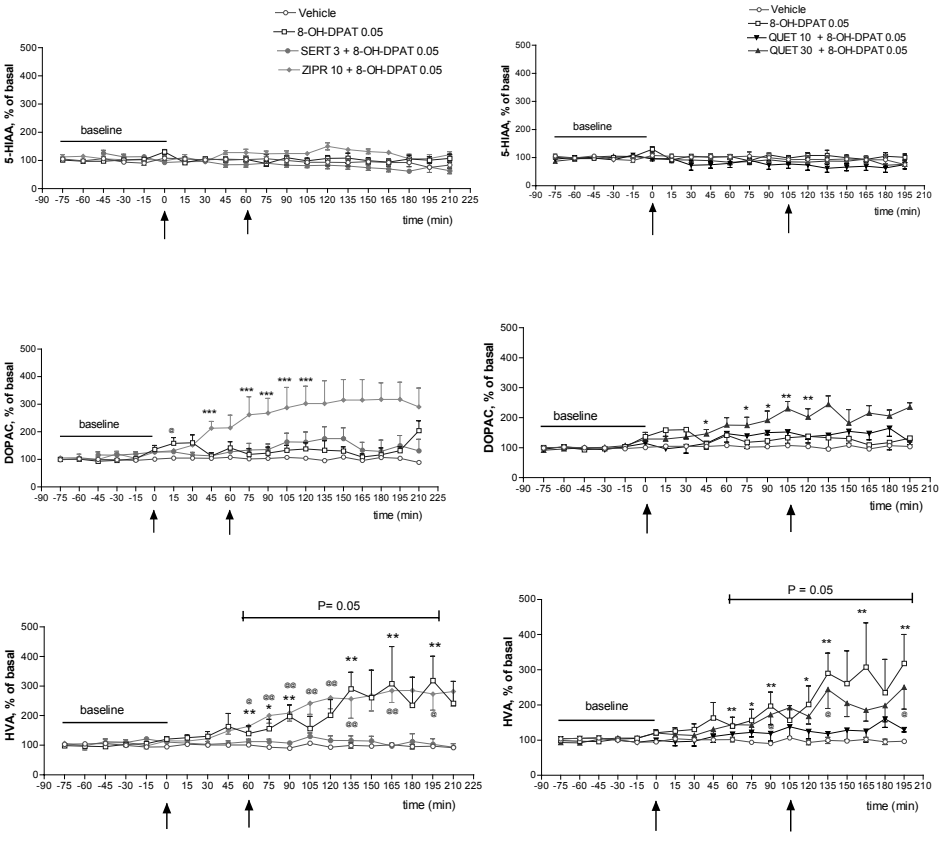

Figure 11. The effects of 8-OH-DPAT, atypical antipsychotics, and the combinations of them on dialysate concentrations of dopamine metabolites (DOPAC and HVA) and the metabolite of serotonin (5-HIAA) in the prefrontal cortex of freely moving rats. Atypical antipsychotics or 8-OH-DPAT as a single treatment were administered i.p. at the time indicated by first arrow. The second arrow indicated the time point of injection of 8-OH-DPAT in case of combined treatment. The results (means±S.E.M.) are expressed as percentages of six consecutive samples collected before any drug treatment (baseline; vehicle and 8-OH-DPAT n=8–9; drug combination group n=3–6).

After chronic treatment with haloperidol (1 mg/kg), melperone (3 mg/kg), and ziprasidone (3 mg/kg), the effect of quipazine was not changed (Fig. 13). In the experiment of chronic administration of quetiapine (10 mg/kg) and sertindole (3 mg/kg), the effect of quipazine was changed for latency and the number of head shakes. Thus, after withdrawal from chronic sertindole treatment, quipazine–elicited wet-dog shakes were still suppressed compared with vehicle and haloperidol treated groups, but after chronic quetiapine the effect of quipazine was significantly enhanced.

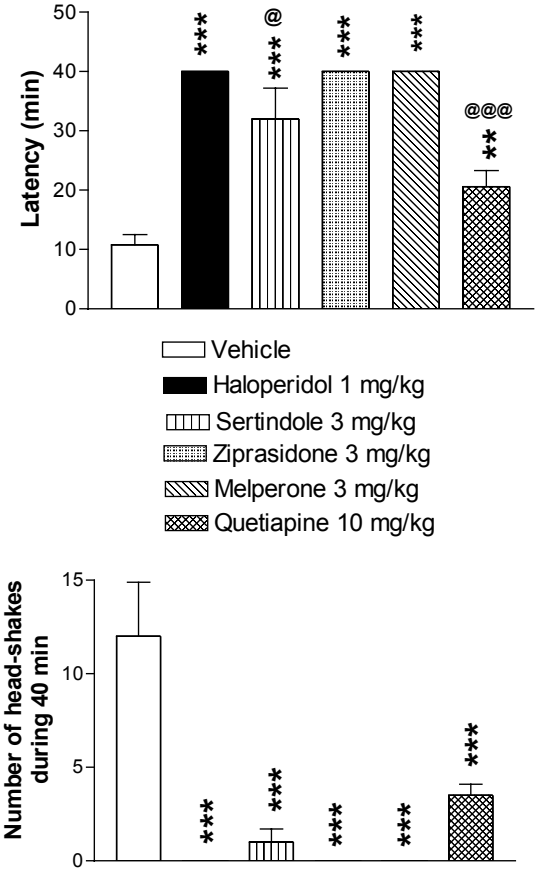

Figure 12. The acute effect of atypical antipsychotics sertindole, ziprasidone, quetiapine and melperone in comparison with typical neuroleptic haloperidol on quipazine-induced wet-dog shakes. Upper panel: latency of wet-dog shakes (minutes), lower panel: number of head shakes durning 40 minutes. N=8 per all groups. All data are expressed means±S.E.M. **p<0.01, ***p<0.001 as compared with control group; $^{@}$p<0.05, $^{@@@}$p<0.001 as compared with haloperidol treatment group (Fisher's LSD test).

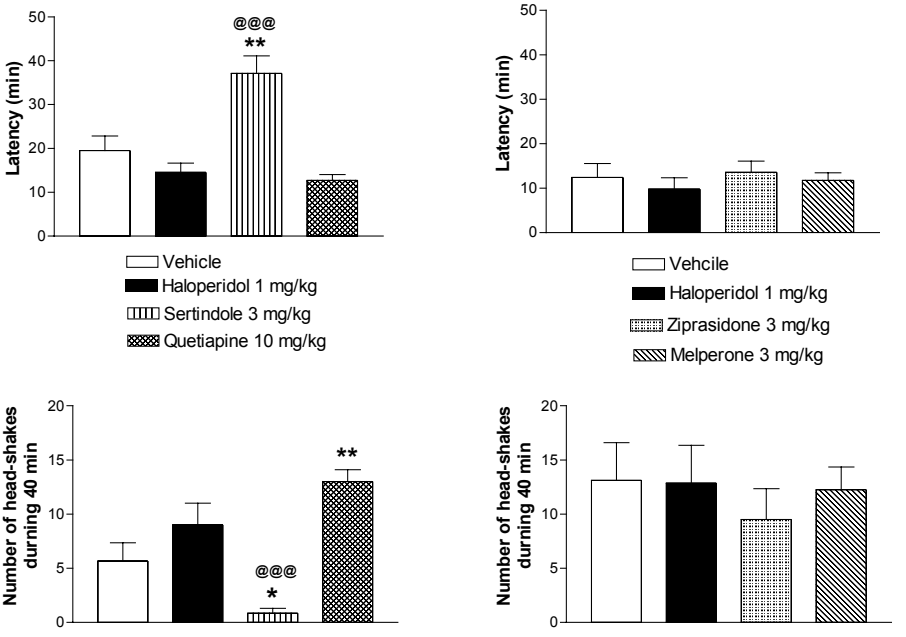

Figure 13. The effect of chronic administration (3 weeks) of atypical antipsychotics sertindole and quetiapine, melperone and ziprasidone in comparison with haloperidol on quipazine-induced wet-dog shakes in 48 hrs after last injection in rats. Upper panel: latency of wet dog shakes (minutes), lower panel: number of head shakes durning 40 minutes. n/control group=6–8, n/drug group=7–8. All data are expressed means±S.E.M. *p<0.05. **p<0.01 as compared with control group; $^{@@@}$p<0.001 as compared with haloperidol treatment group (Fisher`s LSD test).

Locomotor activity in combination with NMDA antagonists MK-801. Haloperidol and all used atypical antipsychotics have differences regarding interaction with glutamatergic neurotransmission. Sertindole (3 mg/kg) and ziprasidone (10–30 mg/kg) inhibited locomotion after MK-801 (0.1 mg/kg) treatment, but quetiapine (30 mg/kg) and olanzapine (3 mg/kg) did not. Haloperidol (0.1–1 mg/kg) induced dose-dependent changes in locomotor activity (VII; Fig. 1–6).

Table 4. Effect of melperone, ziprasidone and co-administration with 5-HT_{1A} agonist 8-OH-DPAT on monoamine turnover

Brain structure and treatment	Noradrenaline	DOPAC	HVA	Dopamine	5-HIAA	Serotonin
Frontal cortex						
Vehicle	380 ± 81	53 ± 14	52 ± 12	23 ± 4	424 ± 69	30 ± 9
Melperone 3 mg/kg	221 ± 80	28 ± 11	28 ± 5	28 ± 6	314 ± 71	41 ± 8
8-OH-DPAT 0.1 mg/kg	438 ± 61	47 ± 16	35 ± 11	30 ± 3	431 ± 58	50 ± 7
Melperone 3 mg/kg + 8-OH-DPAT 0.1 mg/kg	395 ± 59	179 ± 79	77 ± 24	130 ± 86	370 ± 40	44 ± 9
Ziprasidone 30 mg/kg	377 ± 54	117 ± 32	80 ± 18	45 ± 18	360 ± 45	34 ± 4
Ziprasidone 30 mg/kg + 8-OH-DPAT 0.1 mg/kg	496 ± 104	51 ± 15	66 ± 14	33 ± 2	344 ± 21	46 ± 11
Striatum						
Vehicle	623 ± 57	4356 ± 239	673 ± 47	3160 ± 438	645 ± 46	30 ± 5
Melperone 3 mg/kg	529 ± 56	7400 ± 827***	1687 ± 182***	4309 ± 545*	748 ± 35	33 ± 7
8-OH-DPAT 0.1 mg/kg	451 ± 49	3675 ± 591	570 ± 100	3542 ± 631	612 ± 102	40 ± 14
Melperone 3 mg/kg + 8-OH-DPAT 0.1 mg/kg	173 ± 67***	4675 ± 265	942 ± 108	3449 ± 214	613 ± 40	36 ± 6
Ziprasidone 30 mg/kg	245 ± 68***	5382 ± 368	1197 ± 131**	3101 ± 253	539 ± 22	33 ± 8
Hypothalamus						
Vehicle	1281 ± 131	194 ± 62	27 ± 11	479 ± 35	126 ± 20	37 ± 10
Melperone 3 mg/kg	1532 ± 111	269 ± 117	20 ± 12	530 ± 33	172 ± 42	58 ± 20
8-OH-DPAT 0.1 mg/kg	1718 ± 186	257 ± 198	22 ± 13	512 ± 116	214 ± 91	54 ± 6
Melperone 3 mg/kg + 8-OH-DPAT 0.1 mg/kg	1528 ± 148	224 ± 70	31 ± 7	475 ± 42	181 ± 36	47 ± 10
Ziprasidone 30 mg/kg	1462 ± 140	318 ± 112	46 ± 19	539 ± 34	198 ± 50	66 ± 25
Ziprasidone 30 mg/kg + 8-OH-DPAT 0.1 mg/kg	1211 ± 91	742 ± 190**	114 ± 29**	485 ± 36	299 ± 55	55 ± 17

The rats were killed 60 min after acute i.p. administration. All data are expressed as ng/g wet weight tissue (means ± SEM; n/drug group = 6–8 n/control group = 6). *$p<0.05$, ** $p<0.01$, $p<0.001$ as compared with the corresponding vehicle group (Fisher`s LSD test after significant ANOVA)

Table 5. Effect of quetiapine and its co-administration with 5-HT_{1A} agonist 8-OH-DPAT on dopamine and serotonin turnover

Brain structure and treatment	Noradrenaline	DOPAC	HVA	Dopamine	5-HIAA	Serotonin
Frontal cortex						
Vehicle	b.d.l.	52 ± 8	39 ± 5	21 ± 4	868 ± 37	38 ± 5
8-OH-DPAT 0.1 mg/kg	b.d.l.	98 ± 23	36 ± 2	28 ± 3	742 ± 47*	62 ± 18
Quetiapine 30 mg/kg	b.d.l.	110 ± 13	52 ± 5	43 ± 6**	705 ± 42**	67 ± 20
Quetiapine 30 mg/kg+ 8-OH-DPAT 0.1 mg/kg	b.d.l.	130 ± 44	50 ± 8	37 ± 6	466 ± 19***	51 ± 8
Striatum						
Vehicle	125 ± 114	2386 ± 349	549 ± 91	2576 ± 1174	626 ± 109	46 ± 33
8-OH-DPAT 0.1 mg/kg	237 ± 111	2196 ± 269	439 ± 39	1616 ± 400	472 ± 48	15 ± 4
Quetiapine 30 mg/kg	176 ± 58	3356 ± 333	802 ± 67*	1839 ± 226	498 ± 27	13 ± 3
Quetiapine 30 mg/kg+ 8-OH-DPAT 0.1 mg/kg	122 ± 30	2596 ± 195	690 ± 91	1711 ± 163	442 ± 27	16 ± 4
Hypothalamus						
Vehicle	784 ± 213	142 ± 37	26 ± 9	105 ± 14	404 ± 14	32 ± 7
8-OH-DPAT 0.1 mg/kg	863 ± 85	129 ± 40	51 ± 26	102 ± 24	281 ± 17***	19 ± 3
Quetiapine 30 mg/kg	932 ± 75	100 ± 8	17 ± 2	78 ± 11	321 ± 17***	22 ± 4
Quetiapine 30 mg/kg+ 8-OH-DPAT 0.1 mg/kg	647 ± 56	108 ± 14	20 ± 6	93 ± 15	243 ± 10***	24 ± 5

The rats were killed 60 min after acute i.p. administration. All data are expressed as ng/g wet weight tissue (means ± SEM, n/control group=6, n/drug group=6–8), b.d.l. – below detection limit, *$p<0.05$, **$p<0.01$, ***$p<0.001$ as compared with vehicle-treated group (Fischer`s LSD test after significant ANOVA).

Table 6. Effect of sertindole and its co-administration with 5-HT_{1A} agonist 8-OH-DPAT on monoamine turnover

Brain structure and treatment	Noradrenaline	DOPAC	HVA	Dopamine	5-HIAA	Serotonin
Frontal cortex						
Vehicle	452 ± 48	88 ± 36	42 ± 12	31 ± 6	332 ± 27	270 ± 27
8-OH-DPAT 0.1 mg/kg	428 ± 33	115 ± 31	27 ± 5	34 ± 5	395 ± 40	155 ± 34***
Sertindole 10 mg/kg	421 ± 28	123 ± 36	27 ± 5	44 ± 7	325 ± 20	138 ± 7***
Sertindole 10 mg/kg + 8-OH-DPAT 0.1 mg/kg	325 ± 38	80 ± 22	42 ± 8	46 ± 7	301 ± 6	143 ± 9***
Striatum						
Vehicle	422 ± 28	2532 ± 193	607 ± 61	6861 ± 407	362 ± 57	295 ± 14
8-OH-DPAT 0.1 mg/kg	339 ± 59	2004 ± 230	494 ± 64	7217 ± 661	391 ± 35	277 ± 30
Sertindole 10 mg/kg	331 ± 35	2513 ± 326	617 ± 88	7528 ± 311	387 ± 17	296 ± 11
Sertindole 10 mg/kg + 8-OH-DPAT 0.1 mg/kg	342 ± 29	2582 ± 150	801 ± 65	7161 ± 615	387 ± 35	305 ± 25
Hypothalamus						
Vehicle	882 ± 67	270 ± 50	20 ± 2	260 ± 16	487 ± 35	403 ± 14
8-OH-DPAT 0.1 mg/kg	1267 ± 74***	233 ± 49	29 ± 11	260 ± 26	467 ± 9	450 ± 15
Sertindole 10 mg/kg	1129 ± 83*	159 ± 12	21 ± 2	238 ± 13	426 ± 19	428 ± 16
Sertindole 10 mg/kg + 8-OH-DPAT 0.1 mg/kg	1015 ± 34	273 ± 41	39 ± 7	441 ± 98*	375 ± 22**	529 ± 24***

The rats were killed 60 min after acute i.p administration. All data are expressed as ng/g wet weight tissue (means±SEM, n/control group=8, n/drug group=8), *p<0.05, **p<0.01, ***p<0.001 as compared with vehicle-treated group (Fischer`s LSD test after significant ANOVA).

DISCUSSION

Characterization of apomorphine-induced aggressive behaviour

One of the main objectives of the present study was to characterize the apomorphine aggressiveness model with regard to its behavioural specificity and underlying neurochemistry.

It is well known that acute administration of an unselective direct agonist dopamine receptor apomorphine in high doses (>5 mg/kg) causes aggressiveness in rats (Lapin and Samsonova, 1968; Schneider, 1968; Senault, 1970). Repeated treatment (10–15 days) with small (0.15–0.2 mg/kg) or moderate doses (0.5–1 mg/kg s.c. once or twice daily) induces spontaneous defensive-aggressive behaviour in male rats (Allikmets and Vasar, 1982; Porreca *et al.,* 1982).

Similar to our previous experiments (Matto and Allikmets, 1998; Matto *et al.,* 1999; Skrebuhhova-Malmros *et al.,* 2001) as well as in other studies (Kostowski *et al*., 1986; Kask and Harro, 2000), the repeated administration of a low dose of apomorphine induced sensitization as evidenced by increased aggressiveness. Thus the experimental conditions were appropriate for further neurochemical studies. Likewise, as in the experiments of Lang *et al*., (1994; 1995), the first signs of aggressive behaviour in animals were already observed on the 3–6th day of apomorphine administration, whereas onwards from the 9th day of the experiment, the majority of the tested animals became clearly aggressive. Similarly, the time of latency before the first attack was shortened test-to-test. On the other hand, in female rats the development of aggressive behaviour was not observed in the conditions of the present study. Thus, apomorphine-induced aggressive behaviour cannot be used in studies on females, at least with a similar design as in males.

Our results demonstrated that apomorphine-induced aggressiveness developed with repeated low dose apomorphine treatment only with fighting experience, which is in good agreement with previous results of Gotsick *et al.,* (1975).

The neurochemical basis of apomorphine-induced aggressiveness is not clear. Apomorphine is known to have only a very weak affinity to other receptors except those of the dopamine receptor subtypes. The development of apomorphine-induced sensitization may involve, besides the dopaminergic (Võikar *et al.,* 1999), also serotonergic neurotransmission (Matto *et al.,* 1998) and adrenergic system (Troncone and Tufik, 1991).

Though the neuropharmacology of DSP-4 has been well characterized already in eighties by the Ross' group (Jonsson *et al*., 1981; Hall *et al.,* 1984), only in 1996 Harro and Oreland proposed that the DSP-4 treated rats might be considered an animal model corresponding to human affective disorders (Harro and Oreland, 1996). Since aggressive and autoaggressive behaviour are often

symptoms of affective disorders, the DSP-4 pre-treatment could provide additional information on the neurobiology of `pathological` aggressiveness. Administration of DSP-4 (50 mg/kg i.p.) causes a very selective, long-lasting and massive (*ca* 90%) reduction in noradrenergic terminals in the locus coerelus projection areas (Ögren *et al.,* 1980). Locus coereleus neurons play an inhibitory role in apomorphine-induced aggressiveness (Pucilowski *et al.,* 1986).

In the present study DSP-4 pre-treatment accelerated the development of aggressive behaviour. Because DSP-4 does not directly impair the dopaminergic neurotransmission, the accelerated development of aggressive behaviour may be implicated in the dysregulation of adrenergic receptors. The latter idea is supported by the fact that desipramine, a selective noradrenaline reuptake inhibitor, has been reported to act as a proaggressive drug in this paradigm (Kostowski *et al.,* 1986; Matto *et al.,* 1998).

Apomorphine, a dopamine receptor agonist, has been found to induce a dose-dependent decrease in the content of dopamine metabolites DOPAC and HVA in the striatum of rats (Bacopoulos and Roth, 1981). Apomorphine decreased both extracellular dopamine and its metabolites in the rat striatum (Saller and Salama, 1985; Imperato *et al.,* 1988; Ozaki *et al.,* 1989; Liu *et al.,* 1996, Saul`skaya, 1997).

Our results demonstrated that repeated apomorphine treatment moderately reduced the *post mortem* dopamine but increased the DOPAC and HVA and 5-HIAA contents in striatum. Other monoamines or their metabolites were unchanged. While our results are consistent with other studies regarding dopamine levels, the increase in the levels of dopamine metabolites is at variance with previous findings. This inconsistent effect of apomorphine can explain with differences of measuring methods *in vivo* microdialysis after acute administration and measuring of tissue level after repeated administration, respectively.

Our experiments revealed that repeated apomorphine treatment reduced the dopamine content in all four brain regions studied, when the animals were decapitated immediately after the last test of aggressiveness. The concentration of dopamine in the frontal cortex was two fold higher in the apomorphine-nonaggressive animals and in the animals which had been decapitated 24 hours after the last injection, as compared with the control animals or apomorphine-aggressive animals or with animals which had been decapitated immediately after last apomorphine injection. Further, the dopamine metabolism was intensified at this point in time as evidenced by the increased *post mortem* DOPAC and HVA contents. However, in other brain regions this effect was opposite. It can be speculated that such bi-directional effects are complex compensatory reactions to the repeated apomorphine treatment or might be considered as a rebound effect developed in some brain structures, which masked the actual changes of monoamine content during the aggressive contacts. It should be borne in mind that the dose of apomorphine (1 mg/kg s.c.) was sufficiently high to activate both pre- and postsynaptic D_2 receptors in rats

(Knapp and Kornetsky, 1996; Bartoszyk, 1998). Therefore, it is possible that the monoamine content 1 day after the last apomorphine injection might vary between different brain regions due to differences in action on the respective receptor subpopulations. The overall finding is that repeated apomorphine treatment dysregulates the catecholaminergic, but not serotonergic, neurotransmission.

The sensitivity of dopaminergic signal transduction system is determined by the efficacy of receptors coupling with G-proteins. Here the affinity of GDP for G-proteins is a key parameter in the determination of signal transduction (Rinken *et al.,* 2001), and changes in this may cause changes in receptor sensitivity. For example, the 6-hydroxydopamine induced unilateral lesions of the nigrostriatal system, which cause prolonged loss of dopamine nerve terminals, caused a decrease in the affinity of GDP for the G-proteins. It is probable that when the affinity of GDP is lower, less receptors are required to activate the same number of G-proteins, causing the higher sensitivity of the receptors (Terasmaa *et al.,* 2000). In the present study, the increase in the affinity of GDP appears to reduce the dopamine receptor sensitivity, resulting in lower dopamine-stimulated [^{35}S]GTPγS binding. Thus, chronic administration of dopamine receptor agonist downregulated D_2 receptor sensitivity by changes in GDP affinity to G-proteins. Interestingly, this downregulation of sensitivity was not present when the animals had the possibility to fight and had developed aggressiveness. This means that the development of apomorphine aggressiveness is caused by the absence of desensitizaton of D_2 receptors due to the missing changes at the level of G-proteins. What limits the development of this alteration when the animals have the regular fighting experience remains to be elucidated.

In our experiments, repeated apomorphine treatment had no effect in the open field test, quipazine-induced wet-dog shakes test or the forced swimming test, irrespective of whether the animals had developed aggressiveness or not.

In the study of Võikar *et al*. (1999) the authors found, using the same Wistar rat line from Kuopio National Animal Centre, that the changes in the stereotyped behaviour as a consequence of repeated apomorphine treatment (0.5 mg/kg) do not correlate with the increased locomotor activity. Because increased locomotor activity is one of the most important components, which may precipitate the development of aggressiveness, it should be borne in mind that the changes in the monoamine content found in our present study are valid only for the “high apomorphine responders”.

Furthermore, it should be kept in mind that psychosis and psychotic behaviour in humans are not only a result of hyperactivity of the dopaminergic system but rather a complex of neurochemical and -biological changes in CNS only partially known yet (for a review, see Harrison, 1999). With this regard, especially the serotonergic system should be taken into consideration.

Possible role of 5-HT receptors in the neurobiology of apomorphine-induced aggressive behaviour

5-HT$_1$ receptors. In the present study, the 5-HT$_{1A}$ receptor partial agonist buspirone had a weak or no antiaggressive effect at low dosages (0.5 and 1 mg/kg), but at higher doses (2.5 and 5 mg/kg) it completely blocked the apomorphine-induced aggressiveness, which is in line with a previous report of Matto *et al.* (1998). Sanchez *et al.* (1993) have also demonstrated the anti-aggressive effect of buspirone, but in isolation-induced aggressiveness paradigm in male mice. Another 5-HT$_{1A}$ receptor partial agonist gepirone, which shares with buspirone the 5-HT$_{1A}$ agonistic properties but blocks D$_2$ receptors only weakly, was considerably less active in the apomorphine aggressiveness test. The maximal dose of gepirone (10 mg/kg) used in our study is sufficient to have a maximal activity for the 5-HT$_{1A}$ receptors. With this regard, our study is in good agreement with the experiments of Piercy *et al.* (1994), where it was found that buspirone binds to the D$_2$ receptors with considerably higher affinity as compared with gepirone or 8-OH-DPAT. Furthermore, it has been reported that the partial agonists of 5-HT$_{1A}$ receptors act predominantly at the pre-synaptic and/or autoreceptor level. It can be excluded that the tendency of the lowest dose of 8-OH-DPAT used in our study (0.1 mg/kg) to abolish the apomorphine-induced aggressive behaviour can also be associated with the presynaptic/autoreceptors of the 5-HT$_{1A}$ receptor type.

5-HT$_2$ receptors. We did not specially observe the development of the stereotyped behaviour in rats, but from earlier experiments it is known that drugs which attenuate the apomorphine-induced aggressive behaviour, attenuate also stereotyped behaviour (Allikmets and Vasar, 1982). Trazodone seems to have a pharmacological profile similar to those compounds, therefore it is not surprising that it decreases the development of apomorphine-induced aggressive behaviour. However, the environmental cue (or test context) and Pavlovian conditioning are not the major determinants of this test — otherwise the aggressiveness would be elicited in sensitised rats without apomorphine challenge. Furthermore, because the repeated quipazine challenge did not have any major effect on the development of apomorphine-aggressiveness, while the acute quipazine treatment was effective in the pre-sensitized rats, it is likely that other neurobiological mechanisms besides D$_2$ receptor blockade are different between expression of apomorphine aggressiveness and its development. It could be inhibition of dopaminergic terminals via 5-HT$_2$ heteroreceptors.

In our apomorphine–induced aggressive behaviour experiments, 5-HT$_{2A/2C}$ receptor agonist DOI had a strong tendency toward the enhancement of the intensity of aggression and shortening of the time of latency before the first attack. However, both of these effects were statistically not significant, but the latter was quite close to the significance level. An effect in this direction would

have been expected, because from one hand, as a consequence of repeated apomorphine administration the [^{3}H]ketanserin-sensitive 5-HT_{2A} receptors are upregulated (Matto *et al.,* 1999), and on the other hand, the 5-HT_{2A} receptor antagonists act as antipsychotic drugs in humans (Conley, 2000). The failure to demonstrate a statistically significant effect might be explained with the relatively high intensity of aggressiveness of the vehicle-treated animals, *i.e.* the aggressiveness was close to the maximum and could not be further enhanced by a 5-HT_{2A} receptor agonist. DOI increased isolation-induced aggressive behavior (Sakaue *et al.,* 2002). It has been demonstrated that the 5-HT_2 agonist DOI has an antiaggressive effect only at high doses (Sanchez *et al.,* 1993). In our experiments, neither ketanserin nor ritanserin alone (5-HT_2 antagonists) showed to have an antiaggressive effect in apomorphine-induced aggressiveness.

5-HT_3 receptors. 5-HT_3 receptor agonist mCPBG did not have a gross effect on the apomorphine-induced aggressive behaviour. The attenuation of the aggressive behaviour as a consequence of the other 5-HT_3 agonist 1-PBG treatment might be due to the peripheral effect of this drug, and evidently does not reflect the central 5-HT_3 receptor-mediated effect of this compound.

Our experiments have failed to demonstrate the antiaggressive effect of 5-HT_3 antagonist ondansetron, which is in good agreement with previous studies (Sanchez *et al.,* 1993). The 5-HT_3 receptor antagonists MDL-72222 and, to lesser extent, tropisetron, reduced the intensity and increased the latency of apomorphine-induced aggressiveness. Already years ago it has been demonstrated that neither MDL-72222 nor tropisetron have affinity to the dopamine D_2 receptors (Hamik and Peroutka, 1989). Thus, the antiaggressive effect of MDL-72222 and tropisetron is due to the 5-HT_3 receptor antagonism. In our experiments, the doses of these drugs were chosen on the basis of reference experiments (Higgins *et al.,* 1993; Hong and Menses, 1996) where the behavioural tests were used.

The effects of atypical antipsychotics on the apomorphine-induced aggressiveness. All examined atypical antipsychotics were found to reduce the expression of apomorphine aggressiveness. In terms of antiaggressive properties, melperone appeared to be more potent as compared with sertindole, ziprasidone and quetiapine. In conclusion, the atypical antipsychotics were quite weak antagonists of apomorphine-induced aggressiveness as compared to the effect of haloperidol. Risperidone and haloperidol have high affinity for dopamine D_2 and D_3 receptors. The last compound also has high affinity for α_2-adrenergic receptor and moderate affinity for the serotonin 5-HT_{1A} receptor, while risperidone possesses considerable affinity for the dopamine D_1 receptor (White *et al.,* 1991). Based on the affinity data it can be proposed that risperidone and haloperidol inhibited apomorphine-induced aggressiveness by dopaminergic but not serotonergic mechanism.

The more pronounced antiaggressive effect of melperone may be due to a number of reasons. The ratio of D_2/5-HT_{2A} equilibrium dissociation constants

are for sertindole, ziprasidone, and quetiapine in the range 20–30, but for melperone only *ca*. 1.8 [calculated from values originally given in the reference Richelson and Souder (2000)]. This indicates a relatively stronger dopamine D_2 vs serotonin 5-HT_{2A} blocking effect of melperone, which would be of importance if 5-HT_{2A} receptor blockade reduces the effect of D_2 receptor blockade against apomorphine aggressiveness. Rowlett and collaborators (1997) found that after repeated apomorphine treatment the basal dopamine synthesis is enhanced, but the role of the serotonergic neurotransmission in this paradigm remains unclear. Alternatively, melperone can be more efficient because of its considerable dopamine D_4 receptor blocking properties (Lahti *et al.,* 1993). The weaker 5-HT_2 blockade does not allow the increased release of dopamine. Neither the differences in the antimuscarinic or histamine H_1 receptor blocking properties appear to be involved in the regulation of the apomorphine-induced aggressiveness.

Risperidone in doses 0.5 and 1 mg/kg attenuated significantly the intensity of aggressive behaviour and prolonged the time before the first attack as well. Risperidone and DOI coadministration still elicited a significant decrease of aggressiveness and increased the time of latency in comparison with respective vehicle group. The expected effect of DOI in combination with risperidone would have been the antagonism at 5-$HT_{2A/2C}$ receptors, but in our experiment, no such effect was found. In the case of risperidone and haloperidol co-administration, already minimal effective dose 0.03 mg/kg, of haloperidol potentiated the antiaggressive effect of risperidone and this behaviour in rats was blocked completely. This additive effect of haloperidol and risperidone further confirms the idea that D_2 receptor antagonism is the most important mechanism of action in antiaggressive profile of risperidone. Nevertheless, since the repeated apomorphine treatment may induce dysregulation of both pre- and postsynaptic dopamine receptors, it cannot be ruled out that the additive effect of risperidone and haloperidol might be, at least in part, associated with non-specific motor-suppressive properties of haloperidol.

Effects of atypical antipsychotics on exploratory behaviour and monoamine levels in brain

Spontaneous locomotor activity is regulated by several neurotransmitters (Panksepp, 1986). Consistently with this, in the present study the acute locomotor response to a brief exposure to a novel environment was influenced by different pharmacological mechanisms (e.g., dopamine antagonism, glutamate antagonism, 5-HT_{2A} agonism and antagonism etc). Behaviour in the open field is influenced by a number of factors including cognitive, affective and motivational activity induced by novelty (Harro *et al.,* 1995). The interference of these activities, and their simultaneous modulation by psychoactive drugs, can

result in inconsistent responses to specific treatments or complex dose-dependency in the effects of specific drugs.

Typical neuroleptics such as haloperidol are known to potently reduce locomotor activity. However, in low dosage (<0.1 mg/kg), haloperidol has been found to significantly increase locomotor activity in rodents (Strombom, 1997; Frussa-Filho *et al.,* 1997; Mori *et al.,* 2004), even though this is not reproduced in all conditions (Mele *et al.,* 1996). In the present study, haloperidol increased locomotor activity at a low dose (0.1 mg/kg), lost this effect when the dose was increased, and produced a reduction in locomotion at even higher doses.

Depending on baseline-activity of rats, ziprasidone produced either increase or decrease of spontaneous locomotor activity. Sertindole had no effect on locomotor activity in open field test.

It has been found in many studies that acute administration of typical antipsychotic drugs increased dopamine turnover, and that repeated administration induces tolerance to the enhanced dopamine level and metabolism in the striatal tissue (Burki *et al.,* 1974; Sayers *et al.,* 1975; Waldmeier and Maitre, 1976; Bowers and Rozitis, 1976; Lerner *et al.,* 1977; Scatton, 1977; Stanley and Wilk, 1980). Furthermore, Chang *et al.* (1989) found decreased levels of HVA after withdrawal from chronic haloperidol treatment, and Karolewicz *et al.* (1996) found that dopamine metabolism (both metabolites) was significantly depressed in the striatum after 9 days of withdrawal from the chronic treatment with haloperidol. In our experiments, acute treatment with atypical antipsychotics elicited an increase in dopamine metabolism in the striatum as it has been reported for haloperidol. Thus, when given acutely, the neuropharmacological effects of classical and atypical antipsychotics do not differ in this regard. Regarding the effect of haloperidol and atypical antipsychotics upon chronic administration, the results were more ambiguous as the effect of haloperidol was different in the two experiments. This inconsistency of the effect of haloperidol is, as suggested by the literature reviewed above, not surprising, but remains at present unexplained. It could be argued that tissue levels of monoamines are inferior measures to *in vivo* neurochemistry, but indeed the effect of repeated administration of haloperidol on basal extracellular levels of dopamine is similarly inconsistent. It has been reported that basal striatal dopamine release after chronic haloperidol was increased (See *et al.,* 1995), decreased (Ichikawa and Meltzer, 1990; 1992) or unchanged (Yamamoto and Cooperman, 1994). Interestingly, however, it seems that when haloperidol had an effect on tissue monoamine levels, this effect was similarly present with atypical antipsychotics.

Acute administration of antipsychotic drugs blocks dopamine receptors and produces a compensatory increase in dopamine cell firing that causes an elevation in the dopamine release and metabolism (Westerink and Kikkert, 1986; Imperato and Di Chiara, 1988; Arnt and Skarsfeldt, 1989). The effect of atypical antipsychotics is likely to be based on a similar mechanism of action, and thus the dopamine receptor blocking potential at the doses used was comparable to haloperidol.

In microdialysis studies, typical and atypical antipsychotic drugs most commonly produce relatively large increases in dopamine and its major metabolites, DOPAC and HVA, in either the prefrontal cortex or striatum, or in both of these brain regions (Volonte *et al.,* 1997; Westerink *et al.,* 1998; Watanabe and Hagino, 1999). In our experiment sertindole increased DOPAC, ziprasidone increase HVA and quetiapine increased both of the dopamine metabolites. This increase was enhanced after 8-OH-DPAT treatment. Systemic administration of sertindole enhanced dopamine release and extracellular concentration of DOPAC and HVA in the prefrontal cortex (Watanabe and Hagino, 1999; Fink-Jensen, 2000). In our experiments sertindole had no effect on the levels of HVA or on DOPAC.

Earlier studies have quite consistently shown that 5-HT_{1A} receptor agonists elicit dopamine release in the prefrontal cortex (Bantiack *et al.,* 2001). In our experiments 8-OH-DPAT as a single treatment showed no effect on extracellular DOPAC, but increased the levels of HVA.

Effect of atypical antipsychotics on glutamatergic system. MK-801, a non-competitive NMDA receptor antagonist is known to modify locomotor activity in rodents in a dose-dependent manner (Liljequist *et al.,* 1991; Ögren and Goldstein, 1994; Mele *et al.,* 1996; O`Neill *et al.,* 1998; Ninan and Kulkarni 1998; 1999; Andiné *et al.,* 1999). Acute administration of MK-801 in doses up to 0.1 mg/kg has usually no effect on locomotor activity (Carey *et al.,* 1998; Jacobs *et al.,* 2000), which is in agreement with our previous unpublished results, according to which acute administration of MK-801 in doses 0.025–0.1 mg/kg did not affect locomotion in our open field test. In the present study, MK-801 (0.1 mg/kg) was administered in eight independent experiments: it had no effect on horizontal locomotor activity in six experiments (but reduced rears in one and increased in another experiment), and stimulated the vertical activity in two experiments out of eight. Thus, the 0.1 mg/kg dose of MK-801 provides a borderline effect on locomotor activity rather in the direction of stimulation. This is similar to the results of different laboratories which had found in Wistar rats either no stimulation (Druhan *et al.,* 1993) or hyperactivity (Robledo *et al*., 1991; Hargraves and Clein, 1992) in this dose-range. There is no obvious and clear explanation as to what are the reasons for MK-801 at this dose to elicit stimulation only in a few experiments.

Haloperidol has been found to block completely the stimulation produced by MK-801 (Maj *et al*., 1991; Losher and Honack, 1992; Ögren and Goldstein, 1994; Andiné *et al.,* 1999), and this was found also in the present study at a dose level, which by itself, did not reduce locomotor activity. Interestingly, co-administration of MK-801, which in this specific experiment had no independent effect, and the locomotor activating dose of haloperidol further increased locomotor activity. This may suggest that low doses of haloperidol preferentially block a subpopulation of dopamine receptors, which inhibit locomotor activation, and this action is associated with glutamate release, which limits the activation.

Several lines of evidence indicate that the characteristic, stereotypic behavioural stimulation evoked in rats by non-competitive NMDA receptor antagonists such as PCP and MK-801, particularly the hyperlocomotion, is largely mediated via activation of dopaminergic mechanisms through an indirect mechanism, over the reduction of glutamatergic activity (Liljequist *et al.,* 1991) leading to dopamine release (Svensson, 2003).

Sertindole has been found to reduce PCP-induced stereotyped behaviour (Sams-Dodd, 1997) and locomotor activity, whereas the latter effect was slightly stronger than its anti-amphetamine effect (Jackson *et al.,* 1994). We found that sertindole reduced locomotor activation elicited by glutamate receptor antagonism.

Quetiapine has been found to antagonize PCP-induced deficits in sensorimotor gating of the startle response (Bakshi *et al.,* 1994; Swerdlow *et al.,* 1996) and reliably reduced the level of PCP-induced stereotyped behaviour and had distinct effects on PCP-induced social isolation (Sams-Dodd, 1997).

Ziprasidone reversed the impairment caused by PCP (Abdul-Monim *et al.,* 2003) and attenuated the disruptive effect of ketamine on prepulse inhibition (Mansbach *et al.,* 2001). In our study, ziprasidone blocked the MK-801 induced locomotor activation.

Olanzapine (0.5–2 mg/kg) dose-dependently increased spontaneous locomotor activity in mice, whereas this effect does not occur at somewhat higher doses (4 mg/kg) (Gleason and Shannon, 1997; Ninan and Kulkarni, 1999). In our experiments in rats, olanzapine (3 mg/kg) decreased both horizontal and vertical locomotor activity. While it has been found previously that olanzapine blocked both the stereotypy and hyperlocomotion induced by MK-801 in mice (Ninan and Kulkarni, 1999), olanzapine pretreatment had only a minor effect against MK-801 in the present study even though olanzapine-treated rats were markedly sedated.

Thus, while there were signs of antagonistic action between DOI, the 5-HT_{2A} agonist and all antipsychotics, there was no similar consistent interaction between antipsychotics and the blockade of glutamatergic neurotransimission. Sertindole and ziprasidone did interact with administration of MK-801 more clearly than quetiapine and olanzapine. All atypical antipsychotics have a broad spectrum of receptor activity, but the two latter drugs are most clearly distinguished by additional action on H_1 and muscarinic receptors.

Experimental studies have revealed that dopaminergic, glutamatergic and histaminergic action are all connected to the improvement of cognitive functions (Byrely *et al.,* 2001). These mechanisms can be modulated by the blockade of presynaptic 5-HT_2, and D_3 and H_3 receptors, elicited by atypical antipsychotics and resulting in increased release of dopamine and histamine (Ito *et al.,* 1996). Glutamate release depends upon 5-HT_{2A} receptor function. This means that the effect of atypical antipsychotic is associated with indirect glutamatergic effect over 5-HT_{2A} receptors blockade. Taking into consideration that the stimulation of presynaptic 5-HT_2 heteroreceptors on glutamatergic ter-

minals is inhibiting the release of glutamate, the antagonism of atypical antipsychotics on 5-HT_2 receptors could elicit increased release of glutamate and some improvement of cognitive functions.

Effect of atypical antipsychotics on serotonergic system. The behavioural effects of different (putative) neuroleptics were differentially influenced by both 8-OH-DPAT and DOI (Ellenbroek *et al.,* 1994), which are in good agreement with our results, suggesting that there are important differences between the neuronal mechanisms underlying the behavioural effects of these neuroleptic drugs, even within the subclasses of classical and atypical neuroleptics. In rodents, ziprasidone does exhibit 5-HT_{1A} receptor agonist properties (Wadenberg and Ahlenius, 1991; Seeger *et al.,* 1995), inhibited locomotor activity and this effect was reversed by co-administration of WAY-100635 (Carey *et al.,* 2004), which has also demonstrated our experiments. The 5-HT_{1A} receptor antagonist WAY-100635 potentiated the inhibitory action of sertindole, but enhanced if sertindole was used in a higher dose. It can be explained with inverse agonist activity at 5-HT_{1A} receptor of sertindole (Cosi and Koek, 2001). Also WAY-100635 behaves as a strong inverse agonist (Prinssen *et al.,* 2002).

Antipsychotic agents, as a class of drugs, effectively reduced locomotion in DOI-treated animals in our experiments, which is in good agreement with the literature (Wettstein *et al.,* 1999).

To study serotonergic activity we used quipazine-induced wet-dog shake response in rats caused by agonistic effect on 5-HT_{2A} receptors (Sanchez and Arnt, 2000). This response also requires a functionally intact D_1 and D_2 system and is subject to modulatory inhibitory influences by postsynaptic 5-HT_{1A} receptors (Schreiber *et al.,* 1995). Haloperidol (0.2–1 mg/kg i.p.) has been found to reduce significantly and dose-dependently the number of wet-dog shakes (Araki *et al.,* 1988). Consistently, our results demonstrated a similar effect of haloperidol, and acute administration of all used atypical antipsychotics also suppressed the quipazine–elicited wet-dog shakes, which is in a good agreement with previous results with clozapine, risperidone, olanzapine and sertindole (Sanchez and Arnt, 2000; de Angelis, 2002). After 48 h withdrawal from the continuous treatment, with haloperidol, ziprasidone and melperone their action of quipazine effect did not differ from the control, but sertindole inhibited quipazine effect and chronic administration of quetiapine increased the effect of $5HT_{2A}$ receptor stimulation by quipazine. The reason why the effect of sertindole was similar to its acute administration could be in its slow biotransformation (Ereshefsky, 1996; Tamminga, 1997). Thus, after withdrawal from chronic administration of sertindole, its presence in the organism remains likely to account for the effect against quipazine. In contrast, quetiapine was the only antipsychotic, which elicited sensitization of 5-HT_{2A} receptor. Behavioural sensitization in quetiapine-treated rats appears also in spontaneous activity in the open field test, but withdrawal from quetiapine did not elicit wet-dog shakes by itself.

CONCLUSIONS

The experimental results lead to the following conclusions:

1. Repeated apomorphine treatment (1 mg/kg s.c, once daily during two weeks) induces in about 80 per cent of male Wistar rats a gradual development of aggressive behaviour in response to apomorphine injection. Only adult male rats fully fill the criteria of validity for model of "psychotic" aggressive behaviour. Female rats do not fight in response to repeated apomorphine treatment.

2. Sensitization to apomorphine-induced aggressiveness developed only with accumulating experience of fighting during treatment. This behaviour appeared to be mediated by changes at the D_2 receptor — G-protein interaction. Chronic administration of the dopamine receptor agonist apomorphine downregulated D_2 receptor sensitivity by changes in GDP affinity to G-proteins only in animals who had not developed aggressiveness.

3. DSP-4 (noradrenergic neurotoxin, sensitizing noradrenaline receptors) pretreatment significantly accelerated the development of apomorphine-induced aggressive behaviour. Repeated treatment with 5-HT_2 antagonists (trazodone, ketanserin, ritanserin), but not 5-HT_2 agonist (quipazine) challenge slows down the development of apomorphine-induced aggressiveness without having a major impact on the apomorphine-induced enhancement of the dopaminergic neurotransmission.

4. Repeated apomorphine treatment, irrespective of whether aggressiveness developed or not, did not affect exploratory behaviour of rats. In apomorphine — free situation the effects of 5-HT_2 receptor agonist quipazine did not change.

5. Atypical antipsychotics, excluding risperidone, are weak antagonists of dopamine receptors and dopaminomimetics. Contrary to the classical antipsychotics atypical antipsychotics are very weak in suppressing apomorphine-elicited aggressiveness: their inhibiting doses are 20 to 100 times higher compared with haloperidol. According to the effectiveness in suppressing apomorphine aggressiveness the antipsychotics can be put in the following order: haloperidol > risperidone > melperone > sertindole > ziprasidone > quetiapine.

6. Among the atypical antipsychotics used in our study only risperidone and sertindole sensitized dopamine receptors to apomorphine after 3 week chronic administration.

7. Acute administration of the atypical antipsychotics blocks 5-HT_2 agonist quipazine elicited wet-dog shakes, but after chronic administration they sensitize 5-HT receptors to the effect of this agonist.

8. The antagonist of 5-HT_{1A} receptors WAY-100635 potentiates the inhibitory action of atypical antipsychotics to exploratory behaviour: this effect could be explained by increase in the level 5-HT in synaptic cleft and aversive action on 5-HT_2 receptors.

9. Atypical antipsychotics antagonize the behavioural effects of 5-HT_{2A} receptor agonist DOI and in microdialysis experiments increase the output of dopamine in frontal cortex.

10. Acute treatment of atypical antipsychotics had no major effect on monoamine content in any of the three brain regions studied, except that the metabolites of dopamine were increased in striatum. After chronic treatment with atypical antipsychotics the content of dopamine metabolites were decreased in striatum.

11. It could be proposed that in the mechanism of action of atypical antipsychotics the blockade of 5-HT_2 heteroreceptors on dopaminergic terminals and an increase in dopamine release plays an important role: clinically they have promnestic effect and do not elicit motor disturbances as classical neuroleptics.

12. Blockade of glutamatergic neurotransmission by MK-801 differentially influences the behavioural effects of antipsychotics with different complexity of mechanisms of action.

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SUMMARY IN ESTONIAN

Atüüpiliste antipsühhootikumide neurofarmakoloogia ning nende toime loomade psühhoosi mudelis

Antipsühhootikumid kuuluvad keemiliselt heterogeensesse gruppi ja on jaotatud kahte põlvkonda: klassikalised ja atüüpilised antipsühhootikumid. Antipsühhootikumid toimivad peamiselt üle dopamiin D_2 retseptori blokaadi, kuid tänaseks on selge, et nad omavad märkimisväärselt tugevat afiinsust ka serotoniini retseptoritele, mis on atüüpilistel antipsühhootikumidel tugevam kui dopamiini retseptorite blokaad. Glutamaatergilise süsteemi häireid ja sellest tulenevat hüpoglutamaatergilist seisundit peetakse psühhoosi tekke aluseks. Serotoniin-, dopamiin- ja glutamaatergiline süsteem on nii funktsionaalselt kui ka anatoomiliselt interakteerunud.

Uurimistöös püstitati järgmised ülesanded:

– Iseloomustada apomorfiini-indutseeritud agressiivsuse mudelit ja täpsustada selle neurokeemilist mehhanismi.
– Serotoniini retseptori alatüüpide ja glutamaadi retseptori roll käitumiskatsetes atüüpiliste antipsühhootikumide toimes
– Analüüsida atüüpiliste antipsühhootikumide erinevaid käitumuslikke ja biokeemilisi efekte ja võrrelda neid esimese põlvkonna neuroleptikumi haloperidooliga.

Katseloomadeks olid isased ja emased Wistar liini rotid. Käitumiskatsetes kasutati järgmiseid eksperimentaalseid mudeleid: plusspuuri, avarvälja teste, apomorfiini aggressiivsuse ja stereotüüpia määramist, neurokeemilistes katstes aga monoamiinide määramist ajukoe 4 piirkonnas (frontaalkoores, striaatumis, hüpotaalamuses ja hipokampuses) ja *in vivo* mikrodialüüsi frontaalkoorest.

Järeldused

Käesoleva töö kokkuvõttena võib teha järgnevaid järeldusi:

– Korduv apomorfiini manustamine (1 mg/kg s.c) põhjustab 80% isastel rottidel agressiivset käitumist. Emastel rottidel agressiivset käitumist ei täheldatud. Agressiivne käitumine kujunes välja vaid neil loomadel, kes olid saanud katse vältel nö. kaklemise kogemuse. See käitumine on vahendatud D_2 retseptori ja G-valgu vahelisest interaktsioonist. Krooniline apomorfiini manustamine põhjustas loomadel, kel agressiivne käitumine välja ei kujunennud, D_2 retseptorite tundlikkuse allaregulatsiooni ja muutes GDP afiinsust G-valkudel.
– DSP-4 (noradrenergiline neurotoksiin), sensitiseerides noradrenaliini retseptoreid kiirendas apomorfiini agressiivsuse välja kujunemist. Korduv 5-HT_2 retseptori antagonisti trasodooni manustamine aga aeglustas selle fenomeni teket.

– Sõltumatult sellest kas agressiivsus kujunes välja või mitte, ei muutnud see rottide uudistavat käitumist avarväljas.
– Atüüpilised antipsühhootikumid võib järjestada oma efektiivsuselt apomorfiini-indutseeritud agressiivsuse mudelis järgnevalt: haloperidool > risperidoon > melperoon > sertindool > siprasidoon > kvetiapiin, kusjuures agressiivset käitumist inhibeeriv doos võrreldes haloperidooliga on 20–100 kordselt suurem.
– Korduval 3-nädalise manustamise tulemusena olid uuritud atüüpilistest antipsühhootikumidest vaid risperidoon ja sertindool võimelised sensitiseerima dopamiini retseptoreid.
– Akuutsel manustamisel atüüpilised antipsühhootikumid blokeerivad 5-HT_2 agonisti kvipasiini-indutseeritud pea raputusi *(wet-dog shakes),* peale kroonilist manustamist aga sensitiseerivad serotoniini retseptoreid kvipasiini toimele.
– 5-HT_{1A} retseptori antagonist WAY-100635 potentseerib atüüpiliste antipsühhootikumide pärssivat toimet uudistavale käitumisele, mis on seotud 5-HT vabanemisega ja toimega 5-HT_2 retseptoritele.
– Atüüpilised antipsühhootikumid pärsivad ka 5-HT_{2A} retseptori agonisti DOI poolt põhjustatud käitumuslikke efekte ja mikrodialüüsi eksperimendis suurendavad dopamiini metaboliitide vabanemist frontaalkoores.
– Akuutsel manustamisel atüüpilised antipsühhootikumid suurendasid dopamiini metaboliitide taset striaatumis, kroonilisel manustamisel aga langetasid. Teistes uuritud ajupiirkondades atüüpilised antipsühhootikumid ei mõjutanud märkimisväärselt monoamiinide taset.
– Võib oletada, et atüüpiliste antipsühhootikumide toimemehhanismis on oluline koht dopamiinergilistel lõpmetel asuvate 5-HT_2 heteroretseptorite blokaadil ja dopamiini vabanemise suurenemine omab olulist tähtsust promnestilises kliinilises toimes ja on põhjuseks miks nad ei kutsu esile klassikalistele neuroleptikumidele omaseid motoorika häireid.
– Glutamaatergilise närviülekande blokaad NMDA retseptori antagonisti MK-801 poolt mõjustab erinevalt antipsühhootikumide käitumuslikke efekte sõltuvalt nende kompleksest toimemehhanismist erinevatele neuromediatsiooni süsteemidele.

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