

EXPERIMENTAL STUDIES ON NOVEL PHARMACOLOGICAL STRATEGIES IN THE TREATMENT OF SCHIZOPHRENIA

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ABSTRACT

The modified dopamine (DA) hypothesis of schizophrenia suggests a hyperdopaminergic state in subcortical brain regions, which is believed to cause positive symptoms, while cortical regions may show a hypodopaminergic state which is believed to generate negative and cognitive symptoms. Positive symptoms are improved by typical antipsychotic drug (APD) treatment. However, negative and cognitive symptoms remain less affected. Typical APD treatment is often associated with extrapyramidal side effect (EPS) liability due to high DA D₂ receptor blockage (≥80%). In comparison with typical APDs, atypical APDs may show better clinical efficacy, e.g. the atypical APD clozapine shows superior efficacy in treatment-resistant schizophrenia in terms of its ability to improve negative symptoms and some aspects of cognitive impairment with an absence of EPS liability, due to its low DA D₂ receptor occupancy (about 45%). Contrary to typical APDs, clozapine and other atypicals increase cortical DA release in experimental animals. This effect is thought to be underlying its ability to improve negative and cognitive symptoms. However, clozapine may increase the risk for agranulocytosis and also, similar to some other atypicals, cause weight gain and sedation. Since the severity of cognitive impairment in schizophrenia is a critical determinant of treatment outcome, it is important to find alternative pharmacological strategies with better clinical efficacy and fewer side effects than available APDs. Thus, several drug treatment strategies have been applied clinically in order to achieve this goal. For example, earlier clinical studies have shown that adjunctive low doses of L-dopa to typical APDs improve negative symptoms in schizophrenic patients. Recently, it has been reported that adjunctive treatment with the antiepileptic drug topiramate may improve the effect of APDs, especially against negative symptoms, when used in schizophrenic patients maintained on a stable APD medication. Thus, we here examined experimentally these clinical findings, by investigating the effects of adjunctive treatment with either a low dose of L-dopa or topiramate to low doses of the selective DA D₂ receptor antagonist raclopride using the conditioned avoidance response (CAR) paradigm for evaluating antipsychotic activity. EPS liability was assessed by means of the catalepsy test. Using microdialysis, DA output was assessed in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAC), respectively following these drug combinations. We found that adjunct low dose L-dopa or topiramate markedly augmented the antipsychotic-like effect of a low dose of raclopride in CAR. This augmentation was associated with a significant increase in DA output in the mPFC, but with a smaller increase in DA release in the NAC, and without catalepsy. Our experimental results support previous clinical findings that adjunctive treatment with low doses of L-dopa or topiramate to typical APD treatment may augment therapeutic efficacy in schizophrenia and with lower risk of EPS. These results are in line with our previous finding showing enhanced cortical DA output and antipsychotic-like effect of raclopride by adjunct α₂ adrenoceptor blockage and indicate the possibility to augment typical APDs with an absence of severe side effects. Taken together, we conclude that an enhanced prefrontal DA output *per se* may serve to improve the effect of typical APDs in schizophrenia.

Clinical studies have shown that selective serotonin reuptake inhibitors (SSRIs) may, at high doses, induce Parkinsonism in sensitive individuals. However, preclinical studies have revealed that stimulation of 5-HT_{1A} receptors may modulate typical APD-induced catalepsy. Thus, it has been hypothesized that stimulation of 5-HT_{1A} receptors may protect against an inherent potential for parkinsonism of SSRIs. Here, we evaluated the possible risk of EPS following the combined treatment with a high dose (40 mg/kg) of the SSRI citalopram and the selective 5-HT_{1A} receptor antagonist WAY 100635 using catalepsy. We observed significant catalepsy following this drug combination, while the drugs were ineffective when given alone, indicating that catalepsy, even without any direct DA D₂ receptor blockade, can be induced by SSRIs together with 5-HT_{1A} receptor blockade. This effect may be related to enhanced inhibition of dopaminergic, particularly nigrostriatal, activity and may be mediated via 5-HT receptor subtypes other than the 5-HT_{1A}. Since we showed that a high dose of citalopram/WAY100635 in combination produced significant catalepsy; and because catalepsy and suppression of CAR behavior are thought to be mediated via suppression of dopaminergic neurotransmission, we investigated the potential antipsychotic activity of combined treatment with lower doses (10 or 20 mg/kg) of citalopram and WAY100635 in CAR. EPS liability was also evaluated using catalepsy. Combined treatment with citalopram (20 mg/kg) and WAY100635 produced a significant antipsychotic-like effect as revealed by CAR and without producing significant catalepsy. The two drugs were ineffective when given alone. Pretreatment with the selective 5-HT_{2C} receptor antagonist SB242084 completely prevented the citalopram/WAY100635-induced effect on CAR, indicating a specific involvement of the 5-HT_{2C} receptor in the antipsychotic-like effect observed. The effect on CAR by this treatment combination is similar in magnitude to that of a moderate dose of the typical APD haloperidol. Our data suggests a novel treatment option for individuals suffering from e.g. psychotic depression. In conclusion, our studies provide experimental support for previous clinical findings and also suggest novel mechanisms that could be exploited to improve pharmacotherapy of schizophrenia.

Key words: Schizophrenia, L-dopa, topiramate, 5-HT_{2C}, citalopram, WAY 100635, CAR, catalepsy

This thesis is based on the following articles, which are referred to in the text by their Roman numerals.

- I. Eltayb. A, Wadenberg. M-L. G, Svensson. TH:** (2005) Enhanced cortical dopamine output and antipsychotic-like effect of raclopride with adjunctive low-dose L-dopa. *Biological Psychiatry*, 58(4):337-43.
- II. Eltayb. A, Wadenberg. M-L. G, Schilström. B and Svensson. TH:** (2005) Topiramate augments the antipsychotic-like effect and cortical dopamine output of raclopride. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 372(3):195-202.
- III. Eltayb. A, Svensson. TH, Ahlenius. S:** (2001) Catalepsy induced by the 5-HT_{1A} receptor antagonist WAY 100635 in rats pretreated with the selective serotonin reuptake inhibitor citalopram. *European Journal of Pharmacology*, 411(3):275-7.
- IV. Eltayb. A, Wadenberg M-L. G, Schilström. B, Svensson. TH:** Antipsychotic-like effects by combined treatment with citalopram and WAY 100635: involvement of the 5-HT_{2C} receptor. (*Submitted manuscript to International Journal of Neuropsychopharmacology*).

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TABLE OF CONTENTS

1	Introduction.....	1
1.1	Schizophrenia	1
1.2	The dopaminergic system.....	2
1.2.1	Anatomy.....	2
1.2.2	Biochemistry of dopamine.....	3
1.2.3	Dopamine receptors	4
1.2.4	The dopamine hypothesis of schizophrenia.....	5
1.3	The serotonergic system.....	6
1.3.1	Anatomy.....	6
1.3.2	Biochemistry of serotonin.....	8
1.3.3	Serotonin receptors	8
1.3.4	Serotonin and schizophrenia.....	9
1.3.5	Dopamine-Serotonin interactions	10
1.4	Pharmacotherapy of schizophrenia	11
2	Specific aims of the study.....	13
3	Materials & methods.....	14
3.1	Animals	14
3.2	Drugs	14
3.3	Methods.....	15
3.3.1	Catalepsy.....	15
3.3.2	Conditioned avoidance response.....	16
3.3.3	Microdialysis in freely moving rats	18
3.4	Data analysis.....	19
4	Results and discussion	20
4.1	Effects of different add-on treatment strategies on DA D ₂ blockage-induced changes in behavior and central DA output (Paper I-II)	20
4.1.1	Effects of adjunctive treatment with a low dose L-dopa together with raclopride in the CAR and catalepsy tests (Paper I)	20
4.1.2	Effects of adjunctive treatment with topiramate on raclopride-induced changes in the CAR and the catalepsy tests (Paper II)	21
4.1.3	Influence of add-on treatment with a low dose of L-dopa on cortical DA output in the brain in rats treated with raclopride (Paper I)	24
4.1.4	Effects of adjunct topiramate on regional DA output in the brain in rats treated with raclopride (Paper II).....	25
4.2	Effects of serotonergic drugs on extrapyramidal motor functions and antipsychotic activity in the rat (paper III-IV).....	29
4.2.1	Effects of the combined treatment with a selective serotonin reuptake inhibitor and a 5-HT _{1A} receptor antagonist on extrapyramidal motor functions in rats (paper III-IV)	29
4.2.2	Effects of the selective serotonin reuptake inhibitor citalopram alone or in combination with the 5-HT _{1A} receptor antagonist WAY 100635 on CAR (paper IV).....	31

4.2.3 Effects of the selective 5-HT _{2C} receptor antagonist SB242084 on Citalopram/WAY 100635 induced-suppression of the CAR behavior (paper IV)	32
5 General discussion.....	35
6 Acknowledgements	37
7 References	39

1 INTRODUCTION

1.1 Schizophrenia

Schizophrenia is a devastating brain disorder. World wide approximately 1 % of the population develops schizophrenia in their life time. Suicide is a major contributor to the high mortality in patients with schizophrenia. About 10 % of schizophrenic patients commit suicide (Meltzer, 2002). Thus schizophrenia has a severe impact on lives of both patients and the close family members (Stålberg et al., 2004). Schizophrenia is a costly illness for the society in terms of loss of productivity besides costs for the medical care. The illness affects both sexes equally (Aleman et al., 2003) with a relatively early onset which occurs during late adolescence in males and in the late twenties in females (Ohaeri, 1992). The incidence of this disorder is generally claimed to be independent of any economical, geographical, cultural or socio-economical differences (Carpenter and Buchanan, 1994). Family, twin and adoption studies have revealed the importance of heredity in patients with schizophrenia (Asherson et al., 1995). In addition, different susceptibility genes seem to contribute to increase the risk to develop this disease (Harrison and Weinberger, 2005). However, epidemiological reports have revealed a higher risk for schizophrenia in some immigrant minorities such as the Surinamese and Dutch Antillean immigrant groups in Holland (Selten et al., 1997), as well as Afro-Caribbean people in the UK (Boydell et al., 2001). Those observations indicate that environmental factors may also play a role in schizophrenia. Thus, investigators have suggested that schizophrenia involves interactions between both genetic and environmental vulnerability factors.

Schizophrenia is characterized by a complex cluster of symptoms, which vary widely between individuals but are traditionally classified into three main types; positive, negative and cognitive symptoms (Carpenter and Buchanan, 1994). The term positive (psychotic) symptoms refers to the fact that these symptoms are absent in the ordinary population. These symptoms include bizarre behaviors, delusions and hallucinations. However, the negative symptoms usually represent a lack or poverty of normal human behaviors such as speech, emotional arousal, motivation and social activities. The negative symptoms might be present long before a patient experiences his/her first psychotic episode and gets in touch with psychiatric healthcare. The third cluster of symptoms are the cognitive symptoms which include incoherence, impaired attention and memory.

The pathophysiology of schizophrenia still remains elusive. Based on pharmacological, as well as clinical, evidence several hypotheses have been suggested e.g. the dopamine (DA) hypothesis and the glutamate hypothesis of schizophrenia. The DA hypothesis of schizophrenia suggested a general hyperdopaminergic state in brain (Carlsson and Lindqvist, 1963; Carlsson, 1988) and was based on the finding that neuroleptic drugs block DA D₂ receptors. In addition, DA agonists e.g. amphetamine had been found to induce psychosis, in particular paranoid symptoms (Angrist et al., 1974). The glutamate hypothesis is largely based on the finding that administration of non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine (PCP) and ketamine to healthy volunteers may elicit psychosis and negative symptoms as well as worsen existing symptoms in schizophrenic patients (Lahti et al., 1995). Therefore, the psychotomimetic action of the non-competitive NMDA receptor antagonists and their interaction with schizophrenia symptoms provide evidence for a hypoglutamatergic state in brain of schizophrenic patients (Laruelle et al., 2005).

1.2 The dopaminergic system

DA, noradrenaline (NA) and adrenaline belong to a class of compounds called catecholamines. The term catecholamine refers to all organic compounds containing a catechol nucleus and an amine group. In the late fifties, Carlsson and co-workers found that DA, beside its known function as a precursor in the synthesis of NA and adrenaline, is also an independent neurotransmitter in brain (Carlsson et al., 1957; 1958; 1959).

1.2.1 Anatomy

In the beginning of the sixties, Falck and Hillarp introduced a fluorescent histochemical method that enabled visualization of monoaminergic neurons (Falck et al., 1962; Carlsson, 1961) as well as their anatomical projections within different regions in brain. The dopaminergic cell bodies in the rat brain are mainly located in two clusters; the substantia nigra (SN) and the ventral tegmental area (VTA) (Dahlström and Fuxe, 1964). However, other DA neuron clusters also exist in brain, e.g. in the hypothalamus which gives rise to the tuberoinfundibular DA pathway. The SN neurons project to the corpus striatum comprising the nigrostriatal pathway, which includes approximately 75 % of the DA in brain (Rang et al., 2003). This pathway is involved in motor control and its degeneration causes Parkinson's disease. In the VTA, dopaminergic cell bodies send efferents to limbic and cortical areas forming the mesolimbic and mesocortical DA pathways, respectively. The mesolimbic DA pathway terminates in several

limbic areas such as the nucleus accumbens (NAC), the bed nucleus of the stria terminalis, amygdala, the olfactory tubercle, the lateral septal area, the lateral hypothalamus and the hippocampus (see Fig. 1). These pathways are believed to play a role in motivation, drug induced-reward and stress. The mesocortical DA pathway innervates the prefrontal cortex (PFC), the cingulate and entorhinal cortices. Cognitive functions such as working memory are thought to be controlled by the mesocortical DA pathway (Goldman-Rakic and Selemon, 1997).

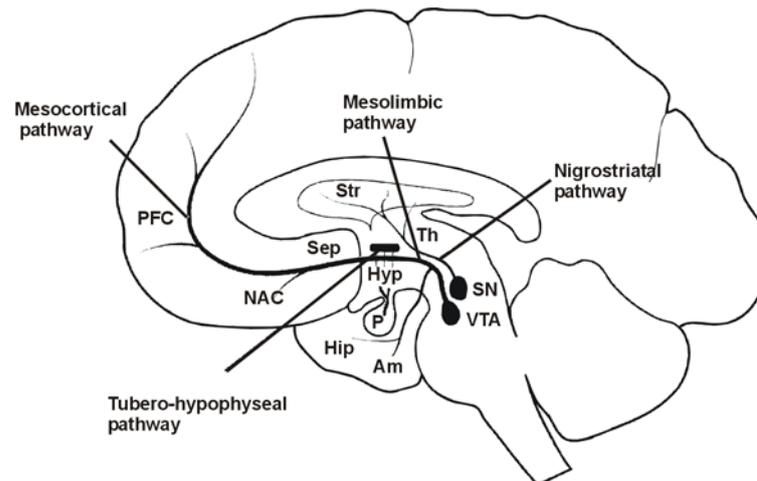


Figure 1: Dopaminergic pathways in the human brain. Abbreviations: PFC-prefrontal cortex, NAC-nucleus accumbens, Sep-septum, Str-striatum, Hyp-hypothalamus, Th-thalamus, P-pituitary gland, Hip-hippocampus, Am-amygdaloid nucleus, VTA-ventral tegmental area, and SN-substantia nigra (Modified from Rang et al., 2003).

1.2.2 Biochemistry of dopamine

The DA precursor tyrosine is a non-essential amino acid. L-tyrosine is taken up from the blood stream by active transport mechanism across the blood brain barrier into the catecholaminergic neuron. The rate-limiting step in DA, NA and adrenaline synthesis, once L-tyrosine has entered catecholaminergic neurons, is the conversion of L-tyrosine to L-dihydroxyphenylalanine (L-dopa) by the enzyme tyrosine hydroxylase. In the dopaminergic neurons, L-dopa is decarboxylated to DA by the enzyme L-aromatic amino acid decarboxylase. DA is stored in vesicles in the nerve terminals. Invasion of the nerve terminal by the action potential is believed to be responsible for DA release in the synaptic cleft via a Ca^{2+} -dependent mechanism. Most extracellular DA is taken back into the nerve terminal by the DA transporter. DA that is recaptured inside the neuron is metabolized to dihydroxyphenylacetic (DOPAC) by the enzyme monoamine oxidase (MAO) (see Fig. 2). Alternatively, DA is recycled in synaptic vesicles. Free DA in the synaptic cleft is converted to homovanillic acid (HVA) through the

sequential action of catechol-O-methyltransferase (COMT) and MAO. In rodents the major DA metabolite is DOPAC, while in humans it is HVA (Cooper et al., 1996).

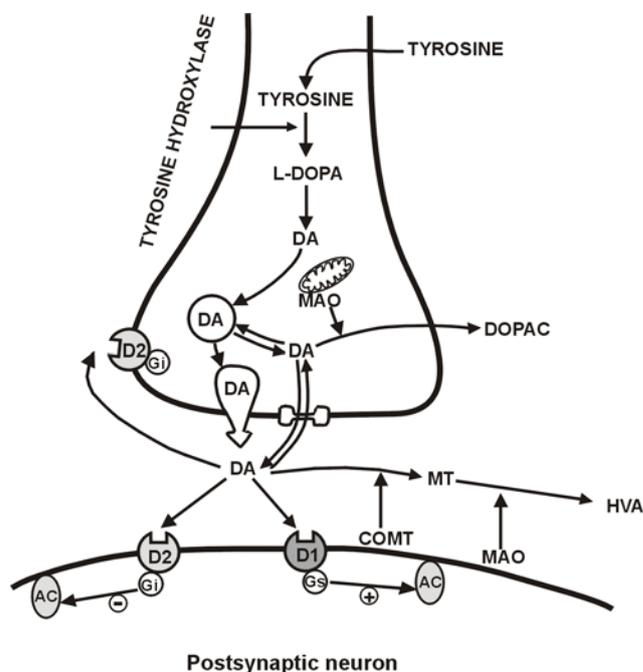


Figure 2: Schematic drawing of a dopaminergic nerve terminal. Abbreviations: DA= dopamine, D₁= D₁-like receptor, D₂=D₂-like receptor, Gi=Gi-protein, Gs= G-protein, AC= adenylyl cyclase, other abbreviations see text (Modified from Cooper et al., 1996).

1.2.3 Dopamine receptors

Dopamine acts mainly at two major types of receptors defined as D₁-like (D₁-D₅) and D₂-like (D₂, D₃ and D₄) receptors based on their distinct biochemical characteristics. Both receptor types are coupled to a G protein controlling the activity of adenylyl cyclase (Spano et al., 1978). D₁ receptor stimulation causes an increase while D₂ receptors stimulation results in a decrease of 3', 5'-cyclic adenosine monophosphate (cAMP) levels (Spano et al., 1978; Missale et al., 1998). Furthermore, D₁ receptors are mainly located postsynaptically outside the synaptic cleft whereas D₂ receptors are located both pre- and postsynaptically. The presynaptic DA D₂ receptors are known as autoreceptors. They are located on the nerve terminals or the somatodendritic area of the DA neuron. The DA D₂ autoreceptors show an inhibitory influence on the rate limiting enzyme in DA synthesis, tyrosine hydroxylase, and are therefore called synthesis-modulating autoreceptors. Presynaptic D₂ autoreceptors may decrease the probability of release by indirectly modulating Ca²⁺ levels in the terminal and are therefore termed release-modulating autoreceptors (Cooper et al., 1996).

The D₁ receptors are predominantly localized in the primary dopaminergic projections of the VTA and SN such as NAC and striatum. In addition, D₁ receptors have also been

detected in the hypothalamus and thalamus (Missale et al., 1998). The D₅ receptors are poorly expressed in the rat brain compared with the D₁ receptors and are restricted to the hippocampus, thalamic and hypothalamic regions (Meador-Woodruff et al., 1992; Khan et al., 2000). The expression of D₂ receptors overlaps with D₁ receptor expression in DA rich areas such as the striatum, olfactory tubercle, NAC, SN and VTA. The D₃ receptors are expressed in high levels in areas such as the island of Calleja, SN, cerebellum, the olfactory tubercle, the ventral pallidum and the NAC but also to some degree in the striatum and the VTA (Stanwood et al., 2000). The D₄ receptors are located mainly in cortex and limbic areas (Van Tol et al., 1991).

1.2.4 The dopamine hypothesis of schizophrenia

The classical DA hypothesis suggests that schizophrenia is a result of a general hyperdopaminergic condition in the brain. This hypothesis was initially based on several lines of indirect pharmacological evidence. In the early sixties, Carlsson and Lindqvist found that haloperidol and chlorpromazine are able to increase the monoamine metabolites, which is an indication of an increase in monoamine turnover (Carlsson and Lindqvist, 1963), and this led to the conclusion that these compounds may block DA receptors. Since that time, a large number of studies in both animals and humans have confirmed Carlsson's conclusion (Andén et al., 1970; Seeman et al., 1975, 1976; Creese et al., 1976; Farde et al., 1988). It is now well known that DA D₂ receptor blockade is a common mechanism for all clinically used antipsychotic drugs (APDs). Furthermore, APDs have been shown to label the DA receptors in patients with schizophrenia (Farde et al., 1988, 1992), and a statistically significant correlation between the degree of D₂ occupancy and APD effect in patients has been demonstrated (Nordström et al., 1993). Other pharmacological evidence supporting the DA hypothesis of schizophrenia is the finding that indirect DA agonists e.g. amphetamine may induce psychotic symptoms that are very similar to paranoid schizophrenia (Angrist et al., 1974) or aggravate already existing psychosis in schizophrenic patients. Furthermore, a number of patients with Parkinson disease who are on L-dopa treatment, suffer from psychotic symptoms as a side effect (Foster and Hoffer, 2004). Direct evidence for a hyperdopaminergic state in schizophrenia has been difficult to demonstrate, given the difficulty of measuring DA transmission in the living human brain. However, recent clinical data have revealed an increased striatal DA metabolism (Lindström et al., 1999) as well as increased central DA release after amphetamine challenge (Laruelle et al., 1996, 2003; Kegeles et al., 2000). These findings suggest that psychotic symptoms may indeed be related to enhanced release of DA. On the other hand, drugs that block DA D₂ receptors or DA

neuronal storage are able to ameliorate positive symptoms but negative symptoms are less responsive and may even get worse (Carpenter, 1996). Moreover, administration of DA agonists such as amphetamine worsens the positive symptoms but may partly improve negative symptoms (Angrist et al., 1982; Van kammen et al., 1988). Based on the above clinical and experimental findings, the classical DA hypothesis has been modified, and a notion of a regional imbalance of central DA systems has emerged (Svensson, 1995; Weinberger and Lipska, 1995). The modified DA hypothesis suggests that hypo- and hyperfunctional dopaminergic states may occur in schizophrenic patients in different regions in brain (Svensson, 1995; Weinberger, 1987). Thus, whereas a hyperdopaminergic state in subcortical regions may trigger positive symptoms, negative and cognitive symptoms may occur as a result of the hypodopaminergic state in cortical regions.

1.3 The serotonergic system

Over fifty years ago Twarog and Page reported the presence of serotonin (5-HT) in the mammalian brain (Twarog and Page, 1953). In 1955, Brodie and co-workers suggested that 5-HT may serve as a neurotransmitter in the central nervous system (Brodie et al., 1955). In the beginning of the sixties, the introduction of a fluorescent histochemical method by Falck and Hillarp (Falck et al., 1962) enabled visualization of serotonergic neurons.

1.3.1 Anatomy

Serotonergic neurons are known to be restricted to 9 discrete serotonergic cell clusters located along the midline of the brain stem. Several branches are projecting from these cell clusters to the whole central nervous system. The caudally located cells send branches to brainstem structures as well as to the spinal cord. The serotonergic neurons that are located more dorsally in the raphe region, i.e. the dorsal and median raphe nuclei (DRN and MRN), send projections to different parts of the forebrain (see Fig. 3). Thus, the frontal cortex and striatum are heavily innervated from DRN and the dorsal hippocampus is mainly innervated by MRN, while the ventral hippocampus receives input from both DRN and MRN (Azmitia and Segal, 1978).

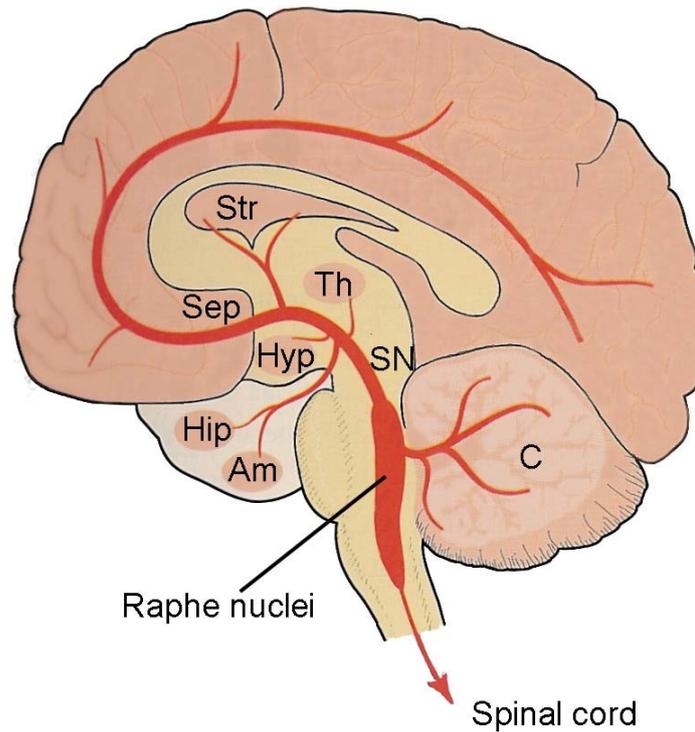


Figure 3: Serotonergic pathways in the human brain. Abbreviations: C-cerebellum, Sep-septum, Str-striatum, Hyp-hypothalamus, Th-thalamus, Hip-hippocampus, Am-amygdaloid nucleus and SN-substantia nigra (Modified from Rang et al., 2003).

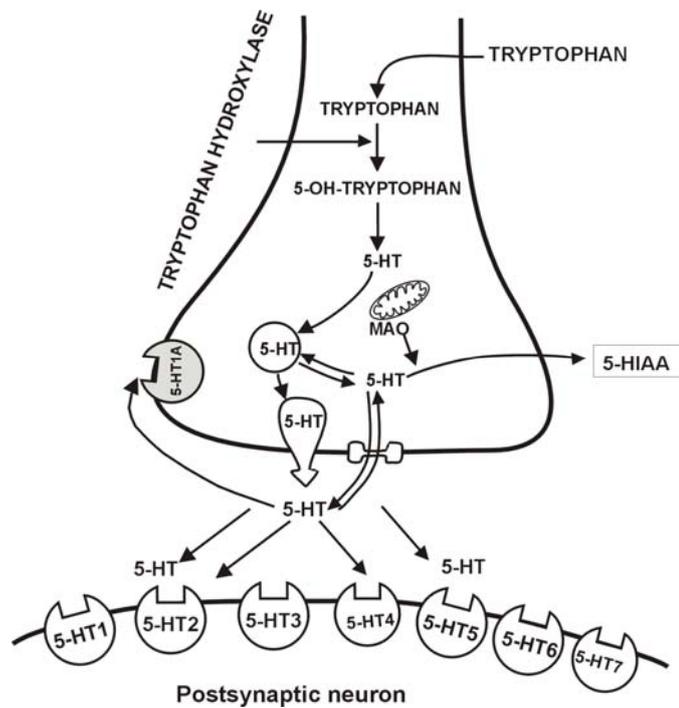


Figure 4: Schematic drawing of a serotonergic nerve terminal. (Modified from Cooper et al., 1996).

1.3.2 Biochemistry of serotonin

Dietary intake of the amino acid tryptophan is important for formation of 5-HT. The availability of tryptophan in the central nervous system seems to control the amount of synthesized 5-HT (Aghajanian and Asher, 1971), and decreasing the intake of tryptophan has been found to reduce the 5-HT synthesis rate in the rat brain (Gessa et al., 1974). Tryptophan is hydroxylated by the enzyme tryptophan hydroxylase to 5-hydroxytryptophan (5-HTP) (see Fig. 4). The newly synthesized 5-HT is either degraded by intracellular MAO and aldehyde dehydrogenase to 5-hydroxyindoleacetic acid (5-HIAA) or taken up in storage vesicles. Similar to catecholamines, 5-HT is released in the synaptic cleft by a Ca^{2+} -dependant exocytotic process when the nerve terminal is invaded by an action potential. Most of the extracellular 5-HT is taken back into the nerve terminal by the 5-HT transporter (Cooper et al., 1996). The serotonergic system is believed to be involved in the regulation of sleep/wakefulness, aggression, anxiety, sexual behavior and mood.

1.3.3 Serotonin receptors

Almost fifty years ago Gaddum and Picarelli (Gaddum and Picarelli, 1957) divided 5-HT receptors into the M and D type. Advanced radioligand binding, biochemical and cloning techniques have now revealed more than 14 different serotonergic receptors, which belong to seven receptor subtypes 5-HT₁-5-HT₇ (Hoyer et al., 1994; Kroeze and Roth, 1998). The 5-HT₁ receptors are coupled negatively to adenylate cyclase and are classified as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F} subtypes (Hoyer et al., 1994). The 5-HT_{1A} receptors are expressed in 5-HT neurons in the raphe nuclei and function as somatodendritic autoreceptors regulating 5-HT cell firing. The 5-HT_{1A} receptors are also expressed postsynaptically in structures such as hippocampus and the cerebral cortex. The 5-HT_{1B} and 5-HT_{1D} are located presynaptically along the nerve terminals and axons of the 5-HT neurons (Riad et al., 2000). The 5-HT₂ receptors include the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. These receptors mediate their effect via activation of protein kinase C and an increased phosphoinositide metabolism (Hoyer et al., 1994). While, the 5-HT_{2B} receptors are expressed mainly in the peripheral neurons system, the 5-HT_{2A} and 5-HT_{2C} receptors are highly expressed in different brain regions such as the cerebral cortex, the hippocampus and the basal ganglia (Abramowski et al., 1995; Cornea-Hébert et al., 1999). The 5-HT₃ receptor is a ligand gated cation channel, which is highly expressed in the lower brainstem. A low density of the 5-HT₃ receptor is also expressed in areas such as the cerebral cortex, amygdala and hippocampus (Hoyer et al., 1994). The 5-HT₄, 5-HT₅, 5-HT₆ and 5-HT₇ receptors are positively coupled to adenylate cyclase (Hoyer et al., 1994). Abundant expression of the 5-HT₄ receptor mRNA has

been observed in the olfactory system, striatum and the hippocampus (Ullmer et al., 1996). The 5-HT₅ receptor is located in the cerebral cortex, hippocampus and cerebellum (Pasqualetti et al., 1998). The 5-HT₆ receptor is expressed in olfactory tubercle, the striatum, NAC and the hippocampus. Fewer 5-HT₆ receptors are expressed in cerebellum, the amygdala, and the cortex (Ward et al., 1995). The highest receptor densities of 5-HT₇ receptor are present in the thalamus, hypothalamus, the hippocampus and cortex (Hedlund and Sutcliffe, 2004).

1.3.4 Serotonin and schizophrenia

The involvement of 5-HT in schizophrenia was suggested by Woolley and Shaw (Woolley and Shaw, 1957) based on the psychotomimetic effects of lysergic acid diethylamide (LSD) (Gaddum, 1953). At that time, LSD was considered to be a 5-HT receptor antagonist. Therefore investigators proposed the hypothesis that schizophrenia may result from a hyposerotonergic state in brain. One of the major problems with this hypothesis was the finding that the primary effect of LSD was to produce visual hallucinations, which are relatively rare in schizophrenia, but not auditory hallucinations, which are the most common among perceptual disturbances in schizophrenia (Iqbal and Van Praag, 1995). In addition, paranoid delusions, conceptual disorganization, and the wide range of cognitive impairments characteristic of schizophrenia e.g., disturbances in working memory and executive function, are generally absent during LSD administration. Furthermore, LSD is a full or partial agonist rather than an antagonist at many 5-HT receptors (Marek and Aghajanian, 1998). These reasons together with the emergence of the DA hypothesis, led to a declining interest in the hyposerotonergic hypothesis of schizophrenia. Moreover, clinical trials of serotonergic precursors, agonists, or non-specific antagonists were generally disappointing, with no evidence comparable to the observation of DA agonists and antagonists causing exacerbations or decreasing psychosis, respectively. The major breakthrough restoring the interest in the role of 5-HT in schizophrenia was the identification of several 5-HT receptor subtypes and their extensive impact on multiple neurotransmitters and behaviors. In addition, it was thought that at least some of the extraordinary ability of clozapine to improve schizophrenic symptoms more effectively and with fewer side effects than typical APDs could be attributed to its ability to block 5-HT_{2A} receptors (Meltzer et al., 1989, 2003; Silver, 2004).

1.3.5 Dopamine-Serotonin interactions

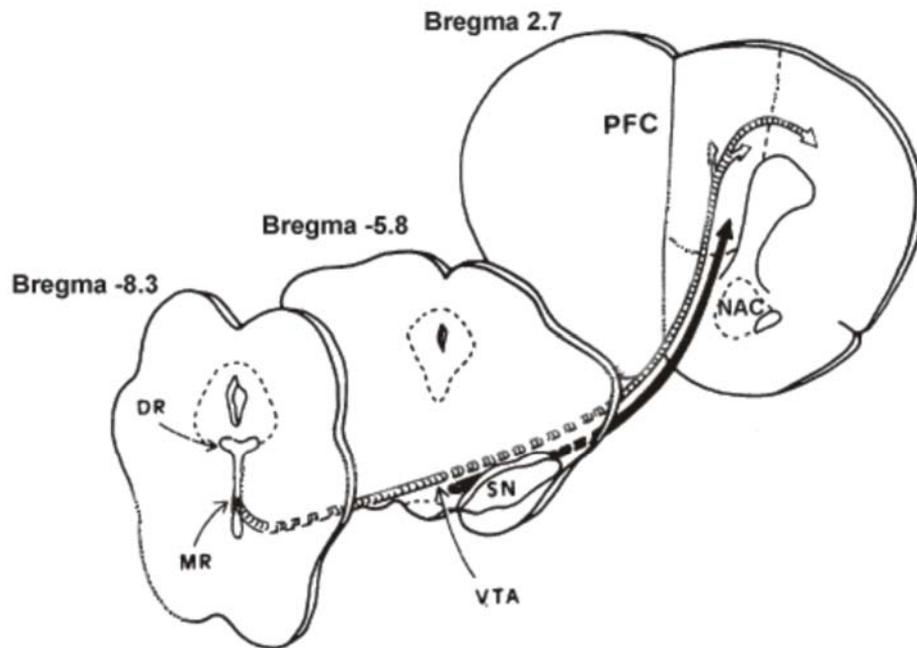


Figure 5: Schematic presentation, on coronal sections of the brain, of the serotonergic pathway from the dorsal and median raphe nuclei (DR, MR), and the mesocortical dopaminergic pathway from the VTA; all three innervate the PFC (Modified from Fuster, 1997).

The serotonergic neurons in both MRN and DRN send projections to the VTA and SN (Dray et al., 1976; Nedergaard et al., 1988; Hervé et al., 1987) (see Fig. 5). The structures that receive dense dopaminergic innervations such as the NAC, striatum and the PFC also receive a prominent serotonergic input from both midbrain raphe nuclei (Azmitia and Segal, 1978). Autoradiography studies in rats have revealed the presence of 5-HT_{1B} and 5-HT_{2A/C} receptors in VTA, SN, NAC, frontal cortex and striatum (Pompeiano et al., 1994). An interaction between DA and 5-HT systems was early hypothesized to mediate disturbances of extrapyramidal motor functions as observed in the catalepsy model in rats (Kostowski et al., 1972). Thus, pretreatment with quipazine, a serotonin agonist, and clomipramine, a selective serotonin reuptake inhibitor (SSRI), was found to potentiate the cataleptic effect of haloperidol in rats (Balsara et al., 1979). Moreover, there is evidence that SSRIs can trigger parkinsonism in vulnerable patients (Gerber and Lynd, 1998; Balsara et al., 1979).

1.4 Pharmacotherapy of schizophrenia

Pharmacological treatment for schizophrenia can be dated to the introduction of chlorpromazine in 1952 by Delay and Deniker (Bennett, 1998; López-Muñoz et al., 2005; Delay et al., 1952). They classified drugs such as chlorpromazine and reserpine with similar effect on mentally ill patients as neuroleptic drugs. A few years later, another potent neuroleptic, haloperidol, was introduced. The classical neuroleptics, that generally induced extrapyramidal side effect (EPS), e.g. parkinsonism and dystonia, were subsequently called typical APDs and later also first generation APDs. At the beginning of the sixties, Carlsson and Lindqvist suggested that both chlorpromazine and haloperidol might act in schizophrenia by blocking brain catecholamine receptors (Carlsson and Lindqvist, 1963). The development of receptor binding techniques led to the demonstration that the clinical potency of neuroleptics in treating psychotic behavior is correlated to blockade of DA D₂ receptors, rather than DA D₁ receptors (Seeman et al., 1976; Creese et al., 1976). Therefore, in the beginning of the eighties a number of typical or first generation APDs that shared the common property of DA D₂ receptor blockade with a substantial EPS liability were released. Even though typical APDs are effective in ameliorating positive symptoms of schizophrenia, negative and cognitive symptoms are less responsive and might even be exacerbated (Carpenter, 1996; Andreasen, 1995). In contrast, the APD clozapine, which was first released in 1965, did not produce EPS but because of the risk of agranulocytosis, clozapine was withdrawn from the market in most countries. However, because of its superior therapeutic effect in treatment-resistant schizophrenia as well as better efficacy against negative symptoms and cognitive impairment (Kane et al., 1988, 2001; Meltzer, 1992; Meltzer and McGurk, 1999) clozapine was defined as an atypical APD and re-introduced in 1990. Following the re-introduction of clozapine, a series of new atypical or so called second generation APDs were developed, but despite a beneficial profile of these drugs compared to typical APDs, clozapine remains the most efficacious. The unique profile of clozapine has been explained by its preferential effect on the mesolimbocortical DA system. Preclinical studies have shown that atypical APDs, but not typical APDs, selectively produce an increase in DA output in the cortical areas of brain but not in subcortical areas (Nomikos et al., 1994; Kuroki et al., 1999). Using positron emission tomography (PET) technique, Farde and co-workers have shown that for typical APDs, a high occupancy of DA D₂ receptors is important for a sufficient clinical effect, which is usually associated with incidence of EPS. In contrast, they found that atypical APDs may occupy a lower percentage of DA D₂ receptors which result in a lower risk of EPS and in some cases better clinical effect than typical APDs (Farde et al.,

1988, 1992). Compared to typical APDs, atypical APDs often show a broader spectrum of receptor affinities which may contribute to their unique limbic selectivity profile. For example, clozapine possesses high affinity for other receptors than D₂ such as D₃ and D₄ receptors, α_1 and α_2 receptors, 5-HT₂ receptors, muscarinic and histaminergic receptors. Due to their location within the limbic system, both D₃ and D₄ receptor subtypes have received considerable attention as targets for APDs (Park et al., 2003; Wang et al., 2006). However, clinical trials have reported that D₄ antagonists are ineffective (Corrigan et al., 2004). More research is warranted in order to understand the contribution of the above listed receptors in the action of atypical APDs.

Although clozapine still seems to be superior to typical as well as atypical APDs in terms of efficacy (Davis et al., 2003), it carries an increased risk of agranulocytosis and also, like other atypicals, produces significant weight gain and substantial sedation. Therefore, in order to develop drug treatments with better clinical efficacy and with fewer side effects, several add-on drug treatment strategies have been tried clinically. For example, a number of antiepileptic drugs have been found to possess beneficial effects in schizophrenic patients. Because of its reported effect on lowering triglycerides and causing weight loss, the recently developed antiepileptic drug topiramate was tested in medicated schizophrenic patients. Besides reducing weight gain, this add-on therapy was also found to improve negative symptoms (Drapalski et al., 2001). Controlled clinical studies have now shown improvements of deficit symptoms in schizophrenia by adjunctive treatment with the antiepileptic drugs topiramate and lamotrigine of patients who are maintained on APD treatment (Dursun and Deakin, 2001; Tiihonen et al., 2005). Moreover, addition of an SSRI in APD treated schizophrenic patients with depressive symptoms was found to improve also negative symptoms (Kasckow et al., 2001; Silver, 2004). Another treatment strategy that has recently been introduced clinically is the use of drugs with mixed DA agonist/antagonist properties (Tamminga and Carlsson, 2002) which also generates interest in earlier studies where direct or indirectly acting DA agonists such as L-dopa was used as add-on treatment to typical APDs (Inanaga et al., 1975). In order to understand the mechanisms underlying the observed beneficial clinical effects by add-on treatments in schizophrenia we wanted to evaluate experimentally some of these novel pharmacological combinations.

2 SPECIFIC AIMS OF THE STUDY

- To investigate whether addition of a low dose of L-dopa, with the peripheral decarboxylase inhibitor benserazide, to the selective DA D₂ receptor antagonist raclopride may enhance cortical DA output and antipsychotic-like effect of the D₂ antagonist.
- To examine the ability of the anticonvulsant drug topiramate, when added to raclopride, to enhance cortical DA output as well as to evaluate the antipsychotic-like effect of the combined treatment with topiramate and raclopride.
- To evaluate the EPS liability of combined treatment with the selective serotonin reuptake inhibitor citalopram and the selective 5-HT_{1A} receptor antagonist WAY 100635.
- To explore whether the combination of citalopram and WAY 100635 may produce an antipsychotic-like effect.

3 MATERIALS & METHODS

3.1 Animals

Adult male Wistar rats (BK Universal, Sollentuna, Sweden), with an average weight of 300 g upon arrival, were used in the present studies. The animals were housed, three or four per cage under standard laboratory conditions with a temperature of $21.0 \pm 0.4^{\circ}\text{C}$, relative humidity of 55-65 %. Food (R34, Ewos, Södertälje, Sweden) and tap water were available ad libitum. The animals were acclimatized for at least one week before behavioral or biochemical experiments started. Animals used for behavioral tests were housed on a reversed light-dark cycle 12:12 h, (lights off at 07:00 am) and transferred from the animal quarters to the laboratory 1 h before the experiments started, and were kept in a ventilated cabinet between observations. Animals used for microdialysis experiments, were housed as above but on a normal light-dark cycle (lights on at 07.00 am). After surgery animals were housed individually for two days prior to experiment. All experiments were performed between 8:00 am and 6:00 pm. The studies were approved by the Local Animal Ethics Committee, Stockholms Norra Djurförsöksetiska Nämnd (ethical approval n. N232/97, N233/00, N216/00, N211/00, N340/02, N129/03, N419/03 and N93/05).

3.2 Drugs

The following drugs were used:

Dopaminergic drugs

Raclopride DA D₂ receptor antagonist S-(-)-3, 5-dichloro-N-[(1-ethyl-2-pyrrolidiny) methyl-2 hydroxy-6-methoxybenzamide(+)-tartrate (Astra Zeneca, Södertälje, Sweden)

L-dopa DA precursor (L-3, 4-dihydroxyphenylalanine methylester) (Sigma-RBI, Stockholm, Sweden)

Benserazide Peripheral decarboxylase inhibitor (DL-serine 2-[2, 3, 4-trihydroxybenzyl]-hydrazide hydrochloride) (Sigma-RBI, Stockholm, Sweden)

Anticonvulsants

Topiramate Anticonvulsant (2, 3:4, 5-bis-0-(1-methylethylidene)- β -D-fructo-pyranose sulphamate) (Johnson & Johnson, Pharmaceutical Research & Development, L.L.C. USA)

Serotonergic drugs

Citalopram Selective serotonin reuptake inhibitor (RS)-1-[3-(dimethylamino) propyl]-1-(p-fluorophenyl)-5-phthalanarbonitrile, hydrobromide (Lundbeck, Denmark)

WAY 100635 5-HT_{1A} receptor antagonist 3HCl N-[2-[4(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-2-pyridinyl) cyclohexane carboxamide (Wyeth-Ayerst, Princeton, USA)

SB242084 5-HT_{2C} receptor antagonist (6-Chloro-5-methyl-1-[2-(2-methylpyrid-3-yloxy)-pyrid-5-yl) carbamoyl] indoline) 2 HCl (Sigma-Aldrich, St. Louis, USA)

Raclopride, benserazide, citalopram and WAY 100635 were dissolved in 0.9 % saline solution. L-dopa was dissolved in a minimal amount of HCl and made up to volume with physiological saline. Topiramate was dissolved in 50 µl of ethanol and diluted to the correct concentration in 0.9 % saline. SB242084 was dissolved in 8 % of hydroxypropyl-beta-cyclodextrin (Lundbeck, Denmark) in 0.9 % saline. In all experiments, before administration of the drug solution in a rat, pH were adjusted to ≥ 6.0 with 0.06 M NaOH. L-dopa, benserazide, topiramate and SB242084 were administered intraperitoneally (i.p.), while raclopride, citalopram and WAY 100635 were administered subcutaneously (s.c.).

3.3 Methods

3.3.1 Catalepsy

Extrapyramidal motor functions involve the nigrostriatal DA pathway in brain (see Introduction). It is well known that the administration of high doses of typical APDs to humans, when striatal DA D₂ receptor occupancy exceeds 80 % in brain, produce Parkinson-like side effects (Farde et al., 1992). Catalepsy in laboratory animals is thought to be equivalent to APD-induced parkinsonism in humans. Catalepsy is defined as a failure of an animal to initiate a movement in order to correct an uncomfortable position. When a normal rat is placed in an unusual posture, it will change its position within seconds. However, a cataleptic rat will maintain this position for a longer period of time (several minutes or longer) (Sanberg et al., 1988). In the present studies, we used the catalepsy test in order to evaluate the EPS liability of drugs with antipsychotic-like activity in rats. A detailed description of the catalepsy measurement can be found in papers I-IV. Briefly, in the present studies, rats were individually placed on an inclined grid (60°) for 30 s of adaptation before observation started. Observations were performed for a maximum of 2.5 min and the time until the rat initiated a movement by one of its paws was recorded (see Picture. 1). The catalepsy was scored from 0-5 (see

Table 1) according to the time (square root transformation) the rat remained immobile (min) (Ahlenius and Hillegaart, 1986).



Score	Time (min)
0	0-0.08
1	0.09-0.35
2	0.36-0.80
3	0.81-1.42
4	1.43-2.24
5	≥ 2.25

Picture 1: Shows a rat placed on an inclined grid during the catalepsy test. Table 1: Shows catalepsy scores according to the square root transformation of the time the rat remains immobile.

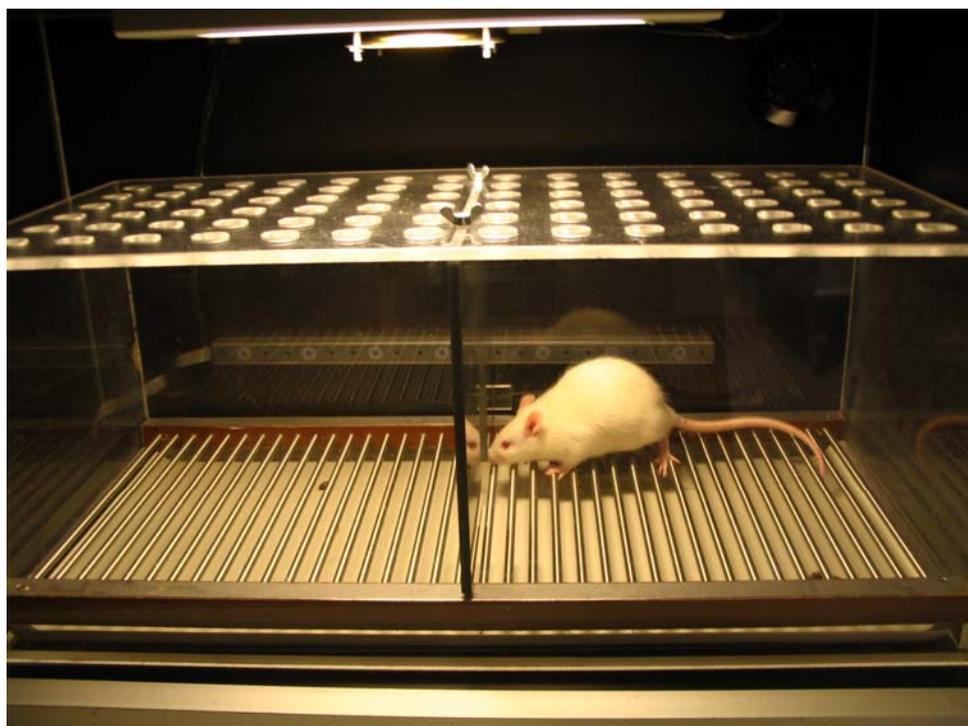
Methodological considerations

Catalepsy is widely used in order to study neuropharmacological effects on extrapyramidal motor functions. Even though tools and procedures used for catalepsy measurement in animals vary between laboratories, the method is considered a reliable model for typical APD-induced EPS (Arnt et al., 1981, see also Wadenberg, 1996). However, although catalepsy is a robust behavioral method many variables may influence the intensity of the cataleptic effect (Sanberg et al., 1988). For example, auditory and visual stimuli are important variables in catalepsy testing. In addition, the importance of handling the animals gently is emphasized by the fact that catalepsy in mice can be produced by neck or tail-pinch (Ornstein and Amir, 1981). Thus, environmental stimuli may potentiate catalepsy by triggering the animal's natural defence mechanism.

3.3.2 Conditioned avoidance response

A detailed description of the CAR test can be found in all papers included in this thesis. Briefly, rats were trained and tested individually in a conventional shuttle-box divided into two equal compartments by a partition with an opening (see Picture. 2). Rats were trained to avoid the unconditioned stimulus (UCS), an intermittent electric shock in the grid floor of approximately 0.2 mA (intershock interval 2.5 s, shock duration 0.5 s), by moving into the opposite compartment within 10 s of presentation of an 80 dB white noise, which is used as conditioned stimulus (CS). White noise is a (hissing-like) type of noise that is produced by combining equal intensity sounds of all different

frequencies to form a broadband spectrum type of sound. The following behavioral variables were recorded: (1) avoidance (response to CS within 10 s); (2) escape (response to CS + UCS); (3) escape failure (if the rat was unable to respond to the shock by moving into the opposite compartment within 50 s the trial was terminated) (Salmi et al., 1994). Suppression of avoidance and a corresponding increase in escape behavior in rats interpreted as an indication of antipsychotic-like activity. However, the appearance of escape failures is indicative of unspecific side effects such as sedation or motor dysfunction suggesting that doses are too high. The animals were trained in one training session which lasted for 15 min per animal and day for 5 consecutive days. Only animals reliably performing at a level of > 90 % avoidance were included in the studies. The same animals were tested repeatedly serving as their own controls in a change-over design (Li, 1964). Experimental days were always separated by at least 2 non-experimental days.



Picture 2
Shows the shuttle-box used in the CAR experiments.

Methodological considerations

The CAR model has been used since the fifties. It has been found to have high predictive validity for detecting potential antipsychotic activity of drugs. Even though the CAR model was established a long time ago, the neural mechanisms underlying the CAR behavior still remain largely unknown. However, local application of a selective DA D₂ antagonist suggested that suppression of CAR is mediated primarily by inhibition of the mesolimbic dopaminergic pathway which innervates

structures such as the NAC (Wadenberg et al., 1990), which also is in line with the notion of a dopaminergic overreactivity, specifically in this region, being responsible for psychotic (positive) symptoms. Furthermore, it was recently shown that antipsychotic-like suppression of CAR by APDs begins to occur at the same drug striatal D₂ occupancy levels (65-70%) that is usually needed for therapeutic response to antipsychotic treatment in schizophrenic patients (Wadenberg et al., 2001; Kapur et al., 2000).

3.3.3 Microdialysis in freely moving rats

Microdialysis is a technique used to monitor or sample the local chemistry of an individual tissue or organ over time. Basically, the microdialysis probe is designed to mimic the function of a capillary blood vessel (Ungerstedt, 1991). In brief, in the present studies, rats were anaesthetized and a concentric type of dialysis probe was implanted in the mPFC or the NAC. After operation rats were kept under standard laboratory condition (see section 3.1) for about 48 h before dialysis procedures started. During experiment, the dialysis membrane of the probe was perfused constantly with a physiological solution which is similar to cerebral spinal fluid but without transmitters and their metabolites. The collected samples were injected into the high performance liquid chromatography (HPLC) system without any further purification. The loading and injecting modes of the injector were directed by a computerized system. After separation the analyte was passed through an electrochemical cell for oxidation and detection. Detailed description of the probe implantation and dialysis procedure, as well as the biochemical analysis, can be found in papers **I** and **II**.

Methodological considerations

Microdialysis is a widely used method in experimental research. The microdialysis technique allows substances in the extracellular fluid to be both recovered from and administered to a tissue. There are many advantages with the microdialysis technique such as the possibility of running experiments in conscious laboratory animals, continuous sampling of substances over long period of time (e.g. hours, day) and decreases number of the animals needed in experiment (Ungerstedt, 1991) as in our case in this thesis. Furthermore, dialysis occurs without a direct contact with the cell tissue which grantee a relatively clean sample for the biochemical analysis. However, the disadvantages include the poor recovery of neurotransmitters over the membrane, which will lead to a poor time resolution (5-30 min). The time resolution depends on the area being dialysed and the type of the monitored neurotransmitter. Furthermore, the big size of the dialysis probe (300 µm) makes it difficult to differentiate between small areas (Ungerstedt, 1991).

3.4 Data analysis

Statistical analysis of behavioral data was performed by means of the Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks test (conditioned avoidance response) or the Kruskal-Wallis one-way ANOVA, followed by the Mann-Whitney U-test (catalepsy) (Siegel and Castellan Jr, 1988) except for **paper III** we used Mann-Whitney U-test for comparison between two treatment groups. Statistical evaluation of microdialysis data was performed by means of a two-way (treatment \times time) ANOVA for repeated measures followed by the Newman-Keuls test for multiple comparisons.

4 RESULTS AND DISCUSSION

4.1 Effects of different add-on treatment strategies on DA D₂ blockage-induced changes in behavior and central DA output (Paper I-II)

Typical APDs are known to improve the positive symptoms of schizophrenia while the negative and cognitive symptoms are less responsive and may even deteriorate (Carpenter, 1996). Due to their high DA D₂ receptor blockage at active clinical doses, APDs may be associated with a relatively high risk of EPS. In contrast to typical APDs, clozapine, shows better efficacy against negative and cognitive symptoms of schizophrenia despite a lower DA D₂ receptor blockage at clinically therapeutic doses and without producing EPS. Although clozapine shows a good efficacy in treatment-resistant schizophrenic patients, it carries an increased risk of agranulocytosis (Naheed and Green, 2001) and also, like other atypicals, produces significant weight gain and sedation. Since the severity of cognitive impairment in schizophrenia is a critical determinant of treatment outcome, it is important to find alternative pharmacological strategies that effectively address these issues. Therefore, a set of *in vivo* studies were undertaken to explore the potential utility of some pharmacological add-on drug treatment strategies that might be beneficial in the treatment of schizophrenia.

4.1.1 Effects of adjunctive treatment with a low dose L-dopa together with raclopride in the CAR and catalepsy tests (Paper I)

All clinically effective APDs share the ability of suppressing avoidance, but not escape behavior in the CAR model, which is indicative of an antipsychotic activity. Previous clinical studies have revealed a beneficial effect of adjunctive treatment with a low dose L-dopa in patients who are maintained on typical APDs (Buchanan et al., 1975; Inanaga et al., 1975). Thus, we here evaluated, experimentally, the potential antipsychotic activity of the addition of a low dose of L-dopa to the selective DA D₂ antagonist raclopride in the CAR model. Raclopride has shown antipsychotic activity in clinical trials (Farde et al., 1989), but is not licensed for clinical use. To evaluate the possible risk for EPS of this drug combination, EPS liability was also assessed by means of the catalepsy test.

We found that the administration of 0.075 mg/kg raclopride in rats produced a statistically significant suppression of the avoidance behavior in CAR. L-dopa (3 mg/kg i.p.) was administered in the presence of 25 mg/kg i.p. of the decarboxylase inhibitor benserazide in

order to avoid peripheral side effects and to allow intact L-dopa to reach the brain. Treatment with this low dose of L-dopa had no effect on the avoidance behavior in CAR when given alone. However, addition of L-dopa dramatically and significantly enhanced raclopride-induced suppression of the CAR behavior (Fig. 6) without concomitant catalepsy (Fig. 11A).

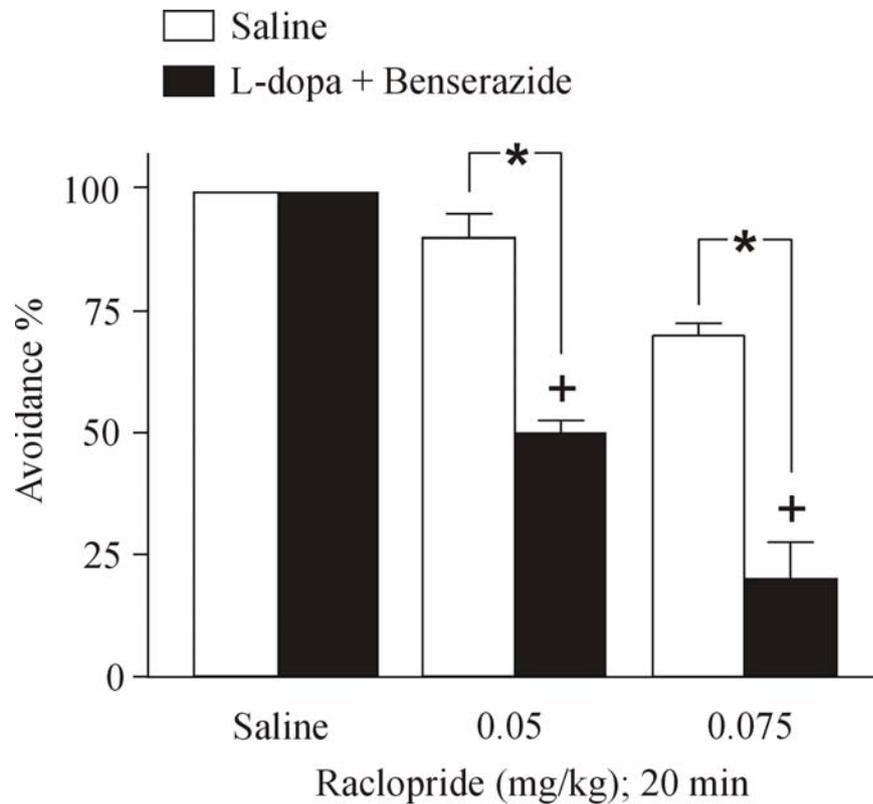


Figure 6

Effects of L-dopa (3 mg/kg) alone or as an adjunct to raclopride on CAR; each bar represents the median (avoidance %) \pm semi-interquartile range based on the observation of the same 7 animals serving as their own controls. ⁺P < 0.05 for comparisons with animals treated with L-dopa + Benserazide alone.

*P < 0.05 for comparisons between the saline/raclopride and L-dopa/raclopride treatment groups.

4.1.2 Effects of adjunctive treatment with topiramate on raclopride-induced changes in the CAR and the catalepsy tests (Paper II)

Several clinical studies have reported advantageous effects of using antiepileptic drugs as an adjunct to APDs in treatment-resistant schizophrenia. For instance, recent studies have revealed that the anticonvulsant drug topiramate may improve deficit symptoms in schizophrenia when added to a stable regimen of an antipsychotic medication (Drapalski et al., 2001; Tiihonen et al., 2005). Topiramate is a new anticonvulsant drug with a complex pharmacological profile. It blocks AMPA-receptors and potentiates GABA-ergic transmission. Previously, we have shown that two AMPA receptor antagonists possess a modest but significant antipsychotic-like effect without producing catalepsy (Mathé et al.,

1999; Svensson and Mathé, 2000). Thus, referring to the clinical studies mentioned above, the AMPA receptor antagonistic action of topiramate may contribute to its antipsychotic effect. Therefore, we here investigated whether addition of topiramate to raclopride may augment its antipsychotic-like effect in CAR. To evaluate the EPS liability of this drug treatment the catalepsy test was undertaken.

In agreement with our previous finding, administration of raclopride (0.075-0.05 mg/kg) was found to induce suppression of CAR. Moreover, pretreatment with topiramate (40 mg/kg) significantly enhanced the raclopride-induced suppression of avoidance behavior in a dose dependent manner (Fig. 7) with no incidence of catalepsy (Fig. 11B).

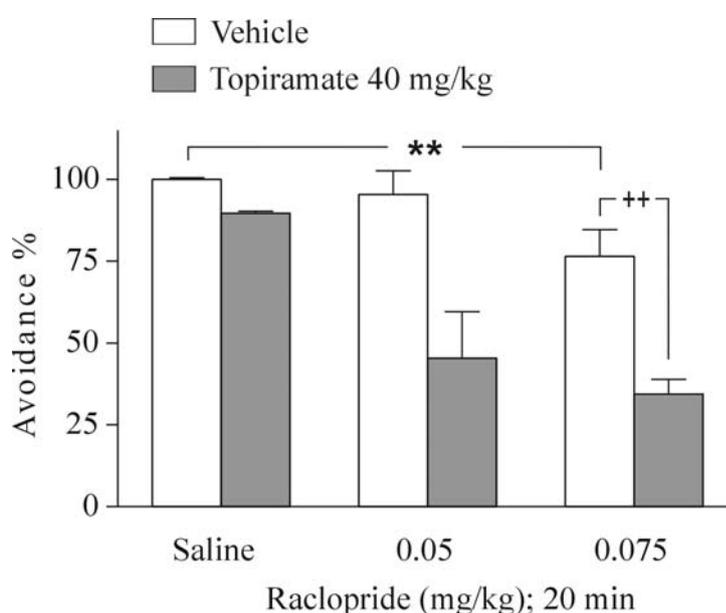


Figure 7

Effects of raclopride alone or in combination with topiramate (40 mg/kg) on conditioned avoidance response; each bar represents the median (avoidance %) \pm semi-interquartile range based on the observation of the same 7 animals serving as their own controls.

* $P < 0.05$ for comparisons with vehicle treated animals.

+ $P < 0.05$ for comparisons between the vehicle/raclopride and topiramate/raclopride treatment groups.

Previous studies in humans have shown that clinically effective doses of typical APDs in schizophrenia produce above 70 % D_2 receptor occupancy in the basal ganglia, whereas EPS are generally seen at occupancies above 80 % (Farde et al., 1992). At 75 % of striatal D_2 receptors occupied, typical APDs produce about 80 % suppression in the CAR test. Thus about 80 % suppression of CAR is predictive of sufficient clinical antipsychotic effect while signs of catalepsy typically are observed at a striatal D_2 receptor occupancy ≥ 80 % (Wadenberg et al., 2000). In the present study, the low dose of raclopride used (0.075 mg/kg, s.c.) caused only a minor suppression of CAR when given alone. However, when raclopride was combined with a low dose of L-dopa, or topiramate, suppression of CAR was achieved that would predict sufficient antipsychotic activity but at a dose of raclopride that should produce a striatal D_2

receptor occupancy of only about 55 % (Wadenberg et al., 2000). Interestingly, this occupancy range is similar to that produced by clozapine in schizophrenic patients (Farde et al., 1992). Thus, the present results indicate that adjunct treatment with either a low dose of L-dopa, or topiramate, together with typical APDs may allow for sufficient antipsychotic effect at lower doses, and thus lower D₂ occupancy, of the APDs than otherwise needed, thereby increasing the therapeutic window and reducing the risk of EPS.

Available data demonstrate that blockade of mesolimbic DA receptors is necessary for the suppression of CAR by typical APDs (Wadenberg et al., 1990). However, the mechanism underlying the augmenting effect of the raclopride-induced suppression of CAR by addition of L-dopa is not clear. According to previous pharmacological findings, a partial agonism can be achieved by combining a direct agonist with a full antagonist (Ebert et al., 1994). Thus the add-on treatment with L-dopa, which could be considered an indirect DA agonist, to the antagonist raclopride may create an analogous treatment to that created by a partial agonist providing an optimal dose for a potent antipsychotic-like action in CAR and below the range of the risk for EPS. In support of such an interpretation, a recent study has shown that treatment with the partial agonist aripiprazole significantly suppresses CAR without producing catalepsy (Natesan et al., 2005). However, it seems that the window for obtaining an optimal dose of L-dopa is rather narrow, since 5 mg/kg of L-dopa only slightly affected the raclopride-induced suppression of CAR. The mechanism by which topiramate augmented raclopride-induced suppression of CAR remains elusive. Topiramate has multiple mechanisms of action as mentioned earlier (see section 4.1.2), including increasing GABA-ergic transmission and antagonism at AMPA-receptors. A previous study has revealed that high doses of benzodiazepines are needed in order to suppress CAR. However this effect was correlated with pronounced sedation and motor disturbances (Arnt, 1982). Since we did not see any motor disturbances in terms of escape failures or catalepsy, potentiation of GABA action seems to play a negligible role in the observed suppression of CAR. On the other hand, our data are in agreement with our previous finding, as mentioned above, that the AMPA receptor antagonist LY326325 produced an antipsychotic-like effect in CAR (Mathé et al., 1999). Moreover, it has been shown that AMPA receptor antagonists, similar to the typical APD haloperidol, attenuate amphetamine and dizocilpine-induced hyperactivity, which is indicative of an antipsychotic profile (Vanover, 1998). Therefore, the AMPA receptor antagonistic properties of topiramate may be involved in its augmentation of the antipsychotic effect of raclopride on CAR.

4.1.3 Influence of add-on treatment with a low dose of L-dopa on cortical DA output in the brain in rats treated with raclopride (Paper I)

The positive symptoms of schizophrenia are believed to result from hyperactivity of the mesolimbic DA system in the brain (Carlsson, 1988) while the negative and the cognitive symptoms are thought to be due to hypoactivity of the mesocortical DA system (Svensson et al., 1995; Weinberger et al., 1994). Preclinical studies have shown that clozapine as well as other atypical APDs, in contrast to typical APDs, preferentially increase DA release in cortical areas such as the PFC but less so in subcortical areas such as the NAC in the rat (Nomikos et al., 1994; Kuroki et al., 1999). Therefore, it has been suggested that the clinical profile of atypical APDs may be attributed to a preferential action on the mesocortical DA system (see section 4.1.1.). Thus, we here investigated whether addition of a low dose of L-dopa to raclopride may facilitate cortical DA output. For this reason, microdialysis experiments were conducted to measure the effect of this drug treatment on the DA output in different brain regions such as the mPFC and the NAC.

Administration of raclopride alone had no significant effect on mPFC DA release, while it caused a small, but significant, increase in DA output in the NAC. L-dopa (+ benserazide), when given alone produced an increase in both mPFC and NAC DA output. However, when added to raclopride, treatment with L-dopa produced a further increase in mPFC DA output (Fig. 8A). Addition of L-dopa to raclopride caused a significant increase in DA output in the NAC (Fig. 8B), but in contrast to the mPFC where the increase was due to a synergistic interaction between raclopride and L-dopa, the increase in the NAC was an additive effect.

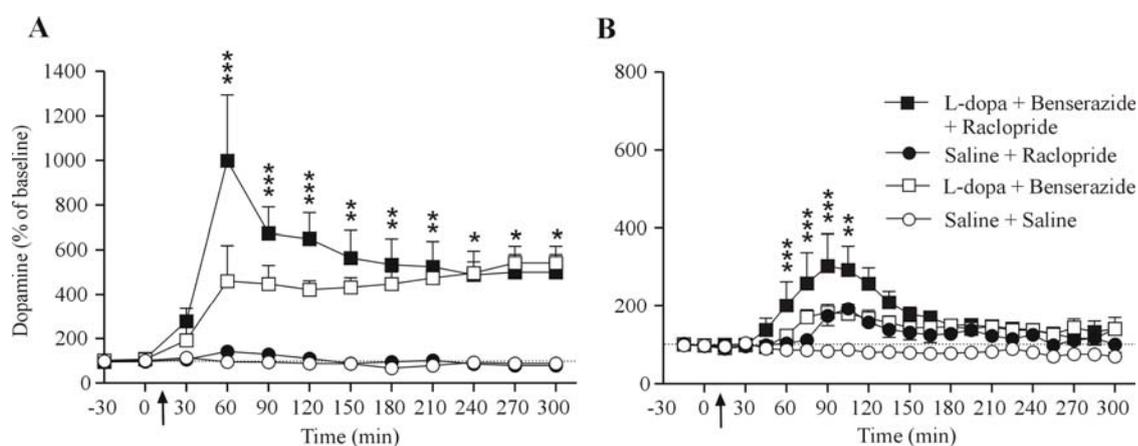


Figure 8 Effects of saline or raclopride (0.075 mg/kg) administration on DA output in (A) mPFC and (B) NAC in rats pretreated with saline or L-dopa (3 mg/kg)/benserazide (25 mg/kg). Arrows indicate time of L-dopa injection. Each point represents the mean (\pm SEM) percent change from baseline ($n \geq 5$) in all groups). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for comparisons between saline/raclopride and L-dopa/raclopride treatment groups.

4.1.4 Effects of adjunct topiramate on regional DA output in the brain in rats treated with raclopride (Paper II)

In analogy with the L-dopa experiments, we also wanted to test whether the addition of topiramate may influence the effect of raclopride on cortical and subcortical DA output. We therefore measured the effect of the drug treatment on DA output in the mPFC and the NAC using microdialysis.

Administration of topiramate or raclopride (0.075 mg/kg) alone had slight enhancing effects on mPFC DA output, which did not reach statistical significance. However, prefrontal DA output was significantly increased following combined treatment with topiramate and raclopride. In the NAC, topiramate by itself did not cause a significant increase in DA output. In contrast, administration of raclopride alone caused a significant increase in DA output in the NAC (Fig. 9B). When the two drugs were combined there was no difference in the total amount of DA in raclopride treated rats versus topiramate/raclopride treated rats (Figure 9B, insertion), but the effect of the combination showed a faster onset than raclopride alone.

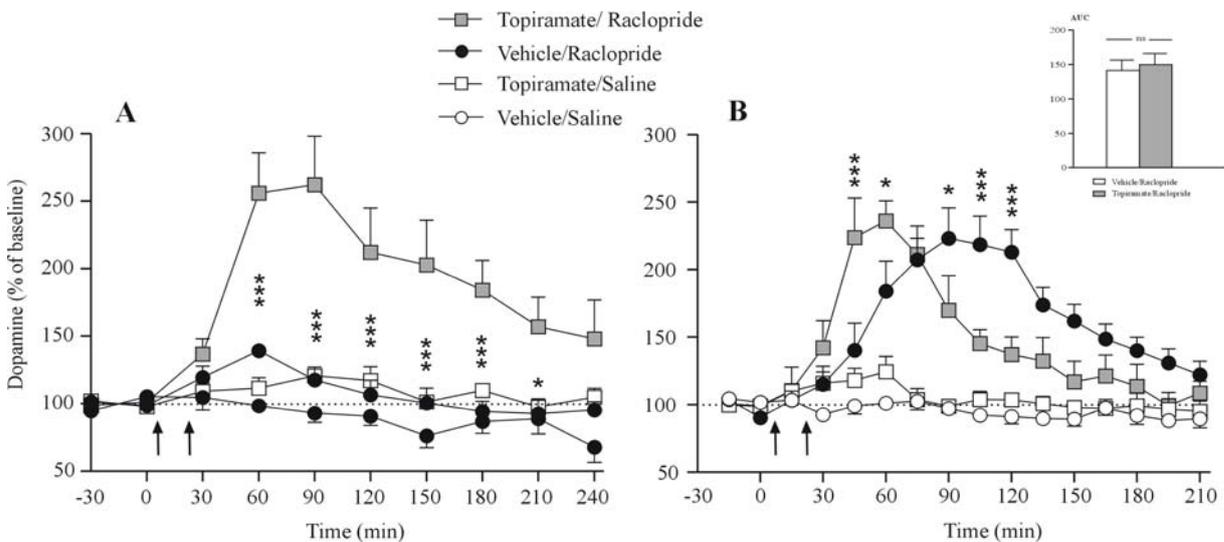


Figure 9

Effects of raclopride (0.075 mg/kg) administration on DA output in (A): mPFC and (B) NAC in rats pretreated with vehicle or topiramate (40 mg/kg). Arrows indicate time of topiramate and raclopride injections, respectively. Each point represents the mean (\pm SEM) percent change from baseline ($n \geq 6$ in all groups). * $P < 0.05$, *** $P < 0.001$ for comparisons between vehicle/raclopride and topiramate/raclopride treatment groups.

Insertion in panel (B) represents cumulative increase (%AUC \pm SEM) following administration of raclopride alone and in combination with topiramate.

Similar to other typical APDs, the effect of the typical APD raclopride on prefrontal DA output is negligible. However, the add-on treatment with either a low dose of L-dopa, or topiramate, to raclopride produced a marked facilitation of prefrontal cortical DA in similarity with clozapine and other atypical, but not typical, APDs. The mechanism behind the observed

increase in prefrontal cortical DA output following L-dopa and raclopride is unclear. As previously discussed in section 4.1.2, addition of L-dopa to the full antagonist raclopride may lead to a partial agonism situation provided the dosing is optimal. There is a considerable interest in the therapeutic potential of partial agonists concerning their use as an alternative treatment against schizophrenia (Tamminga and Carlsson, 2002; Bolonna and Kerwin, 2005). Interestingly, the recently developed partial agonist aripiprazole (Burriss et al., 2002; Fleischhacker, 2005) was shown to cause a marked increase in prefrontal DA output, similar to clozapine and the other atypical APDs (Li et al., 2004).

L-dopa is a precursor of both DA and NA and its administration would be expected to increase the level of both transmitters. In 1990 Carboni and co-workers suggested that in a NA-rich area such as the PFC (Carboni et al., 1990) DA is taken up both by DA and NA terminals. Since, there is a competition between DA and NA at the NA transporter, the rate of DA clearance by the NA transporter would be expected to decrease when NA is increased leading to an increased extracellular level of DA. Thus, the role of NA transporters in the clearance of DA from the extracellular space in the cortex (Yamamoto and Novotney, 1988) may also contribute to the increased levels of DA observed in the mPFC following combined administration of L-dopa and raclopride. Another factor contributing to the increased level of DA could be derived from the recent demonstration that DA is also released from NA terminals (Devoto et al., 2003).

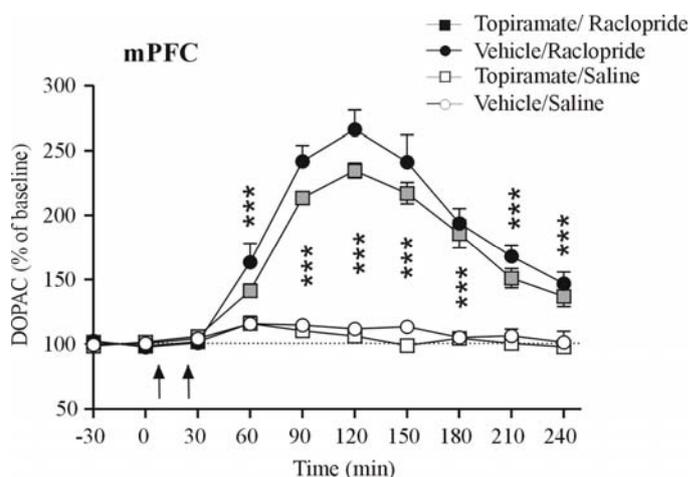


Figure 10

Effects of raclopride (0.075 mg/kg) administration on DOPAC output in mPFC in rats pretreated with saline or topiramate (40 mg/kg). Arrows indicate time of topiramate and raclopride injections, respectively. Each point represents the mean (\pm SEM) percent change from baseline ($n \geq 6$ in all groups). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for comparisons between saline/raclopride and topiramate/raclopride treatment groups.

The mechanism underlying the increase in DA output in the mPFC following combined treatment with topiramate and raclopride is also not clear. The fact that none of these drugs increased prefrontal DA outflow when administered alone, suggests a synergistic interaction between the drugs. The significant increase in prefrontal DOPAC levels that was achieved by the low dose of raclopride (0.075 mg/kg) suggests that the low dose of raclopride used may increase the turnover of prefrontal DA. (see Fig.10). This finding is in agreement with a previous finding from our laboratory showing that raclopride at slightly higher doses may increase extracellular levels of DOPAC in the mPFC without any significant increase in DA (Linnér et al., 2002). Topiramate alone had no effect on DA outflow, but since a low concentration of the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) was shown to increase DA release in the PFC (Wu et al., 2002), indicating that glutamate may exert an inhibitory effect on DA release via AMPA receptors. Therefore, it seems possible that the AMPA receptor antagonistic properties of topiramate may contribute to a synergistic effect. Thus, the enhancement of DA release in the PFC observed in the present study may be due to a synergistic interaction between the antagonistic effect of topiramate on AMPA receptors and an increased turnover of DA by raclopride. Since negative and cognitive symptoms in schizophrenia have been thought to be due to hypoactivity of the mesocortical DA system (Weinberger et al., 1994), the enhanced prefrontal DA output obtained by atypical APDs, as well as with addition of L-dopa or topiramate to a DA D₂ receptor antagonist, may contribute to improve these symptoms (Svensson, 2003). Working memory is thought to be mediated largely through stimulation of DA D₁ receptors in the prefrontal cortical region. Thus facilitation of prefrontal DA may improve cognitive symptoms in schizophrenia (Castner et al., 2000). Our results show that treatment with raclopride alone significantly increased NAC DA output similar to typical APDs. This effect is mostly explained by the ability of APDs to block the DA D₂ autoreceptors, which is thought to trigger a negative feedback response and thereby produce an increase in mesolimbic DA release. In contrast to the markedly increased prefrontal DA output induced by adjunct L-dopa to raclopride, a much smaller increase in DA output in NAC has been detected following the same drug treatment. Conversely, the relatively modest increase in mesolimbic DA output by combined L-dopa and raclopride treatment might reflect the concomitant stimulation of dopaminergic autoreceptors by DA formed from exogenous L-dopa. Not surprisingly, topiramate, alone or in combination with raclopride, did not affect the level of DA released in the NAC. Thus, the output of DA, as induced by raclopride in the NAC, was not significantly different from that obtained by a combination of raclopride and topiramate. The DA-releasing effect of the APDs may thus be seen as indicative of the degree

of D₂ occupancy in the NAC. Consequently, the lack of effect of adjunctive topiramate on the raclopride-induced mesolimbic DA output suggests that adjunct topiramate did not alter the degree of D₂ blockage in the present experiments.

Adjunctive treatment with either L-dopa or topiramate and raclopride did not produce catalepsy. In fact, a slight tendency for a cataleptic response following raclopride (0.075 mg/kg) administration alone was abolished when this dose of raclopride was administered in combination with L-dopa/benserazide or with topiramate (Fig. 11A-B). Within this context it is interesting to note that increased DA release in the PFC following forced swimming, as well as local application of DA into the PFC, can temporarily reverse catalepsy in rats induced by the typical antipsychotic haloperidol (Tucci et al., 1994).

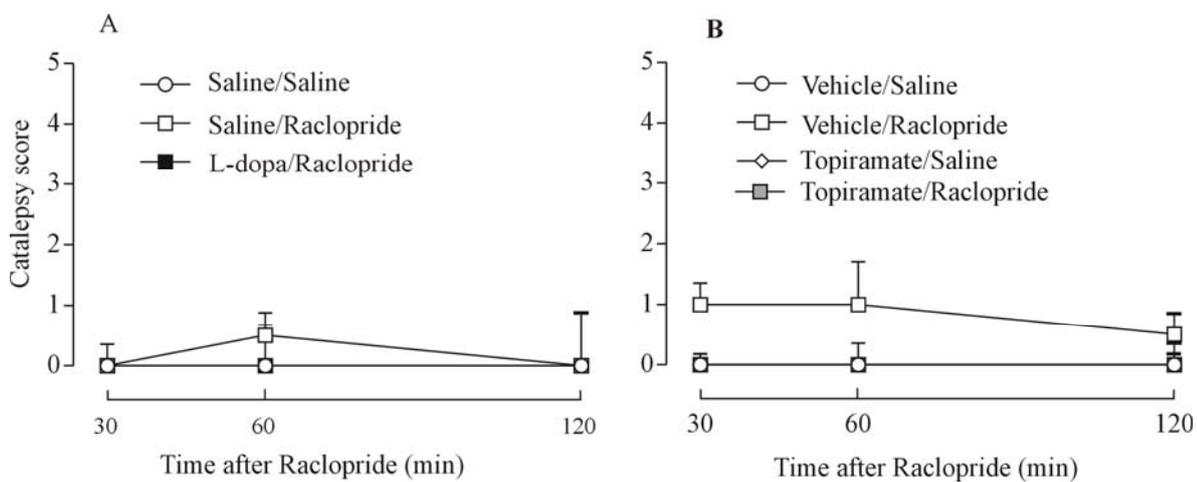


Figure 11 Effects of (A) L-dopa (3 mg/kg) or (B) topiramate (40 mg/kg) when administered alone on the catalepsy test. The results are presented as medians \pm semi-interquartile range, based on the observation of 8 animals per treatment group.

In summary, our results provide experimental support for previous clinical findings showing that addition of L-dopa or topiramate to schizophrenic patients who are maintained on typical APD treatment might be beneficial, especially against negative and cognitive symptoms. These data lend further support to our previous conclusion (Hertel et al., 1999) that an enhanced prefrontal DA output *per se* may serve to improve the effect of typical APDs in schizophrenia.

4.2 Effects of serotonergic drugs on extrapyramidal motor functions and antipsychotic activity in the rat (paper III-IV)

4.2.1 Effects of the combined treatment with a selective serotonin reuptake inhibitor and a 5-HT_{1A} receptor antagonist on extrapyramidal motor functions in rats (paper III-IV)

All selective serotonin reuptake inhibitors (SSRIs) share the ability of increasing synaptic availability of 5-HT in brain (Carrasco and Sandner, 2005). Previous findings have revealed that treatment with high doses of SSRIs may trigger parkinsonism in susceptible individuals (Gerber and Lynd, 1998). In addition, the stimulation or blockade of the 5-HT_{1A} receptor has been reported to modulate DA D_{2/3} receptor-induced catalepsy in rats (Prinssen et al., 2000; Wadenberg and Ahlenius, 1991). It has also been shown that the 5-HT_{1A} receptor agonist 8-OH-DPAT stimulates locomotor activity in rats treated with reserpine, which depletes DA from storage vesicles, suggesting a role for 5-HT_{1A} receptors in extrapyramidal modulation of motor functions (Ahlenius and Salmi, 1995). Thus, it has been hypothesized that stimulation of brain 5-HT_{1A} receptors may protect against an inherent potential parkinsonism of the SSRI treatment. Therefore, in order to investigate this hypothesis, we here evaluated the possible risk of EPS following treatment with low or relatively high doses of citalopram together with the selective 5-HT_{1A} receptor antagonist WAY 100635 using the catalepsy test.

Neither citalopram (10, 20 and 40 mg/kg, s.c.) nor WAY 100635 (0.05, 0.1 and 0.2 mg/kg, s.c.) when administered alone, produced any catalepsy in rats. However, combined treatment with a high dose of citalopram (40 mg/kg) and WAY 100635 (0.05-0.2 mg/kg) produced significant catalepsy (Fig. 12). In contrast, combined treatment with a lower dose of citalopram (20 mg/kg) and 0.2 mg/kg of WAY 100635 did not produce significant catalepsy (Table. 2).

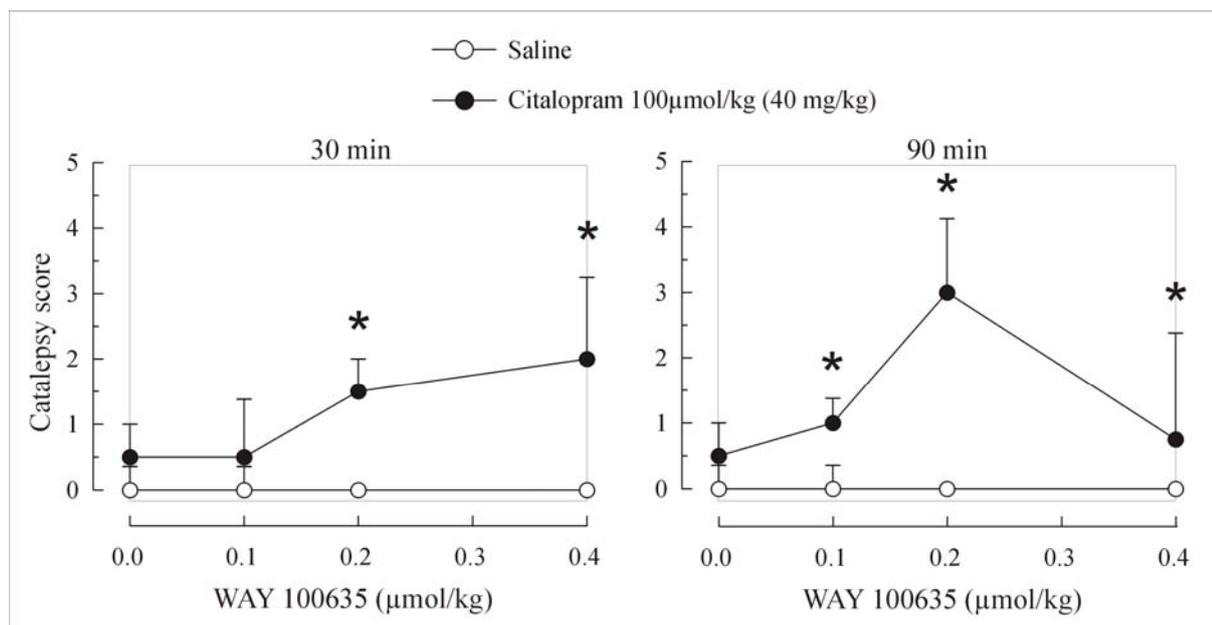


Figure 12

Effects of WAY 100635 on catalepsy in rats pretreated with citalopram. Results are presented as medians (\pm semi-interquartile range) based on repeated observations of 4 animals per treatment group.

* $P < 0.05$ for comparisons between saline and citalopram in the presence of different doses of WAY 100635.

Table 2

Effects of WAY 100635 (0.2 mg/kg) alone or in combination with citalopram (20 mg/kg) on catalepsy in rats.

Shown are medians \pm semi-interquartile range based on observations of 8 animals per treatment group. Statistical analysis, performed by means of Kruskal-Wallis one-way ANOVA, showed no statistical significance.

<i>Treatment (mg/kg)</i>	<i>Time (min)</i>			
	<i>30</i>	<i>60</i>	<i>120</i>	<i>n</i>
<i>Saline/Vehicle</i>	<i>0.0 \pm 0.0</i>	<i>0.0 \pm 0.0</i>	<i>0.0 \pm 0.0</i>	<i>8</i>
<i>Saline/WAY (0.2)</i>	<i>0.0 \pm 0.0</i>	<i>0.5 \pm 1.0</i>	<i>0.0 \pm 0.5</i>	<i>8</i>
<i>Citalopram(20)/Vehicle</i>	<i>0.0 \pm 0.0</i>	<i>0.0 \pm 0.5</i>	<i>0.0 \pm 0.5</i>	<i>8</i>
<i>Citalopram(20)/WAY (0.2)</i>	<i>0.0 \pm 0.25</i>	<i>1.0 \pm 0.75</i>	<i>1.0 \pm 1.0</i>	<i>8</i>

Catalepsy often occurs following a high blockage of the DA D₂ receptor ($\geq 80\%$) by APD treatment. However, the mechanism behind the catalepsy that we observed following citalopram and WAY 100635 in combination is not clear. These drugs are both known to possess some affinity for DA D₂ receptors, but not enough to produce catalepsy. Treatment with citalopram and WAY 100635 would be expected to increase the 5-HT level in brain. It seems possible that a high level of 5-HT may directly or indirectly affect the nigrostriatal DA system and thereby produce catalepsy. Both possibilities have been supported experimentally. For example, while it has been reported that infusion of 5-HT in the neostriatum may facilitate DA release in this region (Benloucif et al., 1993), another study has shown that administration of the SSRI fluoxetine might cause a decrease of DA release. Earlier studies have shown that a reduction in serotonergic neural transmission attenuates neuroleptic-induced catalepsy (Gumulka et al., 1973), while increased availability of synaptic 5-HT potentiates catalepsy induced by inhibition of DA neural transmission (Fuenmayor and Vogt, 1979; Balsara et al., 1979). In consonance with these findings, it has been suggested that a high level of 5-HT may exert an inhibitory effect on dopaminergic neural transmission. Thus, an increase of the 5-HT level in brain may inhibit nigrostriatal DA release and subsequently suppresses extrapyramidal motor functions. Since 5HT_{1A} receptors are blocked by WAY 100635, it seems possible that the enhanced inhibition of dopaminergic, in particular nigrostriatal activity, is mediated via a 5-HT receptor subtype other than the 5-HT_{1A}. It should be noted that the maximal catalepsy obtained by a high dose of citalopram and WAY 100635 appears less pronounced than the catalepsy obtained in experiments with DA D_{2/3} receptor antagonists such as haloperidol or raclopride. Taken together our data indicate that catalepsy can, even in the absence of any direct DA D₂ receptor blockade, be triggered by a high dose of SSRIs treatment together with 5-HT_{1A} receptor blockade via increasing synaptic availability of 5-HT.

4.2.2 Effects of the selective serotonin reuptake inhibitor citalopram alone or in combination with the 5-HT_{1A} receptor antagonist WAY 100635 on CAR (paper IV)

As mentioned above we found that treatment with a high dose of citalopram (40 mg/kg) together with WAY 100635 produced significant catalepsy in similarity with typical APDs. Thus, this drug combination, might also show antipsychotic-like effect. Therefore, we evaluated the possible antipsychotic action of citalopram (10 and 20 mg/kg) and WAY 100635 (0.2 mg/kg) using the CAR model.

Treatment with citalopram (10, 20 and 40 mg/kg) when given alone had no effect on CAR. However, combined treatment with citalopram (20-40 mg/kg), but not 10 mg/kg, and

WAY 100635, produced a statistically significant suppression of CAR (Fig. 14). The combined treatment with citalopram (40 mg/kg) and WAY 100635 did not further suppress CAR compared with the suppression effect that observed following citalopram (20 mg/kg) and WAY 100635 treatment.

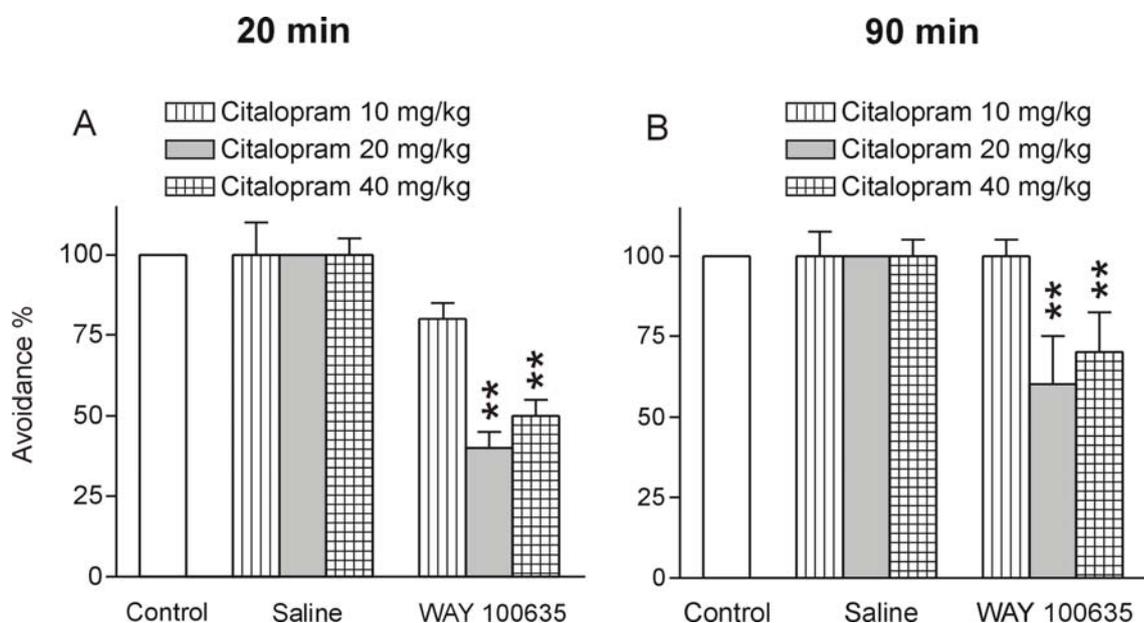


Figure 13

Effects of citalopram alone or in combination with WAY 100635 (0.2 mg/kg) on conditioned avoidance response (A) 20 min (B) 90 min after administration of citalopram; each bar represents the median (avoidance %) \pm semi-interquartile range based on the observation of the same 8 animals serving as their own controls.

* $P < 0.05$, ** $P < 0.01$ for comparisons with vehicle treated animals.

4.2.3 Effects of the selective 5-HT_{2C} receptor antagonist SB242084 on Citalopram/WAY 100635 induced-suppression of the CAR behavior (paper IV)

Our previous result showed that combined treatment with citalopram (20 mg/kg) /WAY 100635 induced a significant suppression of CAR behavior. Other 5-HT receptor subtypes than 5-HT_{1A} receptor are likely to mediate the antipsychotic-like effect of citalopram and WAY 100635 in CAR. Several studies have indicated the involvement of 5-HT₂ receptors in the antipsychotic action of atypical APDs such as clozapine. While a 5-HT_{2A} receptor agonists have been shown ineffective in an animal model of antipsychotic activity such as CAR (Wadenberg, personal communication), recent data indicate that 5-HT_{2C} receptor agonists may possess an antipsychotic activity as shown in preclinical models for antipsychotic activity (Browning et al., 1999). Accordingly, we here evaluated the role of the 5-HT_{2C} receptor in the citalopram/WAY 100635-induced suppression of CAR behavior using the selective 5-HT_{2C} antagonist SB 242084. We found that pre-treatment with SB 242084 (0.5 mg/kg), which, by itself, had no

effect on CAR, completely prevented the citalopram/WAY 100635-induced suppression of CAR (Fig. 14).

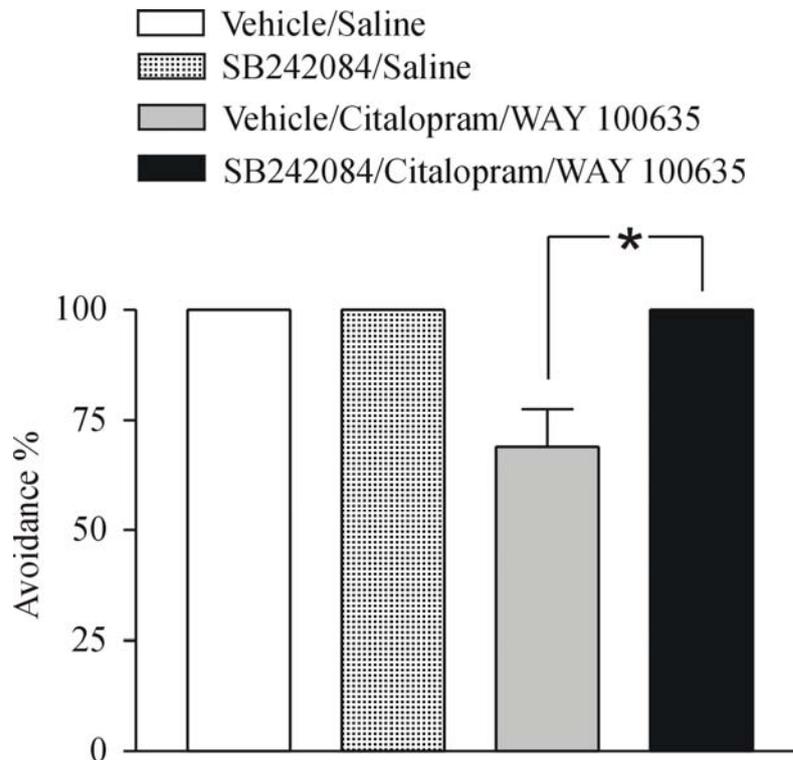


Figure 14

Bars represent the median (avoidance %) \pm semi-interquartile range based on the observation of the same 8 animals serving as their own controls.

* $P < 0.05$ for comparisons between animals treated with citalopram/WAY100635 and animals treated with SB 242084/citalopram/WAY100635.

As mentioned previously, selective suppression of CAR, but not escape behavior is a unique effect for all APDs (Arnt, 1982). These drugs also generally produce suppression of CAR in doses that correlate well with clinically effective doses (Seeman, 1992). Furthermore, as mentioned earlier, blockade of mesolimbic DA transmission in particular, seems important for suppression of CAR by APDs (Wadenberg et al., 1990). In the present study, we found that the combined citalopram/WAY 100635 treatment suppresses the avoidance behavior in CAR although the underlying mechanism is not clear. Neuroanatomical studies indicate that both the SN and the VTA, as well as their projections, receive innervation from 5-HT containing axon terminals originating in the raphe nuclei (Azmitia and Segal, 1978), indicating possible interactions between serotonergic and dopaminergic systems. Thus, our present findings would be compatible with the notion that the combined treatment with citalopram/WAY 100635 may act indirectly by modulating a serotonergic input that inhibits the mesolimbic DA system, thus causing a suppression of CAR behavior. In all probability, this combination would be expected to cause an increase in synaptic levels of 5-HT that would be able to stimulate various

serotonergic receptor subtypes, except for 5-HT_{1A}. Pre-treatment with the selective 5-HT_{2C} receptor antagonist SB242084 completely prevented the suppression of CAR by combined citalopram/WAY 100635 treatment suggesting that the effect is mediated through 5-HT_{2C} receptor stimulation. An involvement of the 5-HT_{2A} or the 5-HT_{1B/1D} receptors is unlikely, since the selectivity of SB242084 for the 5-HT_{2C} receptor is at least 100-fold over these receptor subtypes (Kennett et al., 1997).

Here we found that citalopram (20 mg/kg) in combination with WAY 100635 significantly suppressed CAR but did not produce significant catalepsy (Table 2). Combined treatment with citalopram (40 mg/kg) and WAY 100635, however, did produce significant catalepsy. Therefore, addition of WAY 100635 to a high dose (40 mg/kg) of citalopram may increase the level of 5-HT to the point that it may inhibit nigrostriatal dopaminergic activity thereby causing catalepsy. On the other hand, a lower dose of citalopram together with WAY 100635 may instead potentially result in a more selective modulation of the mesolimbic dopaminergic activity leaving the nigrostriatal DA pathway relatively unaffected. Accordingly, it should be noted that increasing the dose of SSRI did not lead to any additional effect on CAR of the combined treatment.

As mentioned previously, the suppression of CAR by combined treatment with citalopram and WAY 100635 was similar in magnitude to the effect of a low dose of the typical antipsychotic haloperidol, that in this dose range would be expected to produce a striatal DA D₂ receptor occupancy around 55-65% (Wadenberg et al., 2001). Striatal DA D₂ receptor occupancy of around 65-70% is needed for a sufficient therapeutic response in acutely psychotic schizophrenics (Kapur et al., 2000). However, patients in remission, and other categories of patients with mild psychotic symptoms, may do well also at a D₂ occupancy of about 65%, compatible with the magnitude of effect on CAR seen by combined citalopram/WAY 100635 in the present study.

In conclusion, our data showed that combined treatment with citalopram and WAY 100635 produced an antipsychotic-like effect in CAR without significant catalepsy. The effect on CAR by this treatment combination was completely prevented by pre-treatment with the selective 5-HT_{2C} receptor antagonist SB242084, indicating a specific involvement of the 5-HT_{2C} receptor. These data suggest, in principle, a novel and potentially clinically useful combinatorial drug treatment for individuals suffering from mild psychotic illness with or without concomitant depressive symptoms.

5 GENERAL DISCUSSION

Given the fact that the severity of cognitive impairment in schizophrenia is a critical determinant of treatment outcome (Green, 1996), it is important to find alternative pharmacological strategies that are clinically effective and with less side effects. Cognitive symptoms, particularly deficit in working memory and attention, are thought to be due to insufficient activation of the DA D₁ receptor. For example, it has been shown that schizophrenic patients have an increased DA D₁ receptor availability in the PFC (Abi-Dargham et al., 2002). A negative correlation between the D₁ receptor availability and performance on a task requiring working memory was observed. Thus, it was proposed that DA D₁ receptors are upregulated to compensate for decreased release of DA. Moreover, a significant increase in the transmission of the catechol-O-methyltransferase Val allele, involved in the catabolism of prefrontal DA, has been found in families afflicted by schizophrenia (Egan et al., 2001) supporting the notion that the cortical hypodopaminergic state is not due to deficient or missing receptors but to presynaptic mechanisms controlling release or breakdown of DA. This hypothesis also derives support from several previous clinical studies where it was shown that drugs that increase DA release, e.g. L-dopa and amphetamine, which in high doses may trigger positive symptoms, can improve negative symptoms.

In this thesis, we have demonstrated that adjunctive treatment with either a low dose of L-dopa or topiramate markedly augment the antipsychotic-like effect of raclopride in the CAR model concomitant with a preferential increase in prefrontal DA release. As both these drugs have been used clinically to augment pharmacological treatment in schizophrenia, our results indicate that the clinical effects of these drugs combined with D₂ antagonists can be experimentally reproduced within the CAR model. Moreover, even though it has previously been shown that the performance of CAR behavior is critically dependent on an intact dopaminergic neurotransmission in the NAC or adjacent areas of the ventral striatum (Wadenberg et al., 1990), it should be noted that alterations in cortical DA may also play a role in CAR behavior. A number of studies have proposed that cortical DA may regulate subcortical DA transmission (Saunders et al., 1998; Murase et al., 1993). Therefore, in the present studies, it might be hypothesized that the marked increase in cortical DA release produced by addition of L-dopa or topiramate inhibits mesolimbic DA function and thereby reduces the dose needed of raclopride to cause a suppression of CAR behavior that is sufficient for antipsychotic activity. By inference, our data suggest that an increase in prefrontal DA may

not only improve negative and cognitive symptoms but may indirectly have beneficial effects also on positive symptoms.

A common problem with antipsychotic treatment is the frequent occurrence of EPS. Comparisons between animals and humans have revealed that at DA D₂ occupancies over 80% motor dysfunctions start emerging (Farde et al., 1992). Our results, showing that combined treatment with a low dose of SSRI and the 5-HT_{1A} receptor antagonist WAY 100635 produced significant catalepsy, indicate that nigrostriatal dopaminergic transmission can be inhibited without any significant DA D₂ receptor occupancy. Analogous to the graded behavioral response to DA D₂ receptor occupancy, we hypothesized that by lowering the dose of citalopram in the citalopram/WAY 100635 we might detect an antipsychotic-like effect in CAR. Indeed we found that citalopram/WAY 100635 showed antipsychotic activity in the CAR model indicating that serotonergic mechanisms may contribute to inhibit mesolimbic dopaminergic neurotransmission. Since we found that the combined effect of citalopram and WAY 100635 was prevented by a 5-HT_{2C} antagonist, we suggest that the antipsychotic activity is related to stimulation of 5-HT_{2C} receptors. This notion is supported by recent studies indicating antipsychotic effects of selective 5-HT_{2C} agonists in behavioral models.

In summary, our studies provide neurobiological rationale and framework for previous clinical findings demonstrating augmenting effects of L-dopa and topiramate in the treatment of schizophrenia. They also provide additional support for the modified DA hypothesis of schizophrenia suggesting that besides a hyperactive subcortical DA projection, impaired cortical dopaminergic transmission is a critical factor for the symptomatology (Hertel et al., 1999; Laruelle and Abi-Dargham, 1999). Finally, our findings suggest the important conclusion that while direct blockade of DA D₂ receptors is sufficient to generate an antipsychotic effect, D₂ blockade may not necessarily be a prerequisite.

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