

Dopaminergic stabilizers for the treatment of schizophrenia

**Rat studies focusing on negative symptoms and
mechanisms of action**

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ABSTRACT

Schizophrenia is a severe mental disorder manifested by positive, negative and cognitive symptoms. Current antipsychotic drugs have poor effects on negative and cognitive symptoms, thus necessitating the development of new antipsychotic treatments.

Dopaminergic stabilizers constitute a novel concept for the treatment of schizophrenia. These drugs are claimed to normalize dopaminergic transmission in case of either excessive or deficient signalling. Possibly, such drugs are particularly useful for treating conditions involving both increased and decreased dopaminergic tone, as may be the case in schizophrenia. The present thesis focuses on the dopaminergic stabilizers (-)-OSU6162 and ACR16.

The main objectives of this thesis were to 1) test the effects of (-)-OSU6162 and ACR16 in a rat model for negative symptoms, 2) explore their stabilizer properties and 3) shed light on the mechanisms of action of these drugs. (-)-OSU6162 and ACR16 were found to reverse social withdrawal in rats, induced by the NMDA receptor antagonist (+)-MK-801. These results suggest that these drugs may be effective against negative symptoms of schizophrenia. (-)-OSU6162 and ACR16 both had baseline dependent effects on motor activity in drug naïve rats, inhibiting behaviour in rats with a high motor activity level and stimulating behaviour in rats with a low activity level. These effects may be interpreted as support for dopaminergic stabilization.

Based on the effects on prolactin secretion observed in the present work and results from studies performed in other laboratories, it appears that (-)-OSU6162, and probably also ACR16, have negligible intrinsic activities at the D2 receptor. Thus, the behavioural inhibition caused by these drugs is probably a result of D2 receptor blockade. The present results suggest that (-)-OSU6162 and ACR16 act via at least two D2-receptor associated targets with opposing actions on dopaminergic transmission. A recent *in vitro* study suggests that (-)-OSU6162, apart from blocking the orthosteric site, also facilitates receptor activation by binding to an allosteric site at the D2 receptor. This finding provides a candidate for the activating target for (-)-OSU6162 and ACR16.

LIST OF PUBLICATIONS

This thesis is based on the following publications, which will be referred to in the text by their Roman numerals:

- I **Rung JP**, Carlsson A, Rydén Markinhuhta K, Carlsson ML (2005) (+)-MK-801 induced social withdrawal in rats; a model for negative symptoms of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*, 29(5): 827-832.
- II **Rung JP**, Carlsson A, Rydén Markinhuhta K, Carlsson ML (2005) The dopaminergic stabilizers (-)-OSU6162 and ACR16 reverse (+)-MK-801-induced social withdrawal in rats. *Prog Neuropsychopharmacol Biol Psychiatry*, 29(5): 833-839.
- III **Rung JP**, Rung E, Helgeson L, Johansson AM, Svensson K, Carlsson A, Carlsson ML (2007) Effects of (-)-OSU6162 and ACR16 on motor activity in rats, indicating a unique mechanism of dopaminergic stabilization (*submitted*).
- IV **Rung JP**, Rung E, Johansson AM, Svensson K, Carlsson A, Carlsson ML (2007) The dopaminergic stabilizers (-)-OSU6162 and ACR16 stimulate prolactin release in rats (*manuscript*).

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ABBREVIATIONS

ABBREVIATIONS

D1R	dopaminergic D1 receptor
D2R	dopaminergic D2 receptor
EPS	extrapyramidal side effects
GBL	γ -butyrolactone
GPCR	G-protein coupled receptor
MK-801	(+)-MK-801
NMDAR	glutamatergic N-methyl-D-aspartate receptor
OSU6162	(-)-OSU6162
PCP	phencyclidine

POPULÄRVETENSKAPLIG SAMMANFATTNING

Schizofreni är en allvarlig psykisk sjukdom som kan medföra stort lidande för den som drabbas och för hans eller hennes närstående. Symptomen vid schizofreni brukar delas in i kategorierna positiva, negativa och kognitiva symptom. Positiva symptom syftar på egenskaper som tillförs normaltillståndet och omfattar bland annat hallucinationer och vanföreställningar. Negativa symptom innebär att förmågor har gått förlorade. Bland dessa symptom återfinns isolering från socialt umgänge, utslätat känsloliv, viljelöshet och oförmåga att känna njutning. Kognitiva symptom medför problem med bland annat uppmärksamhet, minne och inläring. De läkemedel som finns idag för behandling av schizofreni har i regel god effekt på positiva symptom. Dessvärre är effekterna på negativa och kognitiva symptom inte tillfredsställande. Många av dessa läkemedel är dessutom behäftade med allvarliga biverkningar. Därför är det viktigt med forskning som syftar till att finna nya, bättre läkemedel mot schizofreni.

Gemensamt för alla nuvarande läkemedel mot schizofreni är att de hämmar hjärnans signalering med signalsubstansen dopamin. Detta ledde en gång till slutsatsen att signaleringen med dopamin är överaktiv i hjärnan hos personer med schizofreni. Det har visat sig att den bilden av dopaminets roll vid schizofreni är kraftigt förenklad. Nu tror man istället att signaleringen med dopamin kan vara förhöjd i vissa delar av hjärnan och sänkt i andra. Därför är en generell hämning av denna signalering troligen inte en optimal strategi vid behandling av schizofreni. Vissa av dagens läkemedel kan till och med förvärra de symptom som har satts i samband med sänkt signalering med dopamin. En annan föreslagen orsak till schizofreni är en felaktig signalering med signalsubstansen glutamat. Denna teori baseras på att droger som blockerar glutamat-signalering orsakar ett tillstånd hos människa som är nästan omöjligt att särskilja från schizofreni. En sådan drog är fencyklidin (angel dust), som har varit föremål för omfattande missbruk i framför allt USA.

Så kallade dopaminerga stabiliserare är en typ av läkemedel under utveckling som kan motverka både förhöjd och sänkt signalering med dopamin i hjärnan. Sådana läkemedel skulle kunna vara speciellt användbara vid schizofreni som har satts i samband med en kombination av överaktivitet och underaktivitet i signaleringen med dopamin. Den här avhandlingen fokuserar på de två dopaminerga stabiliserarna OSU6162 och ACR16.

Minskat socialt beteende hos råttor som behandlats med vissa droger anses motsvara negativa symptom vid schizofreni. Den här avhandlingen visar att både OSU6162 och ACR16 kan återställa socialt beteende till normala nivåer hos dessa råttor. Detta tyder på att OSU6162 och ACR16 kan vara effektiva mot negativa symptom.

Droger som stimulerar signalering med dopamin gör råttor mer aktiva, medan droger som hämmar signalering minskar deras aktivitet. Även utan behandling med droger varierar signaleringen med dopamin i olika situationer och över dygnet, vilket avspeglas i råttornas aktivitet. Det här arbetet visar att OSU6162 och ACR16 kan öka aktiviteten hos inaktiva råttor, och dessutom minska aktiviteten hos råttor som är aktiva. Det kan tolkas som att OSU6162 och ACR16 kan öka signaleringen med dopamin när den är låg och hämma signaleringen när den är hög. Således tycks OSU6162 och ACR16 verka som dopaminerga stabiliserare.

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Schizophrenia

Schizophrenia is a severe, often life-long, mental disorder which causes considerable suffering for those affected and a great cost for society. There are three main groups of symptoms associated with schizophrenia, i.e. positive, negative and cognitive symptoms. Positive symptoms are traits added to normality and comprise hallucinations and delusions. The negative and cognitive symptoms both involve loss of function. Negative symptoms include social withdrawal, flattened affect, apathy and anhedonia. Cognitive symptoms involve impairments in learning, memory, attention and executive functions. It should be mentioned in this context that schizophrenia is a very heterogeneous condition; there are great variations among schizophrenics with respect to the relative severity of different symptoms. This makes it necessary to question the concept of schizophrenia as being one single disease. The lifetime prevalence of schizophrenia is approximately 1% and does not vary across sexes, nationalities, cultures or ethnic groups. Schizophrenia is usually said to appear in late adolescence or early adulthood, commonly later among women compared to men. This presumed age of onset refers to the first psychotic episode. However, this is now believed to be preceded by a long prodromal stage of negative and cognitive symptoms (Cannon et al., 2002; Carpenter, 2006; Gross, 1997; Mueser and McGurk, 2004).

Pathophysiology of schizophrenic symptoms

The aetiology of schizophrenia remains unclear. As mentioned above, schizophrenia is a heterogeneous condition which points to a heterogeneity in both aetiology and pathophysiology (Cardno and Farmer, 1995; Tamminga and Holcomb, 2005). As to the pathophysiology, schizophrenic symptoms have been put in connection with dysfunctional transmission with a variety of different neurotransmitters, e.g. dopamine, glutamate, serotonin, GABA and acetylcholine (e.g. Davis et al., 1991; Tamminga, 2006). In this thesis, focus is on dopaminergic and glutamatergic transmission which are those most commonly discussed in the context of schizophrenia.

The dopamine hypothesis

All antipsychotic drugs on the market inhibit dopaminergic signalling to some degree, and the effects of antipsychotic drugs on positive symptoms often correlate with affinity for dopaminergic D2 receptors (D2Rs).

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Furthermore, prolonged administration with dopaminergic agonists, such as amphetamines and L-DOPA, may cause psychotic symptoms resembling positive symptoms of schizophrenia (Angrist et al., 1974b; Srisurapanont et al., 2003; Young and Scoville, 1938). In individuals suffering from schizophrenia, or with mental illness in the family, one single administration of a low dose of amphetamine may be sufficient to temporarily cause or worsen psychotic symptoms (Janowsky et al., 1973; Weiner, 1964). Also, these psychotic symptoms, resulting from dopaminergic stimulation, may be antagonized by antipsychotic drugs (Angrist et al., 1974a; Jha and Fourie, 1999; Misra and Kofoed, 1997; Misra et al., 2000). Early on, such observations led to the dopamine hypothesis of schizophrenia, postulating that schizophrenia is due to excessive subcortical dopaminergic activity (Randrup, 1970; Snyder, 1973). However, negative and cognitive symptoms are not, to any greater extent, induced by dopaminergic agonists or reversed by dopaminergic antagonists. Thus, the dopamine hypothesis, in its original form, does not provide any plausible mechanism for negative and cognitive symptoms.

Instead, drugs stimulating dopamine release are reported to alleviate negative symptoms in schizophrenic patients (Benkert et al., 1995; Van Kammen and Boronow, 1988). In healthy volunteers, improved working memory has been observed in response to a mixed dopaminergic D1/D2 receptor agonist, but not after administration of a selective D2R agonist (Müller et al., 1998). Furthermore, in subhuman primates it has been demonstrated that low doses of D1 receptor (D1R) agonists improve cognitive functions, and that D1R antagonists cause worsening of cognitive function. Therefore, a defective dopaminergic input to the prefrontal cortex has been suggested as a cause for negative and cognitive symptoms. It has also been shown however, that excessive D1R stimulation is associated with impaired cognitive performance. D1Rs are by far more abundant than D2Rs in the prefrontal cortex, and are reported to be up-regulated in schizophrenic patients. This, along with the effects of D1R agonists and antagonists, has led to the proposal that cognitive deficits, and possibly also negative symptoms, may be due to a low D1R stimulation in the prefrontal cortex (Abi-Dargham, 2003; Abi-Dargham, 2004; Abi-Dargham and Moore, 2003; Davis et al., 1991). Thus, it seems that striatal hyperdopaminergia and cortical hypodopaminergia could underlie positive and deficit symptoms respectively.

Investigations indicate that inhibition and stimulation of cortical dopaminergic activity induces opposite effects on dopamine levels in the striatum, possibly via glutamatergic and GABA-ergic pathways. This

suggests that subcortical hyperdopaminergia, resulting in positive symptoms, could be at least in part secondary to cortical hypodopaminergia (Abi-Dargham, 2004; Davis et al., 1991; Tzschentke, 2001). It is interesting in this context that negative symptoms and cognitive deficits may exist years before the appearance of positive symptoms (Cannon et al., 2002; Gross, 1997).

The glutamate hypothesis

N-methyl-D-aspartate receptor (NMDAR) antagonists, such as phencyclidine (PCP) and ketamine, are known to induce a state in healthy humans practically indistinguishable from schizophrenia. This state includes positive, negative as well as cognitive symptoms. In schizophrenic patients these drugs may cause relapse or worsening of the symptoms characteristic of the patient's individual psychosis (Krystal et al., 1994; Lahti et al., 2001; Luby et al., 1959; Snyder, 1980; Stone et al., 2007). These observations form the basis of the glutamate hypothesis of schizophrenia, stating that schizophrenic symptoms are a result of dysfunctional glutamatergic signalling. It has been suggested that this could be due to malfunctioning NMDARs (Stone et al., 2007). In support of the glutamate hypothesis are the observed favourable effects of some drugs promoting NMDAR function. Such drugs, added to antipsychotic treatment, are reported to alleviate negative symptoms and, to some degree, cognitive symptoms (Stip and Trudeau, 2005; Tuominen et al., 2005). It is conceivable that any effect on positive symptoms is masked by the patients' treatment with conventional drugs. In children NMDAR antagonists are reported not to cause psychotic symptoms. It seems that susceptibility to the psychotomimetic effects of these drugs appear at an age when schizophrenia typically would appear. Thus, schizophrenic illness and psychotic symptoms caused by NMDAR antagonists could share the same mechanisms (Stone et al., 2007).

Synthesis of the dopamine and glutamate hypotheses

The dopamine and glutamate hypotheses for schizophrenia are by no means incompatible. NMDAR antagonists affect dopamine release both in the prefrontal cortex and in the striatum. In rats, repeated administrations stimulate dopaminergic transmission in the striatum while transmission is decreased in cortex. Thus, a prolonged NMDAR hypofunction could result in the dopaminergic aberrations seen in schizophrenia. However, acute NMDAR antagonist administration has also been reported to increase cortical dopaminergic transmission (Bubser et al., 1992; Jentsch and Roth, 1999). As mentioned above, elevated and reduced dopaminergic activity in the prefrontal cortex may induce opposite effect in the striatum. This regulation of striatal dopaminergic activity is believed to be mediated by glutamatergic

pathways, as these have a central role in cortico-striatal signalling (Carlsson et al., 1999; Laruelle et al., 2003). Thus, both glutamatergic and dopaminergic signalling are undoubtedly altered in schizophrenia. It is uncertain which or if one of these phenomena is downstream to the other.

Antipsychotic treatments

Comments on intrinsic activity at D2-receptors

Antipsychotic drugs were until recently characterized as D2R antagonists, i.e. without intrinsic activity at D2Rs. The terms “intrinsic activity” and agonism are often used synonymously, as if suggesting that all drugs could be characterized as either agonists or antagonists. All known dopaminergic receptors (D1-D5) are G-protein coupled receptors (GPCRs). According to one current simplified model for GPCR function, these receptors are in equilibrium between one active and one inactive state. Any ligand that, upon binding, may shift this equilibrium has intrinsic activity at the receptor; agonists and inverse agonists shift the equilibrium in favour of the active and inactive state respectively. This shift is suggested to reflect the ligand’s relative preference for either state of the receptor. An active ligand acts towards a new equilibrium that reflects its intrinsic activity. An antagonist has no preference for any of the two states of the receptor, and does not affect the equilibrium in absence of other ligands (Strange, 1999). One could conceptualize the intrinsic activities of GPCR ligands as forming a continuum ranging from full inverse agonism to full agonism. According to this model, the probability of a pure antagonist should be infinitely small (Milligan, 2003; Milligan et al., 1995). It is conceivable that the constitutive activity of a receptor is not constant and could be partly dependent on the circumstances. If so, the effects of drugs would depend, not only on intrinsic activity and presence of other ligands, but also on factors determining the equilibrium of the receptor. This could be a mechanism involved in sensitization/desensitization of receptors. A shift in a receptors constitutive activity would not affect the response to a true antagonist. Lately it has been shown that a majority of typical and atypical antipsychotic drugs may be, not antagonists, but reverse agonists at D2Rs (Akam and Strange, 2004; Hall and Strange, 1997; Nilsson et al., 1996; Roberts and Strange, 2005).

Current antipsychotic medications

Antipsychotic drugs are often categorized into typical and atypical antipsychotics. These two groups of drugs differ mainly with respect to their propensity to cause extrapyramidal side effects (EPS). The typical drugs are generally laden with serious EPS, whereas atypical drugs have the advantage

of causing less EPS. These adverse effects are known to occur at D2R occupancies of approximately 80% or higher. It has been suggested that atypical drugs are clinically effective at D2R occupancies below that level. Another suggested mechanism underlying atypicality is a relatively high affinity for serotonergic 5-HT₂ receptors versus D2Rs (Kapur and Mamo, 2003). Yet another suggested advantage of atypical compared to typical drugs is a lower propensity to cause mental side effects, i.e. effects similar to the negative symptoms of schizophrenia (Lublin et al., 2005; Tandon and Jibson, 2002). Some atypical drugs do however cause other severe adverse effects such as weight gain, increased risk for diabetes and, in the case of clozapine, risk of agranulocytosis (Shirzadi and Ghaemi, 2006; Wahlbeck et al., 2000).

Typical and atypical antipsychotic drugs are generally described as equally effective for the treatment of positive symptoms. The typical drugs have little or no effect on negative or cognitive symptoms, whereas atypical antipsychotics are often claimed to be more effective (Stip, 2000). It has been pointed out that head to head comparisons of atypical versus typical agents may be deceptive, due to the mental side effects of typical antipsychotics. A lower propensity for atypical drugs to cause mental side effects could be mistaken for an improvement of negative symptoms (Lublin et al., 2005). The Cochrane reviews on the subject collectively suggest that, among the atypical antipsychotics, only clozapine has a better overall efficacy than typical agents (Duggan et al., 2005; El-Sayeh and Morganti, 2006; Hunter et al., 2003; Lewis et al., 2005; Srisurapanont et al., 2004; Wahlbeck et al., 2000). However, the effect of clozapine on primary negative symptoms, i.e. not secondary to positive symptoms or antipsychotic treatment, has been the subject for animated debate (Carpenter et al., 1995; Carpenter et al., 1996; Meltzer, 1995). One would expect that any antipsychotic drug with convincing effects on negative symptoms and cognitive deficits would by now have received recognition. Thus, the search is on for novel effective treatments for this aspect of schizophrenia.

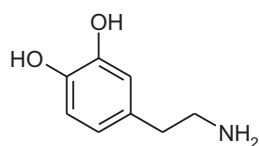
Dopaminergic stabilizers

Conceptually, dopaminergic stabilizers are drugs that normalize dopaminergic signalling in case of either excessive or deficient dopaminergic tone. Such drugs have been proven useful in the treatment of conditions involving dysfunctional dopaminergic signalling, e.g. schizophrenia, Parkinson's disease and Huntington's disease (Gefvert et al., 2000; Petrie et al., 1997; Pirtosek et al., 1996; Tamminga and Carlsson, 2002; Tedroff et al., 1999). Stabilizing of dopaminergic transmission would be particularly useful in the treatment of conditions involving both increased and decreased

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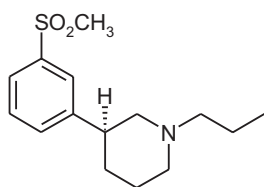
signalling as is suggested in the case of schizophrenia. Also these drugs would counteract hyperdopaminergia with little risk of adverse effects resulting from excessive dopaminergic inhibition. The few drugs which are ascribed dopamine stabilizing effects have been classified as either partial D2R agonists or D2R antagonists. This thesis focuses on the latter of these two groups.

Aripiprazole (Figure 1) is the only claimed dopaminergic stabilizer having reached the market, and is characterized as an atypical antipsychotic agent. Thus, it is not laden with any serious EPS. Like most atypical antipsychotic drugs, aripiprazole has been described as equal to typical drugs with respect to effects on positive symptoms. Individual clinical studies report of reductions of negative and cognitive symptoms (Kane et al., 2002; Potkin et al., 2003). In a recent meta-study, however, aripiprazole does not appear to outdo typical agents in this respect (El-Sayeh and Morganti, 2006). Aripiprazole is a partial D2R agonist with low intrinsic activity (Fujikawa et al., 1996; Kikuchi et al., 1995). (-)-3-PPP (Figure 1) is another D2R partial

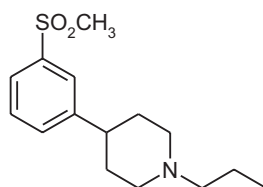


Dopamine

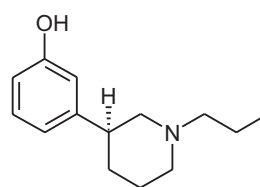
Figure 1. Chemical structures of dopamine and the claimed dopaminergic stabilizers (-)-OSU6162, ACR16, (-)-3-PPP and aripiprazole.



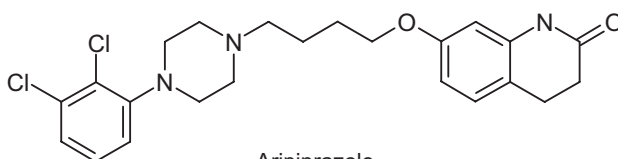
(-)-OSU6162



ACR16



(-)-3-PPP



Aripiprazole

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agonist also discussed in connection with dopaminergic stabilizers (Carlsson et al., 2004). A D2R partial agonist is per definition stabilizing on D2R-mediated transmission at the level of the receptor; it acts towards a level of receptor activation which corresponds to the drug's intrinsic activity. Thus, in case of a low dopaminergic tone, i.e. with a large proportion of receptors at rest, the overall effect will be receptor stimulation. In contrast, in case of a high dopaminergic tone when a large proportion of D2Rs are in the active state, the partial agonist will compete with dopamine and cause an average shift in favour of the inactive state, i.e. acting as an antagonist.

The second group of dopaminergic stabilizers comprise two drugs, (-)-OSU6162 (OSU6162) and ACR16 (Figure 1), which are both structurally similar to the above mentioned partial D2R agonists (-)-3-PPP. When these drugs were first characterized they were shown to reverse amphetamine-induced locomotor activation in rats, and to cause behavioural stimulation in untreated rats. Thus, it was suggested that they could inhibit or stimulate dopaminergic transmission, depending on dopaminergic tone (unpublished data, Dept. of Pharmacology, Göteborg University, summary graph published in Carlsson, 2001; Sonesson et al., 1994). Small clinical studies with OSU6162 suggest antipsychotic effects with respect to both positive and negative symptoms (Gefvert et al., 2000; Tamminga and Carlsson, 2002; O. Gefvert personal communication). When tested in healthy volunteers, OSU6162 was reported to be safe for clinical use (Rodriguez et al., 2004). OSU6162 and ACR16 have been described as D2R antagonists (Carlsson et al., 2004; Sonesson et al., 1994). An early investigation report of a minor affinity of OSU6162 for D2Rs, measured as antagonist and agonist displacement *in vitro* (compound no. 16, Sonesson et al., 1994). Lately, however, both OSU6162 and ACR16 were reported to reach D2R occupancies of up to 90 % *in vivo*, measured as raclopride displacement (Ekesbo et al., 1999; Natesan et al., 2006). In contrast to the partial agonists, there is no obvious mechanism by which D2R antagonists could stabilize dopaminergic transmission. One suggested mechanism was a relative preference for extrasynaptic versus synaptic D2Rs. It was suggested that, in case of low to normal dopaminergic tone, OSU6162 and ACR16 may stimulate dopaminergic signalling by blockade of D2 autoreceptors. This hypothesis also postulated that, in case of an elevated dopaminergic tone, these drugs may dampen transmission mediated extrasynaptic D2 heteroreceptors (Carlsson et al., 2004). One important objective of this thesis was to further study the dopamine stabilizing effects of OSU6162 and ACR16, and to evaluate different conceivable mechanisms by which these compounds may stabilize dopaminergic neurotransmission.

Animal models for schizophrenia

Another objective of this thesis was to test OSU6162 and ACR16 in an experimental model for negative symptoms of schizophrenia. Animal models for schizophrenia are indispensable tools for evaluating potential antipsychotic drugs. These models mostly involve studies of drug induced behaviours believed to correspond to schizophrenic symptoms. The value of a model is determined by its predictive validity, i.e. its power to truthfully predict different responses in schizophrenic patients. Promising antipsychotic drugs should restore behaviour in these animals and validation of a model may be accomplished by testing antipsychotic drugs with known properties. This is complicated by the lack of antipsychotics alleviating negative and cognitive symptoms. Therefore, we must partly rely on face validity, i.e. resemblance to symptoms of schizophrenia.

The amphetamine model

As discussed above, positive symptoms of schizophrenia may be mimicked by administration of dopaminergic agonists, and are also alleviated by D2R antagonists. Amphetamine is an indirect dopaminergic agonist, augmenting transmission by inducing catecholamine release and preventing catecholamine reuptake. Although the effect of amphetamine is not specific for dopamine, the abovementioned observations provide evidence that dopaminergic mechanisms underlie amphetamine psychosis. Amphetamine is used in animal studies to model aspects of schizophrenia. In light of the known effects of amphetamine in humans, the flaws of this model are obvious. Amphetamine psychosis does not mimic the negative or cognitive symptoms of schizophrenia, and hence does not serve to detect effects of potential antipsychotic drugs with respect to these symptoms.

The PCP model

In humans, administration of the NMDAR antagonists PCP and ketamine faithfully mimic most aspects of schizophrenia. This is one observation underlying the glutamate hypothesis of schizophrenia, suggesting a defective glutamatergic transmission possibly involving dysfunctional NMDARs. Consequently, NMDAR antagonists are widely used to model schizophrenia in animals. PCP, ketamine and the hitherto not mentioned drug (+)-MK-801 (MK-801) are non-competitive and use-dependent NMDAR antagonists, binding within and obstructing the ion channel of the receptor. Among these NMDAR antagonists, MK-801 is by far the most potent (Javitt and Zukin, 1991). As NMDAR antagonists truthfully mimic most symptoms of schizophrenia, these drugs may be expected to provide an animal model with superior face validity.

Modelling schizophrenic symptoms

The different symptoms of schizophrenia are believed to correspond with different drug-induced behavioural aberrations in rodents. One major objective of this thesis was to set up a model for negative symptoms, in order to test potential antipsychotics for effect on these symptoms. Social withdrawal is regarded a core negative symptom of schizophrenia. Measurements of social interactions in animals are relatively easy. Other negative symptoms, such as flattened affect or apathy, are difficult or virtually impossible to imitate in animals. Unsuccessful attempts have been made to model anhedonia by measuring reward seeking behaviours. Animals treated with an NMDAR antagonist display marked social withdrawal. Thus, inhibition of social interaction, induced by NMDA-receptor antagonists, is considered a model for negative symptoms (Ellenbroek and Cools, 2000). The model for negative symptoms used in the current work consists of a test modified from Sams-Dodd (1995a), and measures social interactions in MK-801 treated rats.

Locomotor hyperactivity, in response to psychotomimetic drugs, is the most commonly used behavioural model for positive symptoms (van den Buuse et al., 2005). Hyperactivity induced by amphetamine or NMDAR antagonists is reversed by antipsychotic drugs. This indicates both predictive validity and dopaminergic involvement in NMDAR antagonist effects. However, it has been shown that the NMDAR antagonist MK-801 can induce behavioural activation in dopamine depleted mice, indicating that NMDAR antagonist induced hyperactivity is partly dopamine independent (Carlsson and Carlsson, 1989). It should also be mentioned in this context that atypical drugs, with higher degree of serotonergic 5-HT_{2A} receptor antagonism, are more effective in antagonizing PCP-induced hyperactivity (Maurel-Remy et al., 1995). Cognitive deficits may also be modelled in animals, e.g. with different memory tasks (Castner et al., 2004). These will not be discussed in this thesis.

In vivo assessment of D2 receptor effects

In an attempt to shed light on the mechanisms of action of OSU6162 and ACR16, different *in vivo* models were used to study the D2R effects of these drugs.

Dopaminergic tone measured as motor activity

There is an undisputed link between dopamine and behavioural activity. Enhancement of dopaminergic transmission, with amphetamine or D2R agonists, leads to stimulation of motor activity in rodents. Consequently,

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dopaminergic inhibition such as D2R blockade leads to reduced motor activity. Studies indicate a correlation between diurnal variations in motor activity and dopamine release in the nucleus accumbens and the dorsal striatum (O'Neill and Fillenz, 1985). It has been shown that motor activation in a novel environment is associated increased dopamine release in the nucleus accumbens (Rebec, 1998). Thus, motor activity may be used as an indirect measure of dopaminergic tone.

Behavioural model for detecting D2 autoreceptor preference

As mentioned above, preference for extrasynaptic D2Rs is one suggested mechanism for dopaminergic stabilization of OSU6162 and ACR16. D2 autoreceptors are believed to be strictly extrasynaptic and thus exposed to much lower dopamine concentrations compared to synaptic receptors. In consequence of low dopaminergic stimulation, these receptors are both hyper-responsive and available for low concentrations of other ligands. In rats, low doses of D2R agonists such as apomorphine cause marked locomotor inhibition. This is considered an effect of a reduced dopamine release, mediated by D2 autoreceptor stimulation. Reversal of this effect is used to detect D2 autoreceptor blockade (Perrault et al., 1997; Ståhle and Ungerstedt, 1986).

Intrinsic activity at D2 receptors *in vivo*

Secretion of prolactin from the pituitary gland is regulated largely via D2Rs. Thus prolactin release may be used to estimate a drug's intrinsic activity at D2Rs *in vivo*. In rats, systemic administration of D2R antagonists causes marked increase in blood prolactin. Hence, hyperprolactinaemia is a frequent side effect of antipsychotic treatment. Conversely, D2R agonists inhibit prolactin secretion. In order to facilitate detection of partial agonism, it is advisable to minimize the dopaminergic influence on D2Rs on the lactotropic cells in the anterior pituitary. This may be achieved by treatment with e.g. reserpine or γ -butyrolactone (GBL) (e.g. Carlsson et al., 1986; Inoue et al., 1996).

AIMS OF THE THESIS

The objectives during the course of this work were:

- To set up a rat model for negative symptoms of schizophrenia, based on NMDAR antagonist-induced social withdrawal in rats.
- To test the dopaminergic stabilizers OSU6162 and ACR16 in this model for possible effects on negative symptoms of schizophrenia.
- To study base-line dependent effects of OSU6162 and ACR16 on motor activity in rats, to find indirect support for dopamine stabilizing effects of these drugs.
- To shed light on the role of D2 receptors in the mechanism of action of OSU6162 and ACR16.

METHODS

Animals

All experiments were performed with male Sprague Dawley rats weighing approximately 250-350 g. All experiments were approved by the animal ethics committee in Göteborg, Sweden.

Behavioural studies

The video tracking setting

An automatic video tracking system was used to measure behavioural effects of various drug treatments, in rats studied in large rectangular arenas (100 × 150 cm). The rats were released in the arenas and their movements were recorded to videotapes or digital video files using either a colour video camera or a video camera sensitive to infrared light. The recordings were then analysed using a commercially available video tracking software, resulting in tracks describing the animals' movements in the arenas. Behavioural variables were then extracted from these tracks.

The social interaction test

Rats were studied in pairs in the video tracking setting. In order to capture behaviour during the active hours of the rats' diurnal rhythm, the rats were housed in reverse daylight cycle. The arenas were illuminated with ultraviolet light and the rats were marked with fluorescent dyes of different colours. This enabled the tracking software to identify the rats without risking interfering too much with the rats' light-dependent activity level. The two rats were injected simultaneously with an identical combination of MK-801 and test drug. 30 minutes after injection the two rats were introduced into the same arena and filmed for 30 minutes. Social behaviour was measured as percent of the observation time spent in proximity to each other. One "in proximity" period started when the rats came within 20 cm of each other, and ended as the distance between the rats exceeded 25 cm. The rats' motor activity was also measured as mean velocity. In this model, each pair of rats was treated as one object in the statistical analysis. Therefore mean values were calculated for in each pair in an experiment.

Motor activity of active rats

These rats were housed in reverse daylight cycle and motor activity was measured for individual rats in the video tracking system. In these

METHODS

experiments the arenas were illuminated with infrared light. Under these conditions the arenas appear to be in complete darkness. The test drug was injected and 30 minutes later one rat was released into each arena. Behaviour was studied for 30 minutes and motor activity was measured as mean velocity.

Apomorphine-induced hypomotility

Motor activity was measured in infrared light as described above. Apomorphine and test drugs were injected 5 minutes and 30 minutes respectively prior to recording of behaviour.

Motor activity of inactive rats

Rats used for these experiments were housed in normal daylight cycle. Motor activity was measured in small illuminated activity boxes (40 × 40 cm) with 5×5 rows of infrared beams at floor level. A computer connected to the activity boxes registered activity measured as unrepeated beam breaks, i.e. two or several consecutive breakings of one beam were counted as one beam break. The rats were allowed to habituate for 65 minutes to the boxes, and were then injected with test drugs. The rats were returned to the boxes and motor activity was measured for 60 minutes.

Drug effects on prolactin *in vivo*

Test drugs were injected and 60 minutes later the rats were decapitated and trunk blood was collected. Blood samples were centrifuged and plasma prolactin levels were assessed using a commercially available enzyme immunoassay kit specific for rat prolactin.

RESULTS

The social interaction test

The NMDA-receptor antagonist MK-801 induced a substantial and dose-dependent inhibition of social interactions in rats (figure 2). MK-801 also caused stimulation of motor activity. Amphetamine caused marked stimulation of motor activity and appeared to have a weak tendency to stimulate social interactions. These data combined with later observations suggest that there is no relation between these two behavioural variables.

OSU6162 and ACR16 both reversed MK-801-induced social withdrawal (figure 3). This effect was accompanied by inhibition of motor activity. In previously untreated rats neither OSU6162 nor ACR16 had any significant effects on social interactions, but induced marked inhibition of motor activity. Preliminary data indicate that also aripiprazole reverses MK-801-induced social withdrawal and hyperactivity.

Haloperidol dose-dependently added to the social withdrawal induced by MK-801. It also caused a marked inhibition of motor activity. At the highest dose tested the rats' activity was negligible. Clozapine did not induce any statistically significant effects on social interactions or motor activity when added to MK-801. It did, however, have a statistically significant effect on a third variable measured in these experiments, i.e. time in inner zone. All drugs tested in the social interaction setting, except amphetamine, caused the rats to spend less time in a predefined inner zone of the arena.

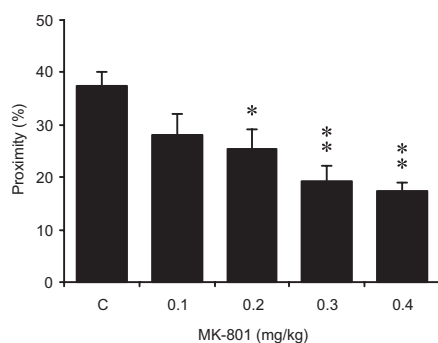


Figure 2. Effects of (+)-MK-801 on social behaviour (proximity). Treatment groups were compared with the control group using ANOVA followed by Dunnett's post hoc test. * $p < 0.5$; ** $p < 0.01$. Data previously presented in paper I.

RESULTS

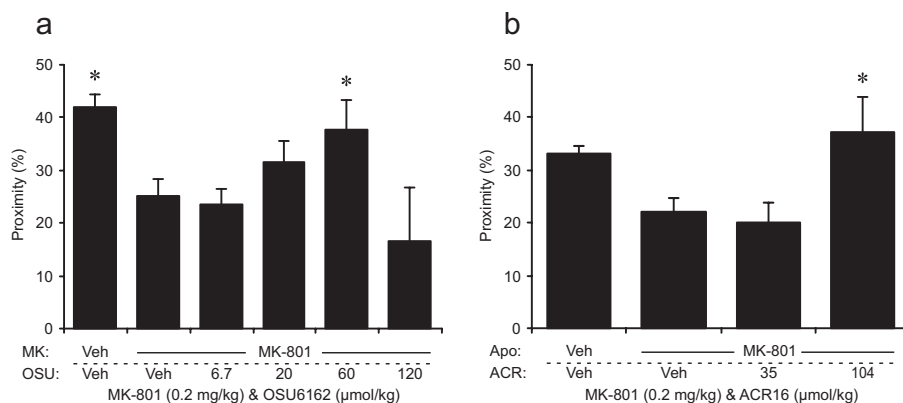


Figure 3. The effects of (a) (-)-OSU6162 and (b) ACR16 on (+)-MK-801 (0.2 mg/kg) induced social withdrawal measured as proximity. Comparisons are made versus the (+)-MK-801 treated group using ANOVA followed by Dunnett's post hoc test. * $p < 0.05$. Data previously presented in paper II.

Baseline-dependent effects on motor activity

Rats housed in reversed daylight cycle and studied in large unlit arenas without any prior habituation to the environment had a high initial activity level. Motor activity dropped markedly, but did not level out during the 30 minutes of observation. OSU6162 and ACR16 induced marked inhibition of activity. ACR16 caused a steeper decline in activity, than did OSU6162 (figure 4). Haloperidol, aripiprazole and (-)-3-PPP induced considerable behavioural inhibition of the same magnitude as OSU6162 and ACR16, whereas amisulpride inhibited motor activity only at a very high dose.

Motor activity was also measured in small illuminated activity boxes. The rats were housed in normal daylight cycle and allowed to habituate to the boxes before the drug was administered. The rats were fully habituated to the boxes at the time of drug injection, in the sense that motor activity was minimal. Apart from a transient activation that resulted from the injection, the control rats remained almost completely inactive. These rats responded to OSU6162 and ACR16 with a distinct behavioural activation (figure 5). This effect was not seen in response to haloperidol, aripiprazole, (-)-3-PPP or amisulpride.

RESULTS

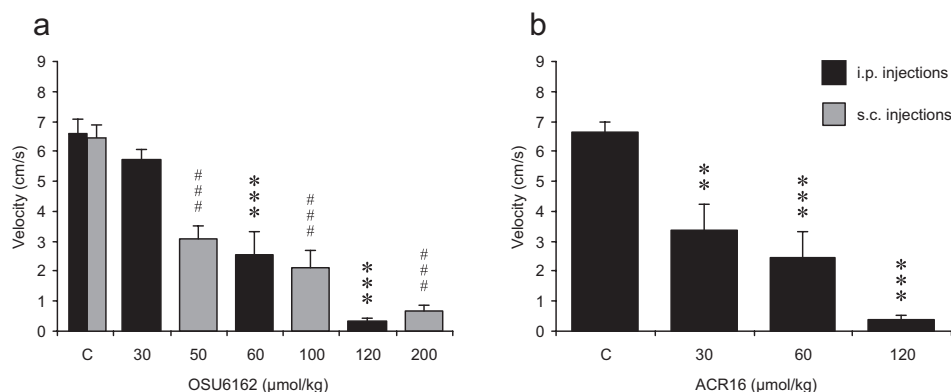


Figure 4. Effects of (a) (-)-OSU6162 and (b) ACR16 on motor activity of active rats. Activity was measured as velocity in the video tracking setting. Statistical comparisons were made versus control with univariate general linear model, followed by Dunnet's post hoc test. ** $p < 0.01$; *** $p < 0.001$ (i.p. injections). #### $p < 0.001$ (s.c. injections). Graphs extracted from paper III.

Apomorphine-induced hypomotility

Apomorphine induced a biphasic inhibitory effect on motor activity. The maximal behavioural inhibition was seen at the dose 0.16 $\mu\text{mol/kg}$ (0.05 mg/kg), which was also used subsequently in these studies.

OSU6162 induced a modest but statistically significant reversal of apomorphine induced hypomotility (figure 6a). ACR16 did not affect motor activity in rats treated with this dose of apomorphine. With a lower dose of apomorphine (0.08 $\mu\text{mol/kg}$), however, treatment with ACR16 tended to add to the apomorphine-induced behavioural inhibition. Amisulpride (figure 6b) restored motor activity of apomorphine-treated rats to a level approaching that of controls. Haloperidol did not have any distinct effect in this model.

Effects on prolactin release

OSU6162 (figure 7a) and haloperidol (figure 7c) induced similar dose-dependent increases of prolactin secretion. ACR16 also stimulated prolactin secretion dose-dependently, but to a lesser extent (figure 7b). Within a reasonable dose-interval, the effect of ACR16 on prolactin release was modest compared to that of OSU6162 and haloperidol. Aripiprazole induced increased prolactin release of a magnitude similar to that of ACR16 (figure 7d).

DISCUSSION

The social interaction test

As mentioned in the Introduction, social withdrawal is one negative symptom of schizophrenia with a measurable counterpart in animal behaviour. Thus, social withdrawal in rats is considered a model for negative symptoms. Paper I describes a setting for automated measurements of social interactions in rats. This setting is similar to one described previously by Sams-Dodd (1995a).

Face validity

Paper I reports of social withdrawal and locomotor stimulation in rats in response to acute administration of MK-801. Similar results had previously been reported in rats after acute or subchronic treatment with MK-801, PCP or ketamine (e.g. Becker and Grecksch, 2004; Sams-Dodd, 1994; Sams-Dodd, 1995b; Sams-Dodd, 1996; Silvestre et al., 1997). PCP and ketamine both cause a schizophrenia-like state in humans which includes negative symptoms. These are observations that contributed to the glutamate hypothesis and the PCP-model of schizophrenia.

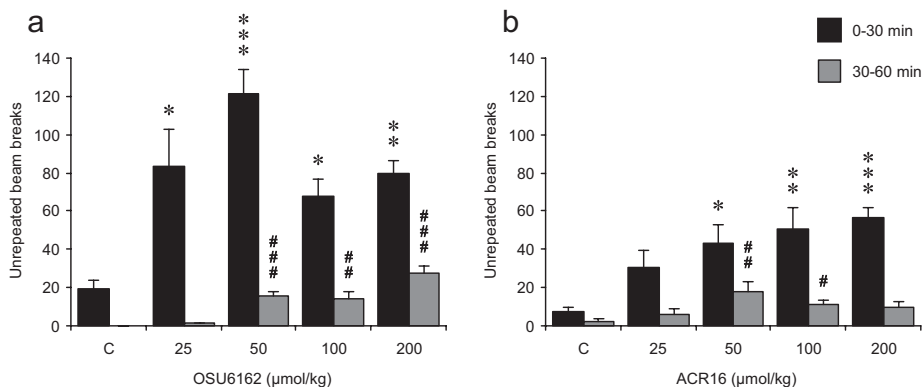


Figure 5. Effects of (a) OSU6162 and (b) ACR16 on motor activity of habituated rats in activity boxes during $t=0-30$ minutes and $t=30-60$ minutes after injection. Statistically significant effects were established with Kruskal-Wallis one-way analysis of variance by ranks, and comparisons versus control were performed as described by Siegel and Castellan (1988). * $p<0.05$; ** $p<0.01$; *** $p<0.001$ ($t=0-30$ min). # $p<0.05$, ## $p<0.01$, ### $p<0.001$ ($t=30-60$ min). Graphs extracted from paper III.

DISCUSSION

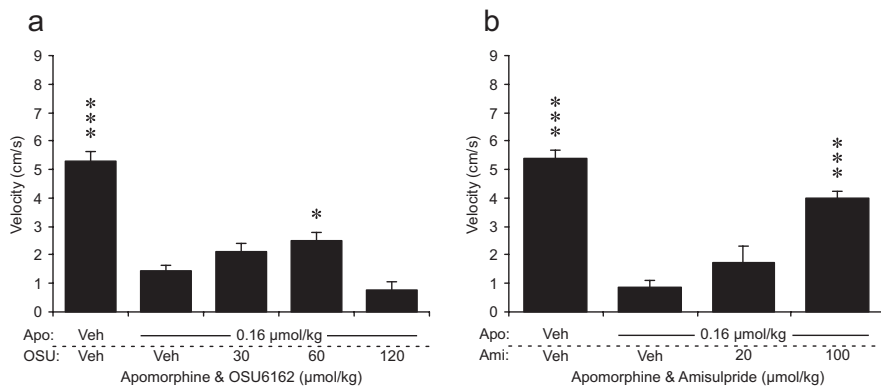


Figure 6. Effects of (a) (-)-OSU6162 and (b) amisulpride on apomorphine induced hypomotility in rats. Apomorphine (0.16 $\mu\text{mol/kg}$) and test drugs were administered 5 and 30 minutes respectively prior to registration. Motor activity was measured for 30 minutes as velocity in the video tracking setting. Statistical comparisons were made versus the apomorphine group with univariate general linear model, followed by Dunnett's post hoc test. * $p < 0.05$, *** $p < 0.001$. Graphs extracted from paper III.

In contrast to the PCP-model, the amphetamine model is not believed to cover the negative symptoms of schizophrenia. In our investigation, rats responded to acute administration of amphetamine with a weak tendency towards increased social interactions. This result agrees with earlier work showing an equivalent response at similar doses (Sams-Dodd, 1995b). In summary, NMDAR antagonists cause marked social withdrawal in rats while amphetamine has only minor effects on social behaviour. Thus, considering the schizophrenia-like effects of NMDAR antagonists, social withdrawal in response to NMDAR antagonists provides a model for negative symptoms with face validity.

Predictive validity

In paper II haloperidol caused a marked decrease in social interactions when added to MK-801, whereas clozapine was without effect. Subchronic administration of some atypical drugs is reported to partly reverse social withdrawal in rats treated subchronically with PCP. These results have been considered as supportive of the predictive validity of this model for negative symptoms. However, with the possible exception of sertindole, these drugs had only marginal effects on social withdrawal and/or they had significant effects in previously untreated rats (Sams-Dodd, 1997). Furthermore, most researchers in the field now seem to question that atypical antipsychotics have advantages over typical agents with respect to effects on negative

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symptoms. Thus, there are currently no drugs that could establish the predictive validity of this model.

Effects of dopaminergic stabilizers

In paper II the dopaminergic stabilizers OSU6162 and ACR16 were compared with the typical antipsychotic drug haloperidol and the atypical agent clozapine with respect to effects on MK-801-induced social withdrawal. As mentioned above, haloperidol caused further inhibition of social interactions whereas clozapine was without effect. In contrast, OSU6162 and ACR16 both restored social interactions to a level similar to that of controls. When tested in drug naïve rats these drugs did not have any significant effects on social interactions. This indicates that the dopaminergic stabilizers OSU6162 and ACR16 specifically blocked MK-801-induced

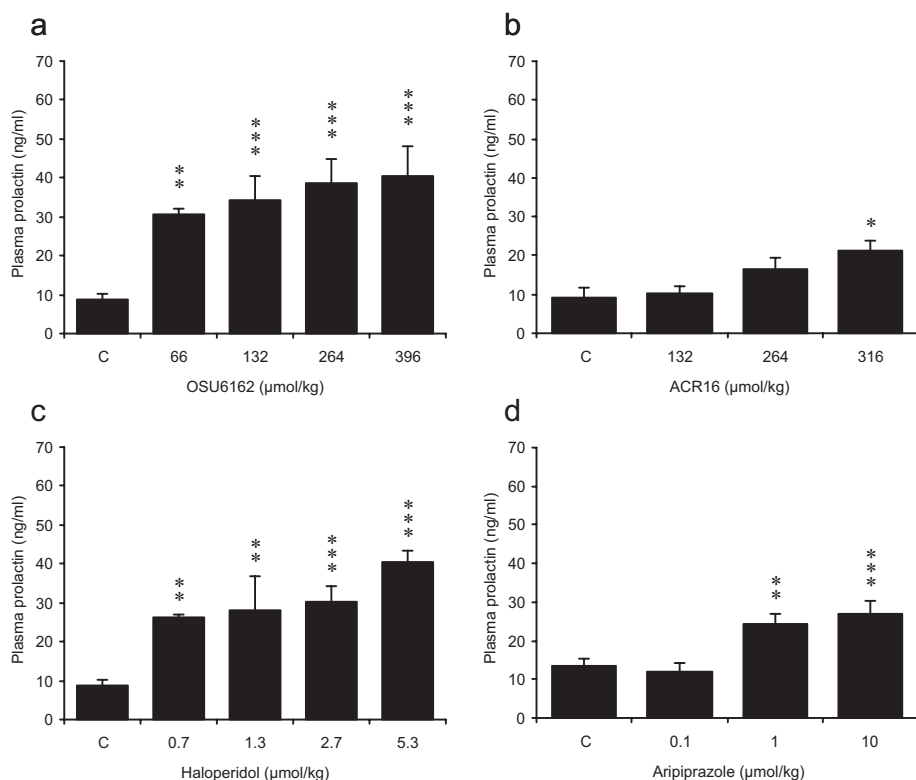


Figure 7. The effects of (a) (-)-OSU6162 (b) ACR16 (c) haloperidol and (d) aripiprazole on prolactin secretion in drug naïve rats. Treatment groups were compared to control group with univariate general model, followed by the Dunnet's post hoc test. * p<0.05; ** p<0.01; *** p<0.001. Graphs extracted from paper IV.

social withdrawal. Unpublished data indicate that aripiprazole too may restore social interactions in this setting. In a recent study aripiprazole was found to reverse PCP-induced social withdrawal in rats (Bruins Slot et al., 2005). Thus, these results indicate that the dopaminergic stabilizers OSU6162, ACR16 and aripiprazole could provide effective treatment for negative symptoms. As mentioned in the Introduction, OSU6162 has produced promising results in small studies with schizophrenic patients. These clinical studies indicate a rapid onset of antipsychotic effects with respect to both positive and negative symptoms. OSU6162 also appeared to be effective in patients with treatment resistant schizophrenia (Gefvert et al., 2000; Gefvert et al, unpublished; Lundberg et al., 2002; Tamminga and Carlsson, 2002). Interestingly, patients are reported to become more sociable in response to OSU6162 (O. Gefvert, personal communication).

Dopaminergic stabilization

Stabilizing effects on motor activity

In paper III we used two different settings in order to measure drug induced effects on spontaneous motor activity in rats with different activity baseline; one in which rats were highly active and one in which the behavioural activity was minimal. We found that the most important factors affecting activity level were habituation to the test equipment and the size of the arenas. Based on the convincing evidence for a strong correlation between dopaminergic tone and behavioural activity, we made the assumption that these differences in arousal reflect differences in dopaminergic activity; the active and inactive rats having high and low dopaminergic tone respectively. This assumption is supported by preliminary biochemistry data showing that active rats, compared to inactive rats, have higher concentrations of homovanillic acid in the striatum, indicating a higher dopaminergic activity.

We found that OSU6162 and ACR16 caused marked locomotor inhibition in active rats, whereas inactive rats were clearly stimulated by these drugs. The inhibition of behaviour in active rats was visible also in the data presented in paper II. In other studies OSU6162 and ACR16 were shown to be either stimulating or without effect on behavioural activity (Natesan et al., 2006; Sonesson et al., 1994). Aripiprazole and (-)-3-PPP caused potent inhibition of motor activity in the active rats, and did not induce any activation in the inactive rats. As increased and reduced motor activity can indicate stimulation and inhibition of dopaminergic signalling respectively, the effects of OSU6162 and ACR16 may be interpreted as activity dependent effects on dopaminergic signalling. OSU6162 and ACR16 have been shown to reverse amphetamine induced hyperactivity in rats, in a setting where these drugs are

otherwise stimulating (Natesan et al., 2006). This confirms that OSU6162 and ACR16 may reverse hyperactivity resulting from a hyperdopaminergic state. Thus, these results show that OSU6162 and ACR16 are probable dopaminergic stabilizers. Our data do not, however, confirm the stabilizing effects of aripiprazole or (-)-3-PPP.

Partial agonism as a mechanism for dopaminergic stabilization

A partial agonist is per definition stabilizing on transmission at the level of the receptor; it acts towards a level of receptor activation reflecting its intrinsic activity, which is below that of a full agonist. The partial D2R agonist properties of aripiprazole and (-)-3-PPP have been demonstrated *in vitro* as sub-maximal activation of D2Rs and, at least for aripiprazole, partial reversal of receptor activation exerted by full agonists. These studies show that aripiprazole's intrinsic activity is clearly below that of (-)-3-PPP (Cosi et al., 2006; Jordan et al., 2007a; Jordan et al., 2007b; Natesan et al., 2007; Urban et al., 2007). The direct effects of these drugs at D2Rs have also been investigated *in vivo*. Both (-)-3-PPP and aripiprazole have been reported to reverse DOPA-accumulation induced by reserpine or GBL (Ahlenius et al., 1989; Kikuchi et al., 1995). Aripiprazole has been shown to stimulate prolactin secretion in drug naïve rats, and to reverse hyperprolactinaemia induced by reserpine (Inoue et al., 1996). In female rats, (-)-3-PPP has been shown to stimulate prolactin release, whereas male rats responded with decreased prolactin secretion. These results were interpreted as a higher sensitivity of D2Rs in the pituitary of male rats, as a result of lower dopamine concentrations at these receptors (Carlsson and Eriksson, 1989). In both male and female rats, however, (-)-3-PPP have been shown to reverse hyperprolactinaemia induced by reserpine or GBL (Carlsson et al., 1986; Hjorth et al., 1985). In female rats with GBL-induced hyperprolactinaemia, it was also shown that (-)-3-PPP partly reversed suppression of prolactin release induced by the its isomer (+)-3-PPP, which has considerably higher intrinsic activity (Carlsson et al., 1987). (-)-3-PPP also partly reverses haloperidol-induced stimulation on prolactin secretion (Svensson et al., 1993). Together, these observations confirm the stabilizing effects of aripiprazole and (-)-3-PPP at receptor level.

The data presented in paper III indicate that, under physiological conditions, the partial D2R agonists aripiprazole and (-)-3-PPP act inhibiting on the dopaminergic system regardless of level of arousal. These results agree with previously reported dose-dependent locomotor inhibition in response to (-)-3-PPP (Hjorth et al., 1983). It has been claimed that the D2R agonist effects of aripiprazole and (-)-3-PPP are visible as yawning behaviour in drug naïve rats (Fujikawa et al., 1996). This interpretation is contradicted by recent work

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suggesting that D2/D3 agonist induced yawning is mediated by D3 receptors, while D2Rs mediate reversal of yawning at high doses of these drugs (Collins et al., 2007; Collins et al., 2005). However, after reserpine-induced dopamine-depletion, rats respond to (-)-3-PPP with a minor stimulation of motoractivity (Hjorth et al., 1983). (-)-3-PPP has also been observed to alleviate symptoms in patients with Parkinson's disease (Pirtosek et al., 1993; Pirtosek et al., 1996; Verhagen Metman et al., 1994). In one investigation, aripiprazole failed to reverse hypoactivity in mice, induced by a lower dose of reserpine (Kikuchi et al., 1995). Thus, aripiprazole and (-)-3-PPP suppress dopaminergic transmission under normal circumstances. Also, (-)-3-PPP can stimulate dopaminergic neurotransmission in case of arrested dopaminergic signalling. This may, however, not be the case for aripiprazole. Furthermore, (-)-3-PPP has been observed to diminish the treatment-induced motor fluctuations in patients with Parkinson's disease (Pirtosek et al., 1993). (-)-3-PPP may thus be considered a dopaminergic stabilizer. Aripiprazole, however, seems to be altogether inhibitory on the dopaminergic system, albeit stimulating at the receptor level under extraordinary circumstances. Nevertheless, aripiprazole has been shown not to cause EPS even at D2R occupancies of up to 95% (Gründer et al., 2003).

In this context it is useful to consider the background for using partial D2R agonists in the treatment of schizophrenia: Low doses of full D2R agonists have been shown to alleviate psychotic symptoms; an effect that fades within days of treatment. This antipsychotic effect is attributed to inhibition of dopamine synthesis and release via D2 autoreceptors. These receptors are believed to be exclusively extrasynaptic and therefore, compared to synaptic heteroreceptors, exposed to minimal amounts of dopamine. As a consequence of the low dopamine concentration at these receptors, they are available for low levels of exogenous ligands and also hypersensitive to D2R agonists. The disruption of the antipsychotic effect of D2R agonists is attributed desensitization of presynaptic receptors; the antipsychotic effect is known to subside more rapidly with higher intrinsic activity of the drug. In the case of (-)-3-PPP the antipsychotic effect wore off over the first two weeks of treatment. Thus, D2R partial agonists with lower intrinsic activities were sought that could achieve suppression of dopamine release, without causing desensitization of autoreceptors. Aripiprazole could be such a partial agonist, since schizophrenic patients are not reported to develop tolerance to this drug (Carlsson and Carlsson, 2006; Tamminga, 2002).

Mechanism for stabilization by OSU6162 and ACR16

Lately it has been shown *in vitro* that OSU6162 has a slight agonist effect at D2Rs (Lahti et al., 2007; Seeman and Guan, 2007). This, however, does not

DISCUSSION

seem to be the case for ACR16; recently published data could be interpreted as a minor inverse agonist effect of this drug (Tadori et al., 2007). In paper IV it was observed that OSU6162 appears to stimulate prolactin release in rats as effectively as haloperidol, which is in agreement with an antagonist action of OSU6162 at D2Rs. ACR16 caused a considerably smaller increase in prolactin secretion within a reasonable dose interval. In our study, as well as in previous studies (Inoue et al., 1996; Natesan et al., 2007), aripiprazole caused a modest increase in prolactin release. Thus, it may be suggested that the intrinsic activity of OSU6162 is negligible, compared to that of aripiprazole. Therefore, the dopaminergic stabilizer properties of OSU6162 and ACR16 cannot be due to partial D2R agonism.

Furthermore, it has been shown that the activating effect of OSU6162 is lost in dopamine depleted rats (Sonesson et al., 1994). Thus, it appears that, in contrast to partial D2R agonists, this drug is dependent on dopamine for its activating effect. As mentioned in the Introduction, one hypothesized mechanism for dopaminergic stabilization of OSU6162 and ACR16 is a preference for extrasynaptic D2Rs. In paper III these drugs were tested for D2 autoreceptor preference, measured as blockade of apomorphine-induced hypomotility. Our results indicate that OSU6162, but not ACR16, has some preference for D2 autoreceptors versus synaptic receptors. The same study also showed that the autoreceptor preference of OSU6162 was modest compared to that of the known autoreceptor preferring drug amisulpride (cf. Perrault et al., 1997). According to the abovementioned hypothesis, dopaminergic activation of OSU6162 and ACR16 would be due to autoreceptor blockade. Therefore, it was expected that amisulpride would also induce behavioural stimulation in inactive rats, but this turned out not to be the case. Thus, taken together, these results contradict the hypothesis of preference for extrasynaptic receptors as the mechanism for dopaminergic stabilization of OSU6162 and ACR16.

The behavioural effects presented in paper III shows that OSU6162 is less inhibitory on activity compared to ACR16. This is what would be expected if OSU6162 were the least potent D2R antagonist of these drugs. However, in paper IV OSU6162 was much more effective in stimulating prolactin release, indicating that OSU6162 is on the contrary more potent than ACR16. This result agrees with a recent *in vivo* study where OSU6162 was shown to more potently than ACR16 displace raclopride in a striatal dopamine D2R occupancy experiment (Natesan et al., 2006). Furthermore, OSU6162 was also the more activating of the drugs in inactive rats, an effect that cannot be attributed to blockade of postsynaptic receptors. These observations indicate that at least two different targets are involved in the stabilizing effects of OSU6162 and ACR16. The most likely mechanism for inhibition is blockade

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of postsynaptic D2Rs. As to the activating effect of these drugs, the results in paper III indicate that this is not mediated by D2 autoreceptors. However, since activation is dependent on the presence of dopamine, the activating target should nevertheless be associated with dopaminergic receptors.

Interestingly, in a recent *in vitro* study, low concentrations of OSU6162 were shown to potentiate the effect of dopamine at D2Rs, whereas higher concentrations inhibited receptor activation. The interpretation of these data was that OSU6162 enhances the effect of dopamine via an allosteric site at the D2R, whereas inhibition results from blockade of the dopamine-site of the receptor (Lahti et al., 2007). This finding lends support for D2 heteroreceptor blockade as the mechanism for inhibition of dopaminergic signalling and motor activity. More importantly, it offers a strong candidate for the activating target of OSU6162 and ACR16.

Summary

NMDAR antagonist-induced social withdrawal is a model for negative symptoms of schizophrenia with face validity. Since current antipsychotics do not appear to have satisfactory effects on these symptoms we do not have the means to verify the predictive validity of this model. The dopaminergic stabilizers OSU6162 and ACR16 were shown to reverse social withdrawal in rats induced by the NMDAR antagonist MK-801. This result suggests that these drugs may be effective against negative symptoms of schizophrenia. OSU6162 and ACR16 were shown to reduce motor activity in highly active drug naïve rats, and to increase activity in inactive previously untreated rats. These effects lend support to the dopamine stabilizer properties of these drugs. OSU6162 partly reversed apomorphine-induced locomotor inhibition, which indicates a relative preference for the extrasynaptic D2 autoreceptors. Since ACR16 did not share this property, and since the known D2 autoreceptor selective drug amisulpride did not induce any behavioural activation, these studies contradict preference for extrasynaptic receptors as a mechanism for dopaminergic stabilization. Nevertheless, the behavioural responses to these drugs suggest that two separate drug targets, both associated with dopaminergic receptors, mediate activation and inhibition of behaviour. Together with recently published data from another lab, these data indicate that inhibition is an effect of orthosteric D2R blockade, whereas the activating target could be an allosteric site of the D2R, mediating an enhanced response to dopamine.

CONCLUSIONS

The studies in this thesis have resulted in the followed main conclusions:

Social interactions

- MK-801-induced social withdrawal in rats is a model for negative symptoms of schizophrenia with face validity
- The claimed dopaminergic stabilizers OSU6162 and ACR16 reversed MK-801-induced social withdrawal, suggesting beneficial effects on negative symptoms

Dopaminergic stabilization

- OSU6162 and ACR16 had base-line dependent effects on motor activity in drug naïve rats, which can be interpreted as support of dopaminergic stabilization.
- Dopamine stabilizing effects of aripiprazole and (-)-3-PPP do not show up in the present behavioural model with drug naïve rats.
- The dopamine stabilizing properties of OSU6162 and ACR16 may consist of:
 - Inhibition by blockade of the orthosteric, i.e. the dopamine binding, site of the postsynaptic D2 receptor
 - Stimulation via interaction with an allosteric site of the D2 receptor

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