Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 84 (2006) 259–265
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Positive modulation of glutamatergic receptors potentiates the suppressive effects of antipsychotics on conditioned avoidance responding in rats

Christina Kurre Olsen [a,*], Mads Kreilgaard [b], Michael Didriksen [c]

[a] Department of Psychopharmacology, Pharmacological Research, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby-Copenhagen, Denmark
[b] Department of Discovery ADME, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby Copenhagen, Denmark
[c] Department of Pharmacology Target Research, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby Copenhagen, Denmark

Received 15 December 2005; received in revised form 19 April 2006; accepted 10 May 2006
Available online 19 June 2006

Abstract

Non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist administration induces a syndrome indistinguishable from schizophrenia including positive and negative symptoms and cognitive deficits. Concordantly, augmentation of the NMDA receptor function by glycine-site agonists such as D-serine and D-cycloserine has been reported to improve negative symptoms and some cognitive deficits in schizophrenia patients when added to conventional antipsychotic treatment, although they appear less effective when combined with clozapine specifically. In contrast, administration of the AMPAkine CX-516 (which positively modulate the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor) as an adjuvant to clozapine, has been shown to exert some beneficial action on the negative symptoms and cognitive deficits in schizophrenia. In the rat, selective suppression of conditioned avoidance response (CAR) behaviour has been widely reported to be a test with high predictive validity for antipsychotic efficacy. We found that D-serine and CX-516, at doses ineffective by themselves, significantly potentiated the suppression of CAR induced by threshold doses of risperidone (0.16 mg/kg, s.c.), olanzapine (0.63 mg/kg, s.c.) and clozapine (1.3 mg/kg, s.c.) without causing additional motor disturbances. Thus, the adjunct enhancement of NMDA or AMPA receptor function observed clinically, appears reflected in the present rat CAR study. Consequently, our data lend further support to the potential use of the CAR test in the investigation of augmentation strategies involving the addition of non-dopaminergic target compounds to existing atypical antipsychotics.

Keywords: Conditioned avoidance response; Antipsychotic drugs; Glycine-site receptor agonist; AMPAkine; Rat

1. Introduction

Non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist administration induces a syndrome indistinguishable from schizophrenia including positive and negative symptoms and cognitive deficits, suggestive that hypofunction of the glutamatergic system may play a role in the pathophysiology of schizophrenia (Coyle, 1996; Javitt and Zukin, 1991; Kim et al., 1980; Krystal et al., 1999; Olney et al., 1999). Concordantly, the interest around manipulating the NMDA receptor as a target for the improved treatment of schizophrenia has increased significantly (Millan, 2005).

Indeed, clinical evidence showing a beneficial effect by the addition of glycine or related agonists of the strychnine insensitive glycine co-agonist site, such as D-serine or D-cycloserine, to existing antipsychotic treatment has been reported recently (Goff et al., 1999b; Heresco-Levy et al., 1996, 1998, 2002, 2004, 2005; Javitt et al., 1994; Tsai et al., 1998). However, this finding is open to controversy as there are also reports showing no significant effect (Duncan et al., 2004; Evins et al., 2000), or even a worsening of symptoms (Cascella et al., 1994; Rosse et al., 1989; van Berckel et al., 1999), following such adjunct treatment. An alternative approach taken, also resulting in the enhancement of glutamatergic transmission, is via the positive allosteric modulation of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor. Use of the AMPAkine CX-516 as an add-on treatment to clozapine was reported to exert a beneficial effect (Goff et al., 2001). Moreover, such augmentation strategies have led to the development of other compounds, including the glycine transport-1 inhibitor (GlyT1) sarcosine, which has also shown a beneficial effect

* Corresponding author. Tel.: +45 36 43 22 73; fax: +45 36 43 82 58.
E-mail address: ckol@lundbeck.com (C.K. Olsen).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.05.006

when given in combination with existing antipsychotics (Lane et al., 2005; Tsai et al., 2004).

The purpose of the present study was to evaluate whether the increased efficacy observed in those clinical studies utilising a co-administration treatment regime of D-serine or CX-516 with an existing antipsychotic, would also translate to the preclinical setting and be reflected as a potentiation of the suppressive effect on conditioned avoidance response (CAR) behaviour in rats. This rodent test is considered to have a high degree of predictive validity for antipsychotic efficacy, and is regarded as a valid assay to detect potentially novel antipsychotics as well as to support various augmentation strategies (Hertel et al., 1999; Linner et al., 2002; Prinssen et al., 1996; Shannon et al., 1999; Wadenberg et al., 2001a;Wadenberg and Ahlenius, 1991).

2. Materials and methods

2.1. Subjects

Male Wistar rats (Møllegaard, Denmark) weighing approximately 200 g at the beginning of training were used. Rats were housed under controlled laboratory conditions (temperature: 21 ± 2 °C; humidity: 55 ± 5% relative) on a 12:12 h light–dark cycle (lights on at 06:00). The animals were kept on a restricted diet in order to keep them to 80% of their free-feeding weight. Water was available ad libitum. All experiments were performed in accordance with the ethical guidelines of H. Lundbeck A/S.

2.2. Drugs

D-serine (molecular weight (MW) 105, Sigma) was dissolved in water. Risperidone (free base, MW 410, Jansen), olanzapine (free base, MW 312, H. Lundbeck A/S) and clozapine (free base, MW 327, Sigma) were dissolved in a minimal amount of diluted HCl acid and saline. CX-516 (Piperidin-1-yl-quinoxalin-6-yl-methanone, MW 241, synthesised at H. Lundbeck A/S) was dissolved in a minimal amount of diluted methansulfonic acid and saline. All solutions were adjusted to physiological pH (6–8) and administered subcutaneously (s.c.) in a volume of 5 ml/kg. Each drug had its corresponding vehicle being either water or saline with a drop of hydrochloric acid or methansulfonic acid, respectively.

2.3. Experimental procedure

Conditioned avoidance behaviour was assessed using four automated shuttle-boxes (42 × 16 × 20 cm) (ENV-010M, MED-Associates) each placed in a sound-attenuated chamber. The boxes were subdivided into two compartments by a partition with one opening. The position of the animal and movement from one compartment to the other were detected by 8 photocells sensitive to infrared light on each side of the dividing wall. Rats were trained to move into the adjacent compartment (response) within 10 s upon presentation of the conditioned stimuli (tone and light) in order to avoid the appearance of the unconditioned stimulus, a 0.5 mA scrambled electric shock in the grid floor of 10 s in maximal duration. The following behavioural variables were recorded: avoidance (response to conditioned stimuli within 10 s) and escape failures (failure to respond to the unconditioned stimuli within 10 s).

Rats were habituated to the shuttle-box 3 min before each test session. During training, each test session consisted of 30 trials with inter-trial intervals varied at random between 20 and 30 s. Training was carried out 5 days/week until the animals had avoidance measures of >80% (24 trials) of the trials on three consecutive days. All experimental sessions consisted of 10 trials with inter-trial intervals varied at random between 20 and 30 s.

2.4. Experimental design

Drug test was preceded by a pre-test (the day before). At least 3 days elapsed between drug injections to prevent cumulative drug effects. Vehicle-treated groups and drug-treated groups were run concurrently. All drugs were administered 30 min prior to test.

2.5. Drug exposures and bioanalysis

Drug exposure was assessed in a parallel group of awake undisturbed rats with catheters implanted in the carotid artery and connected to automated blood samplers (AccuSampler ™, DiLab, Sweden). In a similar design to the CAR test, 100 µl blood samples were drawn 30 min after s.c. administration of risperidone and D-serine in heparinised tubes. Plasma was subsequently separated after centrifugation (2200 ×*g*, 10 min) and stored at −80 °C until time of analysis. Plasma content of risperidone and 9-OH-risperidone was determined by high turbulent flow chromatography/tandem mass spectrometry (HTF-MS/MS) as previously described (Sanchez and Kreilgaard, 2004). MS/MS detection was performed with a Quattro Ultima (Micromass, UK) in positive-ion electrospray ionization mode. Risperidone and 9-OH-risperidone were detected at parent > daughter molecular mass of 411.08 > 191.01 and 427.07 > 207.05 Da, using a cone voltage of 60 and 66 V and a collision energy of 30 eV, respectively. Retention times were 3.24 min for risperidone and 3.26 min for 9-OH-risperidone. The peak areas correlated linearly with the plasma concentrations of risperidone and 9-OH-risperidone ($r^2 > 0.99$) in the range of 0.5–500 ng/ml. The lower limit of quantification was 0.5 ng/ml (S/N > 10).

2.6. Statistics

Data are presented as percentage avoidances compared to preceding pre-test, with each animal serving as their own control. Statistical analysis of percent avoidances was performed using one-way analysis of variance (ANOVA) with post-hoc Student–Newman–Keuls test or two-way (treatment × dose) ANOVA followed by post hoc Dunnett's test where appropriate. Multiple comparisons were performed for each antipsychotic in combination with vehicle and in combination with D-serine and CX-516, respectively.

The plasma exposure of risperidone and 9-OH-risperidone was analysed using a two-tailed *t*-test assuming equal variances in the groups.

3. Results

3.1. Effects of D-serine in combination with risperidone, olanzapine and clozapine on conditioned avoidance responding

Fig. 1 presents the mean percentages of avoidances compared to the preceding pre-test following treatment with D-serine alone or in combination with risperidone, olanzapine and clozapine, respectively. The use of D-serine as an add-on treatment enhanced the effect of risperidone on CAR (Fig. 1, top panel). Both a significant effect of adding D-serine ($F_{1, 47}=12.1$, $P=0.001$) and a significant interaction between D-serine and risperidone treatment were found ($F_{2, 47}=3.27$, $P=0.048$) using a two-way ANOVA. Post hoc analysis revealed that the effect of a threshold dose of risperidone (0.31 mg/kg) was augmented by the addition of D-serine ($P<0.05$). Similarly, the effect of olanzapine was also enhanced by D-serine co-administration (Fig. 1, middle panel). One-way ANOVA showed a significant effect between treatment groups ($F_{10, 119}=22.1$, $P<0.001$). Post hoc comparisons showed potentiation of an ineffective dose of olanzapine (0.63 mg/kg) by D-serine ($P<0.05$). Finally, Fig. 1 bottom panel shows the effect of D-serine in combination with clozapine. Again, one-way ANOVA showed a significant effect between treatment groups ($F_{10, 119}=20.5$, $P<0.001$), and post hoc comparisons showed potentiation of both an ineffective dose (1.3 mg/kg) and a threshold dose (2.5 mg/kg) of clozapine by D-serine ($P<0.05$).

3.2. Effect of CX-516 in combination with risperidone, olanzapine and clozapine on conditioned avoidance responding

Fig. 2 presents the mean percentages of avoidances compared to the preceding pre-test following treatment with CX-516 alone or in combination with risperidone, olanzapine and clozapine, respectively. The use of CX-516 as an adjunct therapy enhanced the effect of risperidone on CAR (Fig. 2, top panel). Both a significant effect of adding CX-516 ($F_{2, 109}=34.7$, $P<0.001$) and a significant interaction between CX-516 and risperidone treatment were found ($F_{4, 109}=10.9$, $P<0.001$) using a two-way ANOVA. Post hoc analysis revealed that the effects of threshold doses of risperidone (0.16 and 0.31 mg/kg) were augmented by the addition of 80 mg/kg CX-516 ($P<0.05$). Furthermore, the effect of 0.31 mg/kg risperidone was augmented by 5 mg/kg CX-516 ($P<0.05$). CX-516 in doses between 5 and 80 mg/kg was without effect when given alone (data not shown). The effect of olanzapine was also enhanced by CX-516 (Fig. 2, middle panel). Both a significant effect of adding CX-516 ($F_{2, 62}=19.4$, $P<0.001$) and a significant interaction between CX-516 and olanzapine treatment were found ($F_{4, 62}=5.90$, $P<0.001$) using a two-way ANOVA. Post hoc analysis revealed that the effect of a threshold dose of olanzapine (0.63 mg/kg) was augmented by the addition of 80 mg/kg CX-516 ($P<0.05$). The combination of 0.31 mg/kg olanzapine with 80 mg/kg CX-516 resulted in a non-specific effect as escape failures appeared (data not shown). Finally, Fig. 2 bottom panel shows the effect of CX-516 in combination with clozapine. Both a significant effect of adding CX-516 ($F_{2, 124}=$

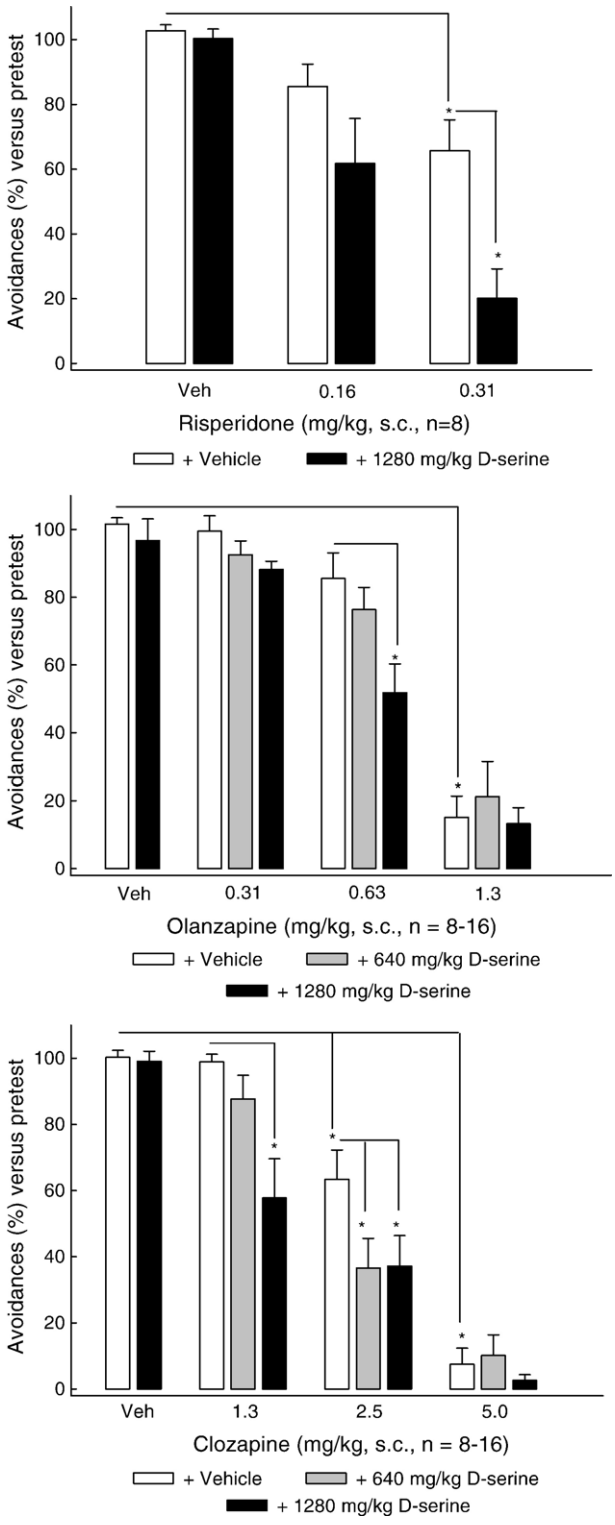

Fig. 1. Dose–response of risperidone (top panel), olanzapine (middle panel) and clozapine (bottom panel) alone, and in combination with D-serine on CAR behaviour in rats. All drugs were administered s.c. 30 min prior to test. Values are mean±sem ($n=8$–16) percentage avoidances compared to the preceding pre-test, with each animal serving as their own control. Statistical analyses were performed using either a one-way ANOVA with post hoc Student–Newman–Keuls test or a two-way ANOVA followed by post hoc Dunnett's test. *$P<0.05$.

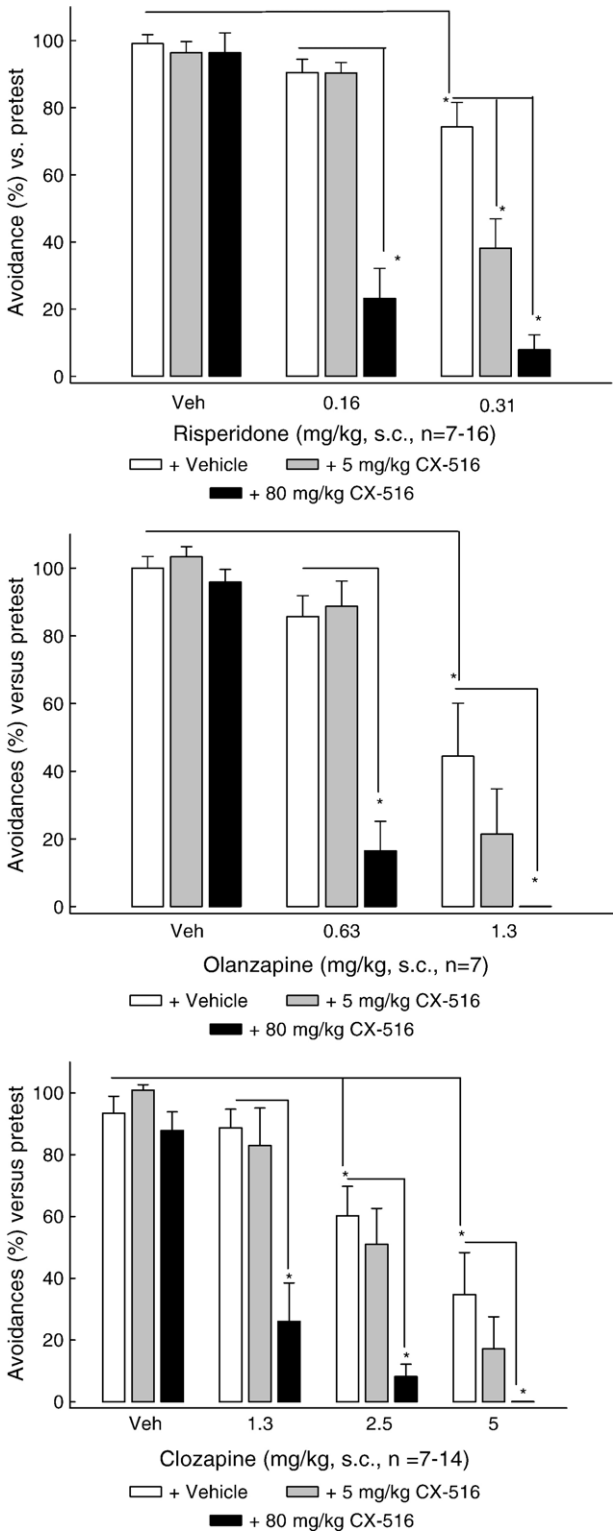

Fig. 2. Dose–response of risperidone (top panel), olanzapine (middle panel) and clozapine (bottom panel) alone, and in combination with CX-516 on CAR behaviour in rats. All drugs were administered s.c. 30 min prior to test. Values are mean±sem (n=7–16) percentage avoidances compared to the preceding pre-test, with each animal serving as their own control. Statistical analyses were performed by either a one-way ANOVA with post hoc Student–Newman–Keuls test or a two-way ANOVA followed by post hoc Dunnett's test. *P<0.05.

Table 1
Mean±sem (n=3) plasma exposure of risperidone and 9-OH-risperidone, 30 min following s.c. administration of 0.31 mg/kg risperidone with and without 1280 mg/kg D-serine

Treatment	Risperidone exp (ng/ml)	9-OH-risperidone exp (ng/ml)	Total risp+9-OH-risp (ng/ml)
Risperidone	98.9±9.5	35.4±3.0	134.3±7.9
Risperidone+ D-serine	131.8±18.6	20.1±5.0	151.9±15.3

22.2, P<0.001) and a significant interaction between CX-516 and clozapine treatment were found ($F_{6,\ 124}$=2.83, P=0.013) using a two-way ANOVA. Post hoc analysis revealed that the effects of threshold doses of clozapine (1.3–5 mg/kg) were augmented by the addition of 80 mg/kg CX-516 (P<0.05). The combination of 2.5 or 5 mg/kg clozapine with 80 mg/kg CX-516 resulted in a non-specific effect as escape failures appeared (data not shown).

3.3. Drug exposures

Plasma exposure of risperidone and 9-OH-risperidone with and without co-administration of D-serine is presented in Table 1. No statistically significant differences were found in risperidone (P=0.18) or total risperidone+9-OH-risperidone (P=0.36) plasma exposure, 30 min after s.c. administration of 0.31 mg/kg risperidone with or without co-administration of 1280 mg/kg D-serine.

4. Discussion

In the present study the CAR paradigm is used to assess antipsychotic activity measured as a suppression of avoidance response to a conditioned stimuli in doses that do not induce escape failures (Arnt, 1982; Ogren and Archer, 1994; Wadenberg and Hicks, 1999). This selective disruption is commonly observed both for classical dopamine D_2 receptor antagonist neuroleptics such as haloperidol and chlorpromazine, as well as for atypical antipsychotics such as olanzapine, risperidone and clozapine (Moore et al., 1992; Wadenberg et al., 2001b). The paradigm is widely used to differentiate antipsychotics from other drug classes such as antidepressants or anxiolytics which act as negative controls, and is also regarded as a valid tool to detect potentially novel antipsychotics in addition to supporting augmentation strategy work (Hertel et al., 1999; Linner et al., 2002; Prinssen et al., 1996; Shannon et al., 1999; Wadenberg et al., 2001a;Wadenberg and Ahlenius, 1991). Several hypotheses have been raised in order to explain the nature behind the suppression of avoidances ranging from a general disruption of motor performance, impaired motor initiation response, deficit in associative learning or, as more recently proposed, a deficit in incentive motivation that drives the animal to actively pursue the goal (Beninger et al., 1980; Li et al., 2004). Nevertheless, irrespective of the exact underlying nature of this task, suppression of CAR is a characteristic effect of all clinically effective antipsychotic drugs. Recent studies show the relationship between dopamine D_2 receptor occupancy and effect in CAR correlates well with the relationship between human D_2

occupancy and clinical effect (Natesan et al., 2005; Wadenberg et al., 2001b). The CAR paradigm therefore is considered to possess a high degree of predictive validity (Wadenberg and Hicks, 1999).

Although the glutamate hypothesis of schizophrenia is widely accepted, the clinical evidence supporting the use of glycine or related agonists of the strychnine insensitive glycine co-agonist site such as D-serine or D-cycloserine to enhance NMDA function, and thus provide a positive treatment effect in schizophrenia, is less clear. Whilst there are many studies showing a beneficial effect by the addition of glycine to existing antipsychotic treatment (Heresco-Levy et al., 1996, 1998, 2004; Javitt et al., 1994), there are also reports demonstrating either no effect when added to clozapine (Evins et al., 2000), or even a worsening of symptoms following co-administration with other antipsychotics (Rosse et al., 1989). Similarly, in treatment resistant schizophrenia, clozapine alone has been reported to exert more of a beneficial effect than when co-administered with glycine (Potkin et al., 1999), although there are reports showing the exact opposite (Heresco-Levy et al., 1999).

These conflicting data are not unique to glycine. A beneficial effect of D-cycloserine as an add-on treatment to existing antipsychotics has been shown in some small clinical studies (Goff et al., 1999b; Heresco-Levy et al., 2002), whereas others have found either no effect (Duncan et al., 2004), or a worsening of the clinical symptoms (Cascella et al., 1994; van Berckel et al., 1999). However, the variable effects noted with D-cycloserine specifically may be due, in part, to its partial agonism.

It is also interesting to note that clozapine appears to differ compared to the other antipsychotics employed in adjunct therapy studies. Typically, no effect (Goff et al., 1996) or an impairment in effect (Goff et al., 1999a) is observed following add-on treatment to clozapine. For example, the addition of D-serine, a full agonist of the NMDA glycine-site, to olanzapine or risperidone revealed an improvement in both positive, negative and cognitive symptoms (Tsai et al., 1998; Heresco-Levy et al., 2005), although no improvement was observed against clozapine (Tsai et al., 1999). One explanation for this differential effectiveness may be clozapine's ability to inhibit the glycine transporter directly (Williams et al., 2004), resulting in elevated glycine levels and, thus, a decreased potential for augmentation (Javitt et al., 2005; Sumiyoshi et al., 2005). An alternative explanation proposed lies with the bias in the patient population as clozapine is not a first line treatment and, instead, is primarily used in non-responder and older chronic patient groups.

Overall, with such conflicting data it has been difficult to reach a general consensus on the benefits of augmentation therapy using glycine, D-serine and D-cycloserine specifically. In a recent study in which the effects of glycine versus D-cycloserine were compared directly (Heresco-Levy and Javitt, 2004), it was proposed that full agonists such as glycine and D-serine are likely to be more effective than partial agonists such as D-cycloserine in augmenting the effect of existing antipsychotics. Certainly this distinction appears to be reflected in preclinical studies whereby the effect of D-cycloserine in rodent tests of antipsychotic efficacy was shown to be inferior compared to those induced by D-serine (Millan, 2005).

The present study also provides support for augmentation treatment using D-serine. Our data demonstrate that positive modulation of the NMDA receptor by D-serine alone is insufficient to suppress CAR behaviour in the rodent. This is in agreement with clinical findings showing no improvement with D-cycloserine administered as a single agent (van Berckel et al., 1996). In contrast, D-serine selectively potentiated the suppression of CAR induced by threshold doses of risperidone, olanzapine and clozapine, supporting the hypothesis that positive modulation of the NMDA receptor complex potentiates antipsychotic efficacy. The differences in effectiveness observed in the clinic with clozapine compared to other antipsychotics were not supported by the present study where the suppressive effect of risperidone, olanzapine and clozapine on CAR was potentiated by D-serine to a similar extent. However, the lack of differential effect in the rat may be expected if the clinical patient population bias argument for clozapine holds true.

A second approach leading to the enhancement of glutamate function is via the positive allosteric modulation of AMPA receptors. As a single agent, administration of the AMPAkine CX-516 was ineffective as a schizophrenia treatment (Marenco et al., 2002). Conversely, a positive clinical effect has been reported when CX-516 was given as an adjunctive therapy to clozapine (Goff et al., 2001). Preclinically, a failure to attenuate both amphetamine and dizocilipine-induced hyperactivity up to doses that affected basal locomotor activity has been demonstrated with solo AMPAkine treatment (Vanover, 1997). Similarly, a lack of effect of CX-516 when given alone was demonstrated in the present study, whereby CX-516 did not suppress CAR in the rat. In contrast, when administered as an add-on treatment, CX-516 selectively potentiated the suppression of CAR induced by threshold doses of risperidone, olanzapine and clozapine. Moreover, this is in agreement with other findings where CX-516 has been described to potentiate the effect of haloperidol, clozapine as well as risperidone in a methamphetamine-induced activity test in rats (Johnson et al., 1999).

One possible explanation of the augmentation induced by D-serine and CX-516 upon existing antipsychotic efficacy may be due to pharmacokinetic interaction. As a consequence, the effect of D-serine on the pharmacokinetic profile of risperidone in rats was included as part of the present study. No significant difference in mean plasma risperidone or total risperidone plus its pharmacological active metabolite 9-OH-risperidone concentration was found, highly indicative that the potentiating effect of D-serine was not due to pharmacokinetic interaction. Similar results have been demonstrated by others evaluating the pharmacokinetic interaction between CX-516 and clozapine (Johnson et al., 1999).

Finally, with the exception of those studies in which combinations of high doses of CX-516 with clozapine or olanzapine also resulted in the presence of escape failures, the potentiation of the CAR suppressive effect induced by antipsychotic treatment is specific. Where animals not responding to the conditioned stimuli did respond to the unconditioned stimuli, one could argue that the sedative effect of the antipsychotic may be responsible. However, it is highly unlikely that these effects could be interpreted as sedation as, firstly, sedatives such as diazepam are ineffective in this assay and, secondly, antipsychotics are able to suppress CAR at doses ineffective at decreasing locomotor activity (unpublished observations; Wadenberg and Hicks, 1999).

In conclusion, therefore, the present rat CAR data support the majority of clinical findings in which existing antipsychotic efficacy can be augmented by the enhancement of glutamate function by positive modulation of either the NMDA or the AMPA receptor. The present study is the first to demonstrate a positive adjunctive effect of a glycine-site agonist (D-serine) and an AMPAkine (CX-516) in the CAR paradigm. Also noteworthy is the unpublished observation that the glycine transport-1 inhibitors NFPS and other disclosed compounds, produced a very similar profile to D-serine and CX-516, namely they appeared ineffective in suppressing CAR behaviour when given alone, but augmented antipsychotic-induced CAR suppression. Overall, these data add to the potential of using CAR to support further augmentation strategy research work, to evaluate the effects of adding non-dopaminergic target compounds to existing atypical antipsychotics.

Acknowledgements

The authors thank Susanne Herskind-Hansen, Lotte Schmidt Marcher, Rikke Madsen, Martin Øvergård, Karina Gerdes and Ulla Østerby Mønsted for their excellent technical assistance, and to Rebecca Dias for critical reading of the manuscript.

References

Arnt J. Pharmacological specificity of conditioned avoidance response inhibition in rats: inhibition by neuroleptics and correlation to dopamine receptor blockade. Acta Pharmacol Toxicol (Copenh.) 1982;51:321–9.

Beninger RJ, Mason ST, Phillips AG, Fibiger HC. The use of conditioned suppression to evaluate the nature of neuroleptic-induced avoidance deficits. J Pharmacol Exp Ther 1980;213:623–7.

Cascella NG, Macciardi F, Cavallini C, Smeraldi E. d-cycloserine adjuvant therapy to conventional neuroleptic treatment in schizophrenia: an open-label study. J Neural Transm Gen Sect 1994;95:105–11.

Coyle JT. The glutamatergic dysfunction hypothesis for schizophrenia. Harv Rev Psychiatry 1996;3:241–53.

Duncan EJ, Szilagyi S, Schwartz MP, Bugarski-Kirola D, Kunzova A, Negi S, et al. Effects of D-cycloserine on negative symptoms in schizophrenia. Schizophr Res 2004;71:239–48.

Evins AE, Fitzgerald SM, Wine L, Rosselli R, Goff DC. Placebo-controlled trial of glycine added to clozapine in schizophrenia. Am J Psychiatry 2000;157: 826–8.

Goff DC, Tsai G, Manoach DS, Flood J, Darby DG, Coyle JT. D-cycloserine added to clozapine for patients with schizophrenia. Am J Psychiatry 1996;153:1628–30.

Goff DC, Henderson DC, Evins AE, Amico E. A placebo-controlled crossover trial of D-cycloserine added to clozapine in patients with schizophrenia. Biol Psychiatry 1999a;45:512–4.

Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA, et al. A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. Arch Gen Psychiatry 1999b;56:21–7.

Goff DC, Leahy L, Berman I, Posever T, Herz L, Leon AC, et al. A placebo-controlled pilot study of the ampakine CX516 added to clozapine in schizophrenia. J Clin Psychopharmacol 2001;21:484–7.

Heresco-Levy U, Javitt DC. Comparative effects of glycine and D-cycloserine on persistent negative symptoms in schizophrenia: a retrospective analysis. Schizophr Res 2004;66:89–96.

Heresco-Levy U, Silipo G, Javitt DC. Glycinergic augmentation of NMDA receptor-mediated neurotransmission in the treatment of schizophrenia. Psychopharmacol Bull 1996;32:731–40.

Heresco-Levy U, Javitt DC, Ermilov M, Silipo G, Shimoni J. Double-blind, placebo-controlled, crossover trial of D-cycloserine adjuvant therapy for treatment-resistant schizophrenia. Int J Neuropsychopharmcol 1998;1: 131–5.

Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein M. Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. Arch Gen Psychiatry 1999;56:29–36.

Heresco-Levy U, Ermilov M, Shimoni J, Shapira B, Silipo G, Javitt DC. Placebo-controlled trial of D-cycloserine added to conventional neuroleptics, olanzapine, or risperidone in schizophrenia. Am J Psychiatry 2002;159: 480–2.

Heresco-Levy U, Ermilov M, Lichtenberg P, Bar G, Javitt DC. High-dose glycine added to olanzapine and risperidone for the treatment of schizophrenia. Biol Psychiatry 2004;55:165–71.

Heresco-Levy U, Javitt DC, Ebstein R, Vass A, Lichtenberg P, Bar G, et al. D-serine efficacy as add-on pharmacotherapy to risperidone and olanzapine for treatment-refractory schizophrenia. Biol Psychiatry 2005;57:577–85.

Hertel P, Fagerquist MV, Svensson TH. Enhanced cortical dopamine output and antipsychotic-like effects of raclopride by alpha2 adrenoceptor blockade. Science 1999;286:105–7.

Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991;148:1301–8.

Javitt DC, Zylberman I, Zukin SR, Heresco-Levy U, Lindenmayer JP. Amelioration of negative symptoms in schizophrenia by glycine. Am J Psychiatry 1994;151:1234–6.

Javitt DC, Duncan L, Balla A, Sershen H. Inhibition of system A-mediated glycine transport in cortical synaptosomes by therapeutic concentrations of clozapine: implications for mechanisms of action. Mol Psychiatry 2005;10:275–87.

Johnson SA, Luu NT, Herbst TA, Knapp R, Lutz D, Arai A, et al. Synergistic interactions between ampakines and antipsychotic drugs. J Pharmacol Exp Ther 1999;289:392–7.

Kim JS, Kornhuber HH, Schmid-Burgk W, Holzmuller B. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. Neurosci Lett 1980;20:379–82.

Krystal JH, D'Souza DC, Petrakis IL, Belger A, Berman RM, Charney DS, et al. NMDA agonists and antagonists as probes of glutamatergic dysfunction and pharmacotherapies in neuropsychiatric disorders. Harv Rev Psychiatry 1999;7:125–43.

Lane HY, Chang YC, Liu YC, Chiu CC, Tsai GE. Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. Arch Gen Psychiatry 2005;62:1196–204.

Li M, Parkes J, Fletcher PJ, Kapur S. Evaluation of the motor initiation hypothesis of APD-induced conditioned avoidance decreases. Pharmacol Biochem Behav 2004;78:811–9.

Linner L, Wiker C, Wadenberg ML, Schalling M, Svensson TH. Noradrenaline reuptake inhibition enhances the antipsychotic-like effect of raclopride and potentiates D2-blockage-induced dopamine release in the medial prefrontal cortex of the rat. Neuropsychopharmacology 2002;27:691–8.

Marenco S, Egan MF, Goldberg TE, Knable MB, McClure RK, Winterer G, et al. Preliminary experience with an ampakine (CX516) as a single agent for the treatment of schizophrenia: a case series. Schizophr Res 2002;57:221–6.

Millan MJ. *N*-Methyl-D-aspartate receptors as a target for improved antipsychotic agents: novel insights and clinical perspectives. Psychopharmacology (Berl.) 2005;179:30–53.

Moore NA, Tye NC, Axton MS, Risius FC. The behavioral pharmacology of olanzapine, a novel "atypical" antipsychotic agent. J Pharmacol Exp Ther 1992;262:545–51.

Natesan S, Vanderspek S, Nobrega JN, McClelland RA, Kapur S. Contrasting loxapine to its isomer isoloxapine—the critical role of in vivo D2 blockade in determining atypicality. Schizophr Res 2005;77:189–99.

Ogren SO, Archer T. Effects of typical and atypical antipsychotic drugs on two-way active avoidance. Relationship to DA receptor blocking profile. Psychopharmacology (Berl) 1994;114:383–91.

Olney JW, Newcomer JW, Farber NB. NMDA receptor hypofunction model of schizophrenia. J Psychiatr Res 1999;33:523–33.

Potkin SG, Jin Y, Bunney BG, Costa J, Gulasekaram B. Effect of clozapine and adjunctive high-dose glycine in treatment-resistant schizophrenia. Am J Psychiatry 1999;156:145–7.

Prinssen EP, Kleven MS, Koek W. Effects of dopamine antagonists in a two-way active avoidance procedure in rats: interactions with 8-OH-DPAT, ritanserin, and prazosin. Psychopharmacology (Berl) 1996;128:191–7.

Rosse RB, Theut SK, Banay-Schwartz M, Leighton M, Scarcella E, Cohen CG, et al. Glycine adjuvant therapy to conventional neuroleptic treatment in schizophrenia: an open-label, pilot study. Clin Neuropharmacol 1989;12:416–24.

Sanchez C, Kreilgaard M. R-citalopram inhibits functional and 5-HTP-evoked behavioural responses to the SSRI, escitalopram. Pharmacol Biochem Behav 2004;77:391–8.

Shannon HE, Hart JC, Bymaster FP, Calligaro DO, DeLapp NW, Mitch CH, et al. Muscarinic receptor agonists, like dopamine receptor antagonist antipsychotics, inhibit conditioned avoidance response in rats. J Pharmacol Exp Ther 1999;290:901–7.

Sumiyoshi T, Jin D, Jayathilake K, Lee M, Meltzer HY. Prediction of the ability of clozapine to treat negative symptoms from plasma glycine and serine levels in schizophrenia. Int J Neuropsychopharmacol 2005;8:451–5.

Tsai G, Yang P, Chung LC, Lange N, Coyle JT. D-serine added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 1998;44:1081–9.

Tsai GE, Yang P, Chung LC, Tsai IC, Tsai CW, Coyle JT. D-serine added to clozapine for the treatment of schizophrenia. Am J Psychiatry 1999;156:1822–5.

Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine transporter I inhibitor, *N*-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 2004;55:452–6.

van Berckel BN, Hijman R, van der Linden JA, Westenberg HG, van Ree JM, Kahn RS. Efficacy and tolerance of D-cycloserine in drug-free schizophrenic patients. Biol Psychiatry 1996;40:1298–300.

van Berckel BN, Evenblij CN, van Loon BJ, Maas MF, van der Geld MA, Wynne HJ, et al. D-cycloserine increases positive symptoms in chronic schizophrenic patients when administered in addition to antipsychotics: a double-blind, parallel, placebo-controlled study. Neuropsychopharmacology 1999;21:203–10.

Vanover KE. Effects of AMPA receptor positive modulators on amphetamine- and dizocilpine-induced locomotion. Eur J Pharmacol 1997;332:115–9.

Wadenberg ML, Ahlenius S. Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: enhancement of antipsychotic-like effects without catalepsy. J Neural Transm Gen Sect 1991;83:43–53.

Wadenberg ML, Hicks PB. The conditioned avoidance response test re-evaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? Neurosci Biobehav Rev 1999;23:851–62.

Wadenberg MG, Browning JL, Young KA, Hicks PB. Antagonism at 5-HT(2A) receptors potentiates the effect of haloperidol in a conditioned avoidance response task in rats. Pharmacol Biochem Behav 2001a;68:363–70.

Wadenberg ML, Soliman A, VanderSpek SC, Kapur S. Dopamine D(2) receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. Neuropsychopharmacology 2001b;25: 633–41.

Williams JB, Mallorga PJ, Jeffrey CP, Pettibone DJ, Sur C. Effects of typical and atypical antipsychotics on human glycine transporters. Schizophr Res 2004;71:103–12.