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Modulation of striatal dopamine release in vitro by agonists of the glycine$_B$ site of NMDA receptors; interaction with antipsychotics

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Abstract

The *N*-methyl-D-aspartate (NMDA) glutamate receptor possesses an obligatory co-agonist site for D-serine and glycine, named the glycine$_B$ site. Several clinical trials indicate that glycine$_B$ agonists can improve negative and cognitive symptoms of schizophrenia when co-administered with antipsychotics. In the present study we have investigated the effects of glycine$_B$ agonists on the endogenous release of dopamine from preparations of rat striatal tissue prisms in static conditions. The glycine$_B$ agonists glycine (1 mM) and D-serine (10 μM), but not D-cycloserine (10 μM), substantially increased the spontaneous release of dopamine, but significantly reduced the release of dopamine evoked by NMDA. The effect of glycine on spontaneous release was abolished by the non-competitive NMDA antagonists 5*R*,10*S*-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cyclohepten-5,10-imine (MK-801, 10 μM) and ifenprodil (5 μM), but was only partially suppressed by the competitive antagonist 4-(3-phosphonopropyl)-piperazine-2-carboxylic acid (CPP, 10 μM). The selective inhibitor of the glial glycine transporter GlyT1 *N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS, 10 μM) significantly increased the release of dopamine in an MK-801-sensitive manner. Interestingly, haloperidol (1 μM), but not clozapine (10 μM), prevented the effects of glycine. This study shows that glycine$_B$ modulators can control dopamine release by interacting with a distinctive NMDA receptor subtype with which some typical antipsychotics can interfere.

Keywords: Glycine$_B$ site; *N*-methyl-D-aspartate receptors; Dopamine; Schizophrenia; Antipsychotic

1. Introduction

The glutamatergic hypofunction hypothesis of schizophrenia has been formulated from clinical and behavioural evidence showing that drugs that block *N*-methyl-D-aspartate glutamate (NMDA) receptor such as phencyclidine, 5*R*,10*S*-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cyclohepten-5,10-imine (MK-801) and ketamine are psychotomimetic. The psychosis induced by these drugs not only causes positive symptoms similar to the action of dopamine agonists, but also negative symptoms and cognitive deficits that are characteristics of schizophrenia (Javitt and Zukin, 1991).

The NMDA receptor possesses two types of binding site that must be occupied by an appropriate agonist in order for the receptor ion channel to open (Thomson et al., 1989). One of these sites is the binding site for the endogenous agonist glutamate, or the specific agonist NMDA, this receptor is located on the NR2 subunit. The other important binding site, located on the NR1 subunit, is for glycine or D-serine, and is named the glycine$_B$ site (Hirai et al., 1996; Laube et al., 1997). Clinical studies on schizophrenic patients have shown the benefits of adjunctive therapy with glycine$_B$ agonists when given with neuroleptics (Javitt, 1999; Tsai et al., 1998, 2004). Interestingly, the adjunctive regime is predominantly effective in improving negative symptoms, against which most classic neuroleptics, but not the atypical ones, are inefficient.

However, it is a matter of some debate as to whether the glycine$_B$ site is saturated under physiological conditions. The concentration of glycine in cerebrospinal fluid is within the micromolar range, yet the affinity of glycine for the glycine$_B$ site is in the high nanomolar range indicating that the glycine$_B$ site may be saturated (Chen et al., 2003). However, potent glycine transporter systems, such as the glycine transporter type 1 and type 2 (GlyT1, GlyT2), located adjacent to NMDA receptors on astroglial cells and glutamate axon terminals, may control glycine concentrations in the vicinity of the

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NMDA receptor (Gadea and Lopez-Colome, 2001; Aragon and Lopez-Corcuera, 2003; Raiteri et al., 2005) and therefore affect the degree of saturation of the glycine$_B$ site. This hypothesis is supported by investigations showing co-localisation of NMDA receptors and GlyT1 proteins (Schell et al., 1995). Several investigators using in vivo (Fedele et al., 1997; Nilsson et al., 1997; Lim et al., 2004) and in vitro electrophysiological or neurochemical paradigms (Berger et al., 1998; Chen et al., 2003) have demonstrated that the addition of glycine$_B$ agonists, or GlyT1 inhibitors, potentiates the effects of NMDA (Bergeron et al., 1998). This suggests not only that the glycine$_B$ receptors are not saturated, but more importantly that GlyT1 is critical in modulating the functioning of NMDA receptors. However, this theory is not supported by some other studies showing that the addition of glycine lacks an effect on NMDA receptor function (Galli et al., 1992; Obrenovitch et al., 1997; Harsing et al., 2001). It is possible that differences in apparent saturation depend on differences in regional receptor subtype expression, local glycine$_B$ agonist concentrations and the expression of specific glycine transporters modulating the concentration in the region of the NMDA receptor.

The aim of our study was to examine the extent to which modulators of the glycine$_B$ site exert control over striatal dopamine neurotransmission, and if this control can be affected by antipsychotics. To this end, we have used an in vitro protocol to assess endogenous dopamine release from rat striatal tissue preparations in static conditions.

2. Methods and materials

2.1. Static release protocol

Sub-adult male Sprague–Dawley rats (Charles River, UK) weighing between 250 and 350 g were used in all experiments. Animals were housed in groups of 2 to 4 in controlled conditions of temperature and humidity. Only experimentally naïve rats were used. All experiments were conducted with permission from the UK Home Office and had approval from the De Montfort University ethics committee.

Animals were sacrificed by cervical dislocation and the brains were then quickly removed and placed on an ice-cold platform for further dissection. The striata were rapidly dissected out and placed in ice-cold oxygenated Krebs buffer containing magnesium (NaCl 125 mM, $MgSO_4$ 1.2 mM, KCl 2.5 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, glucose 10 mM, pH 7.4). Slices were then cut into 350×350 μm striatal prisms using a M^{c}Ilwain tissue chopper, washed three times in fresh, oxygenated Krebs buffer and allowed to recover for 30 min at room temperature, before a 10-min equilibration period in the presence of 10 μM pargyline (a monoamine oxidase inhibitor) and 1 μM nomifensine (a catecholamine reuptake inhibitor) in a magnesium-free Krebs buffer. After this stage, all experiments, unless otherwise stated, were performed in magnesium-free conditions. Oxygenated buffer containing tissue prisms was separated into even portions, suspended in mesh baskets possessing 100 μm pores (CellMicroSieve) and immersed in oxygenated Krebs buffer at 37 °C. During experiments tissue prisms were incubated in 1.5 ml of Krebs buffer (with appropriate drugs as detailed). Solutions were replaced with fresh solutions at 5-min intervals. Following incubation, 1 ml samples of each test solution were removed and mixed with 100 μl of a preservative solution (50 μM EDTA in 1 M perchloric acid) before being centrifuged at 12,000 ×g from which the supernatant was frozen prior to analysis by HPLC. At the end of the experiments, tissues were removed from the mesh baskets and homogenised for 60 s at 20,000 rpm with a Polytron PT-3100 in 1 ml Krebs buffer with 100 μl of preservative.

2.2. Sample analysis

Analysis of samples was performed using high pressure liquid chromatography (HPLC) coupled with electrochemical detection (ecd). Separation was achieved by passing samples through a C-18 reversed phase column (Lichrospher RP-select B 150×4.6 mm, pore size 5 Å, Phenomenex Ltd, UK). The column was flushed at a flow rate of 1.2 ml/min with a mobile phase consisting of 10% v/v methanol, 50 mM KH_2PO_4, 0.25 mM octanesulphonic acid and 0.1 mM EDTA adjusted to pH 3.0 with orthophosphoric acid. Detection was facilitated by a dual glassy carbon working electrode held at a potential of +700 mV (oxidation) versus an Ag/AgCl gel reference electrode (Bioanalytical Systems Ltd, UK). The electrodes were connected to an LC-4C Amperometric Detector (Bioanalytical Systems Ltd, UK) with the range set at 1 nA. Sample traces were compared with authentic dopamine standards with a limit of detection of <500 pM.

2.3. Data analysis

All data are expressed as the mean±standard error of the mean (S.E.M.) of at least 3 sequential samples from the same tissue samples collected in the same experimental conditions and calculated as the percentage of their respective control mean (also determined from 3 to 4 sequential values). Statistical analyses were performed using paired Student's t tests or one-way analysis of variance (ANOVA), if more than 2 sets of data are analysed, followed by appropriate post hoc tests. Probabilities smaller than 0.05 were considered to be significant; n values refer to the number of samples (baskets of tissue prisms) used and not to the number of rats. An average of 3–4 baskets of tissues was obtained from each rat. Unless stated otherwise, the different baskets of tissues from the same animal were used for separate experimental protocols, so that no experiments contained results obtained from a single animal.

2.4. Chemicals

All drugs used were obtained from Sigma-Aldrich (Sigma-Aldrich Company Ltd. Poole, UK), except for clozapine, 5,7-dichlorokynurenic acid (DCKA), haloperidol HCl and tetrodotoxin citrate which were obtained from Tocris (Tocris-Cookson

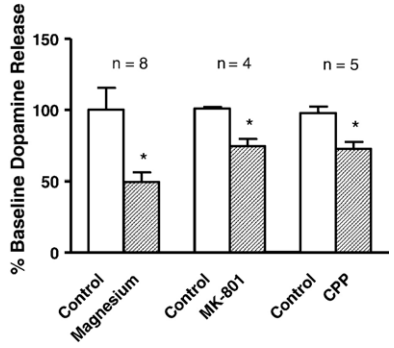

Fig. 1. Effects of magnesium (1.2 mM) and of the NMDA receptor antagonists MK-801 (10 μM) and CPP (10 μM) on the baseline release of dopamine from a preparation of striatal prisms. Values are expressed as a percentage of respective controls obtained from the mean of at least three stable samples measured immediately before applying the antagonists. Separate tissues were used for each different antagonist. In this, and subsequent figures, *n* values refer to the number of samples tested. **P*<0.05 as assessed using Student's paired *t* test versus respective controls.

Ltd, Avonmouth, UK). Water for HPLC analysis (with resistance of ~18 MΩ) was produced on site.

3. Results

3.1. Effects of NMDA receptor modulators

Under our experimental conditions of static release, the variation of release between samples did not exceed 15% in the absence of applied drugs (data not shown). In all experiments, the average baseline dopamine outflow represented less than 0.1% of the total tissue concentration. Release was dramatically reduced to less than 40% of the normal baseline in the absence of calcium in the incubation medium (data not shown). Application of the sodium channel inhibitor tetrodotoxin (1 μM) also produced a significant decrease of 17% in the tonic dopamine outflow (data not shown).

As shown in Fig. 1, the presence of magnesium in the incubation medium significantly decreased dopamine release, as did the application of the NMDA antagonists MK-801 and 4-(3-phosphonopropyl) piperazine-2-carboxylic acid (CPP), suggesting the presence of a tonic activation of NMDA receptors controlling baseline dopamine release in these experimental conditions. The 5-min application of 10 μM NMDA into the incubation medium produced a significant (but sub-maximal) increase in endogenous dopamine release, which was totally calcium-dependent and completely blocked by the competitive NMDA antagonist CPP (10 μM, Fig. 2), applied 15 min before and during the 5-min application of NMDA. On the other hand, the specific antagonist ifenprodil, that antagonises selectively NMDA receptors containing the NR2B subunits, did not induce any change on the baseline and did not alter significantly the release of dopamine elicited by NMDA (Fig. 2).

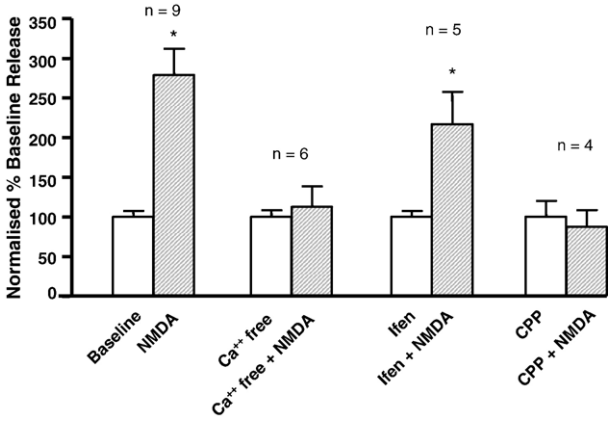

Fig. 2. Effects of calcium and of the NMDA antagonists CPP (10 μM) and ifenprodil (5 μM) on NMDA-evoked dopamine release from striatal prisms. Drugs, or calcium-free buffer, were applied at least 15 min before and during the 5-min application of NMDA (10 μM). Values are expressed as percentage of respective controls obtained from the mean of at least three stable samples measured in the tested conditions immediately prior to the application of NMDA. Separate tissues were used for each series of experiments. **P*<0.05 as assessed using Student's paired *t* test versus respective controls.

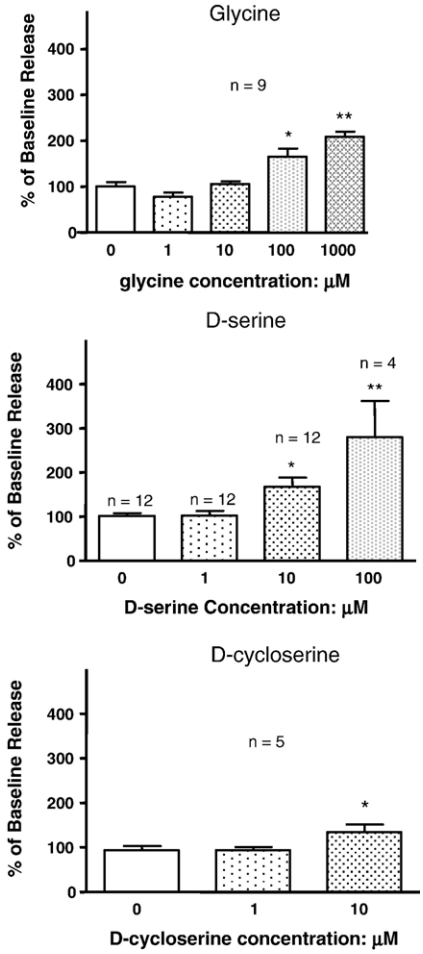

Fig. 3. Concentration of glycine$_B$ agonists versus dopamine release from striatal prisms. Results are presented as percentage of respective controls obtained from the mean of at least three stable baseline samples measured prior to the application of the agonists. Each concentration of agonist was applied for at least 15 min on the same tissue samples in the same sequence as indicated in the graph. **P*<0.05, ***P*<0.01 versus relevant control as assessed using 1-way ANOVA with Tukey's post hoc test.

3.2. Effects of glycine$_B$ agonists

Fig. 3 shows that glycine has a stimulatory effect on tonic dopamine release when applied alone. However, this only becomes significant when applied in relatively high concentrations (at or above 100 μM). By contrast, the other endogenous glycine$_B$ agonist tested, D-serine, showed a significant effect when applied at concentrations of 10 μM and greater (Fig. 3). The non-endogenous partial agonist D-cycloserine, while producing a significant response at a concentration of 10 μM, does not produce an effect of similar magnitude to that of D-serine (Fig. 3). The effects of glycine were calcium-dependent and were completely prevented by tetrodotoxin (in contrast to that which was observed with NMDA whose effects on dopamine release were partially tetrodotoxin-sensitive, data not shown).

When applied 15 min before glycine, the non-competitive NMDA antagonist MK-801, and the selective NR2B subunit antagonist ifenprodil prevented the increase in dopamine release induced by glycine. This was in contrast to what was observed with NMDA (Fig. 4). The competitive antagonist CPP only partially reduced the increase produced by glycine (Fig. 4). However, this increase was completely reversed when the competitive glycine$_B$ antagonist 5,7-dichlorokynurenic acid (DCKA) was introduced to the incubation medium in addition to CPP. These data suggest that, in our experimental conditions, two distinct receptor subtypes may trigger the respective effects induced by the applications of NMDA and glycine$_B$ agonists.

3.3. Effects of inhibition of glycine transport

The fact that glycine requires a much higher concentration to produce an effect on dopamine release than D-serine may suggest that extra-cellular glycine is cleared from the local environment of the NMDA receptor with which glycine interacts. This may occur through the specific glial transporter system consisting of GlyT1. To test this hypothesis the selective and potent GlyT1 inhibitor *N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS) was tested. NFPS by itself produced a significant increase in the baseline amount of dopamine released in the absence of glycine, to a level that appears still somewhat lower than the value obtained in the presence of 1 mM glycine (Fig. 5A). The addition of 30 μM glycine (a concentration which by itself does not alter baseline dopamine release) only slightly further increases the release of dopamine. As indicated in Fig. 5B, the co-application of MK-801 with NFPS totally prevented the effects of NFPS on dopamine release. Sarcosine, an endogenous compound which interacts with low affinity as a substrate for GlyT1, was also tested on a small number of samples from one animal (n=4). It was found that sarcosine produced a significant increase in dopamine efflux if applied at relatively high concentration (300 μM) and only in the presence of 30 μM of glycine (data not shown).

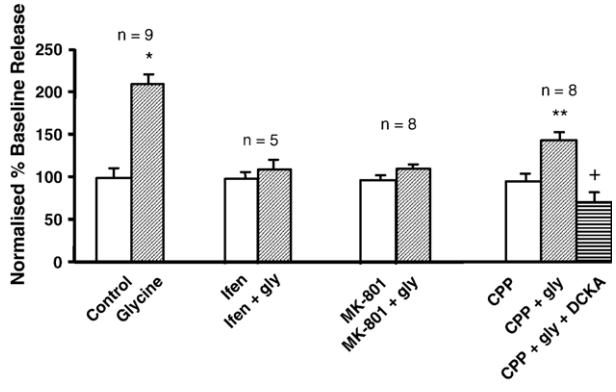

Fig. 4. Effects of the NMDA/glycine receptor antagonists MK-801 (10 μM), CPP (10 μM) and 5,7-dichlorokinurenic acid (DCKA, 10 μM) on glycine-evoked release of dopamine from striatal prisms. Drugs were applied at least 15 min before and during the application of glycine (1 mM). Values are expressed as percentage of respective controls obtained from the mean of at least three stable samples measured in the tested conditions immediately prior to the application of glycine. For each series of experiments, controls and stimulated release were performed on the same tissue samples in the same sequence as indicated in the graph. Separate tissues were used for each different antagonist. *P<0.01 versus respective control as assessed using Student's paired t tests versus respective controls. **P<0.001 versus CPP, +P<0.001 versus CPP plus glycine as assessed using 1-way ANOVA with Tukey's post hoc test.

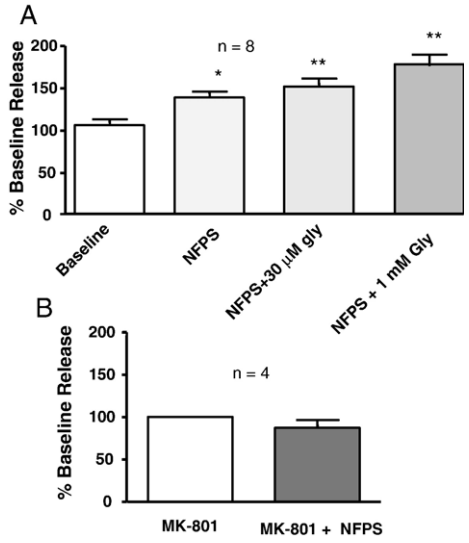

Fig. 5. A: Effect of NFPS (10 μM) alone, and in combination with glycine, on dopamine release from striatal prisms. B: Effect of MK-801 (10 μM) on the NFPS-induced increase in dopamine release. Drugs were applied for at least 15 min in the same sequence as indicated in the graph. Results are presented as percentage of respective controls obtained from the mean of at least three stable samples measured immediately prior to the application of NFPS. *P<0.05, **P<0.01 versus baseline as assessed using 1-way ANOVA with Tukey's post hoc test.

3.4. Effects of glycine$_B$ agonists on NMDA-evoked release

A series of experiments were performed to test whether glycine$_B$ agonists act synergistically, or additively, with NMDA on dopamine outflow. In control conditions, two 5-min applications of 10 μM NMDA (S1 and S2) separated by 30 min of washout time produce very similar stimulations of dopamine release. As shown in Fig. 6A, the ratio (S2/S1) between the second and first stimulations was near 0.9. When

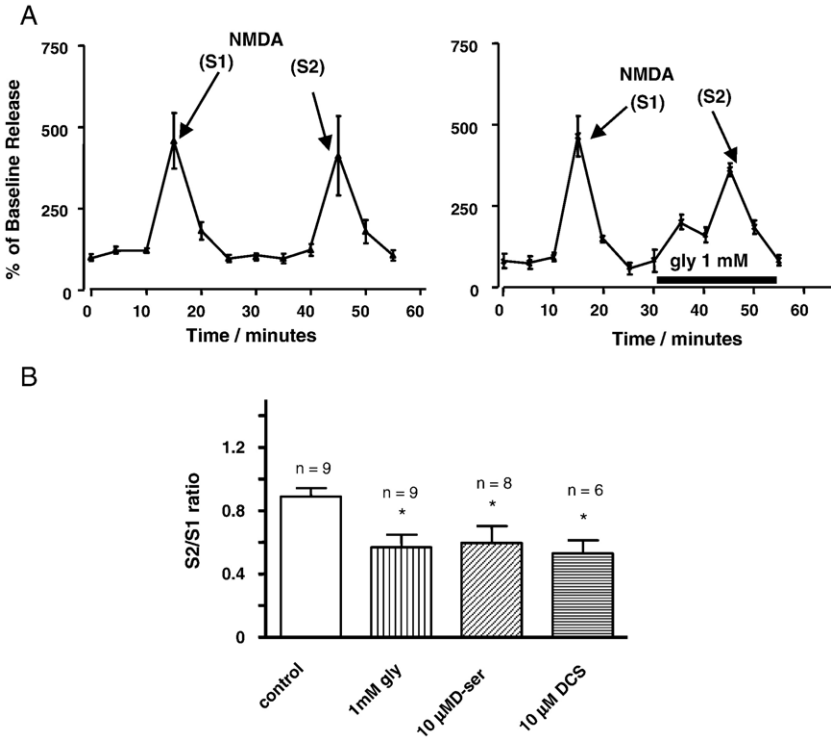

Fig. 6. Effect of glycine$_B$ agonists on dopamine release evoked by NMDA from striatal prisms. A: Left: reproducibility of the magnitude of dopamine release induced by two 5-min applications of 10 μM NMDA, at 15 (S1) and 45 min (S2). Right: reduction of NMDA-induced dopamine release in the presence of glycine (1 mM) applied 15 min before and during the second stimulation. Results are presented as percentage of controls obtained from the mean of at least three stable samples measured immediately before the first NMDA stimulus (S1). B: Effects of different glycine$_B$ agonists on the mean (±S.E.M.) S2/S1 ratios. Each glycine$_B$ agonist was applied onwards of 15 min before the second stimulation (S2). Separate tissues were used for each different agonist (gly: glycine, D-ser: D-serine, DCS: D-cycloserine). For calculating stimulated release (*S*) the peak values obtained from the stimulation were normalised for the averaged baseline obtained during the last 15 min prior to each stimulation. *$P<0.05$ versus control as assessed using 1-way ANOVA with Tukey's post hoc test.

glycine (1 mM), D-serine (10 μM), or D-cycloserine (10 μM) were applied onwards of 10 min after the first NMDA stimulation (10 μM), less dopamine was released in the presence of NMDA during the second stimulation. This is in spite of the glycine$_B$ agonists (except D-cycloserine) inducing an increase in baseline dopamine release (Fig 6A and B). The application of a lower concentration of glycine (10 μM) failed to induce any changes in the NMDA response (data not shown).

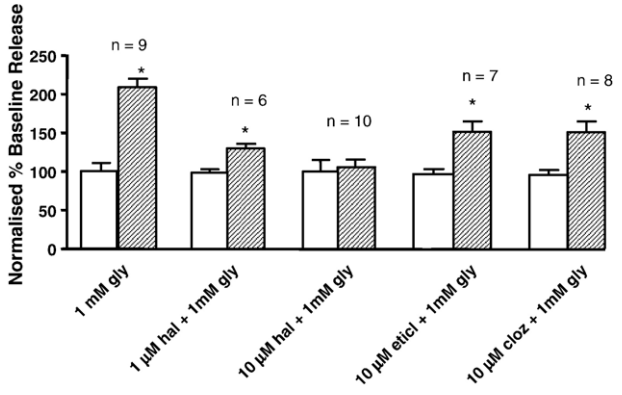

Fig. 7. Effects of different dopamine D_2 antagonists (haloperidol, eticlopride and clozapine) on glycine-evoked dopamine release from striatal prisms. Drugs were applied for at least 15 min before, and during the application of glycine (1 mM). Values are expressed as a percentage of respective controls obtained from the mean of at least three stable samples measured in the tested conditions immediately prior to the application of glycine. Separate tissues were used for each different antagonist. *$P<0.01$ significantly higher than respective control (paired Student's *t* test).

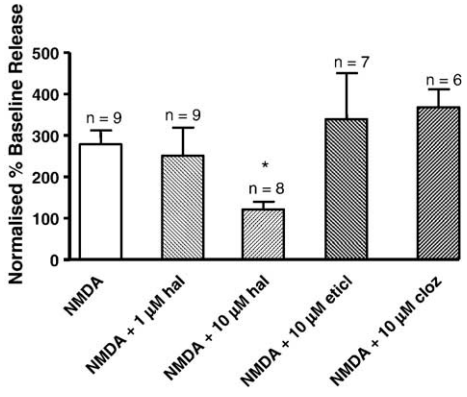

Fig. 8. Effects of different dopamine D_2 antagonists (haloperidol, eticlopride and clozapine) on NMDA-evoked dopamine release from striatal prisms. Antagonists were applied for at least 15 min before, and during the 5-min application of NMDA. Values are expressed as a percentage of respective controls obtained from the mean of at least three stable samples measured in the tested conditions immediately prior to the application of NMDA. *$P<0.05$ as assessed using 1-way ANOVA with Dunnett's post hoc test versus NMDA alone.

3.5. Interactions with antipsychotics

When glycine (1 mM) was added to the incubation medium with slices that were pre-incubated for at least 15 min with 10 μM of haloperidol, there were no effects of glycine on the release of dopamine (Fig. 7). When slices were pre-incubated with 1 μM of haloperidol, the addition of 1 mM glycine was only able to produce a slight, but still significant, increase in dopamine release (29±6% over control). Fig. 8 shows that the stimulatory effect of NMDA (10 μM) on dopamine release is completely prevented by the presence of haloperidol (10 μM). This effect was not demonstrated by the lower dose of 1 μM haloperidol. We have also investigated the effects of the potent and selective D_2 antagonist eticlopride and the atypical antipsychotic clozapine, as a comparison to haloperidol. In the presence of 10 μM eticlopride or of 10 μM clozapine, both glycine (1 mM) and NMDA (10 μM) stimulated the release of dopamine (Figs. 7 and 8). However, the response induced by glycine tended to be attenuated.

4. Discussion

In the presence of high concentrations of glycine (100 μM and 1 mM), or a lower concentration of D-serine (10 μM), endogenous dopamine outflow from striatal prisms in static release conditions was significantly increased. This result differs from numerous other studies that have been performed in perfused conditions using radiolabelled dopamine that have failed to find any stimulatory effects of $glycine_B$ agonists on striatal dopamine efflux (Javitt et al., 2000, 2005a; Nankai et al., 1995). This may be due to differences in methodology. Static release conditions probably allow a greater accumulation of neurotransmitters over a definite period of time and a better tissue penetration of drugs, which amplifies their effects to some extent. In addition, we have measured endogenous release of dopamine, which must include a significant fraction of newly synthesised dopamine. Moreover, nomifensine and pargyline may also contribute to amplify the effects of drug-induced endogenous dopamine release by preventing its rapid clearance. Therefore, the characteristics of dopamine release assessed in our experimental conditions may not show exactly the same properties as in radiolabelled and perfused conditions.

Glycine-induced dopamine release was sensitive to NMDA antagonists suggesting that in these experimental conditions the $glycine_B$ site of at least one subtype of NMDA receptor responsible for modulating striatal dopamine release is not saturated. In vitro, glycine and D-serine bind with similar affinity to the $glycine_B$ site (Miller, 2004). The large difference in potency between these two agonists is probably due to the presence of the very specific, and potent, astroglial transport system that can maintain a tight control of local glycine concentration in the vicinity of the NMDA receptor (Gadea and Lopez-Colome, 2001). As already mentioned, this system has no affinity for D-serine, which explains its action at much lower concentration. Apparently, no similar uptake system exists for D-serine or D-cycloserine, as current theory suggests that D-amino acids are inactivated by the enzyme D-amino acid oxidase (Nagata, 1992; Snyder and Kim, 2000), rather than by an uptake system. The fact that very high concentrations of glycine are necessary to overcome the action of the transporter can be explained if we consider the kinetic characteristics of the GlyT1 transporter. The Michaelis constant (K_m), which reflects the affinity of glycine for this transport system, is in the high micromolar range (70–90 μM) (Kim et al., 1994). This means that saturation of this transport system will occur only in the low millimolar range, which is compatible with the fact that 100 μM of glycine is the minimum concentration observed to produce an effect on dopamine release. To test the hypothesis that the presence of unsaturating concentrations of glycine in our slice preparation is due to the action of GlyT1, we have investigated the effects of two drugs interacting specifically with GlyT1: sarcosine and NFPS. Both drugs were found to increase the outflow of dopamine, indicating that manipulating extra-cellular glycine levels can alter the function of some striatal NMDA receptors that regulate dopamine release. However, these two inhibitors seem to perform slightly differently. With high concentrations of sarcosine, at least 30 μM of exogenous glycine is necessary to activate the dopamine outflow, while NFPS alone is able to stimulate dopamine release in the absence of exogenous glycine. This suggests that NFPS prevents the uptake of the presumably small amount of endogenous glycine already present in the vicinity of the $glycine_B$ sites modulating dopamine overflow. In these conditions the endogenous glycine should accumulate rapidly to a level sufficient to activate these receptors. In addition, it was evident from our results that NFPS exerts its effect via an indirect action on NMDA receptors as, despite this drug having no affinity for the NMDA receptors, its effect was completely prevented by MK-801.

Interestingly, the effects of NFPS alone could be further augmented in the presence of the highest concentration of glycine, as intermediate concentrations of glycine were found to have only a minor additional effect in increasing dopamine outflow. The difference relative to the effect of 1 mM (approximately 20%) may be due to other types of amino acid transporters of lower affinity for glycine, and less sensitive to NFPS. For example, the neuronal system A transporter (Javitt et al., 2005b), which may still be functioning and would start to be saturated by glycine only at the higher concentrations was used in this study. Overall these data clearly demonstrate that dopamine release in the striatum can be indirectly modulated by the glial glycine uptake system involving GlyT1, which appears to be critical for controlling glycine concentrations at some NMDA receptors that stimulate dopamine release.

Interestingly, the responses triggered by the application of NMDA and glycine agonists on dopamine overflow seem to exhibit important differences in their pharmacological characteristics, in particular regarding their sensitivity to the specific NR2B antagonist ifenprodil. In our study, ifenprodil blocks glycine but not NMDA-induced effects, indicating that $glycine_B$ agonists increase dopamine overflow by activating an NMDA receptor that contains the NR2B subunit and that is probably distinct from the receptors that trigger the effects of NMDA alone. In addition, the NMDA-evoked release of dopamine was not potentiated by $glycine_B$ agonists, while these agonists did

stimulate the spontaneous release of dopamine in the absence of NMDA. Therefore, these data may indicate that, in our experimental conditions, two distinct populations of NMDA receptors are involved in the respective effects of NMDA and glycine on striatal dopamine release. For one population, preferentially stimulated by NMDA, and insensitive to ifenprodil, the glycine site would normally be saturated. For the other population, possibly more weakly stimulated by the addition of NMDA, and sensitive to ifenprodil, the local concentration of glycine is critical. Further studies using other selective agents, such as those acting selectively on the NR2A sub-unit (e.g. NVP-AAM07), would be useful to better identify pharmacologically these two populations. It is possible that there exists non-conventional "glutamatergic" NMDA receptors at which glycine (or the other agonists used in the study) are capable of activating the receptor alone. Other investigators have identified some receptors that could be classed in this way (Paudice et al., 1998; Chatterton et al., 2002). Paudice et al. (1998) proposed that the NMDA receptor(s) that control the release of the neuropeptides cholecystokinin and somatostatin from rat neocortical synaptosomes require only glycine agonists for activation as, in common with our results, they were found to be sensitive to ifenprodil and the non-competitive antagonist MK-801, but not to competitive NMDA antagonists, such as CPP.

Contrary to expectation, it was found that the application of $glycine_B$ agonists to the incubation medium induced an attenuation of the NMDA response. One possible explanation for this result could be that the prolonged effect of persistent high levels of $glycine_B$ agonist induces a desensitisation of the NMDA receptor, making it relatively insensitive to further stimulation by exogenous NMDA. Other investigators (Nong et al., 2003, 2004; Martina et al., 2004) have found evidence that provides support for this desensitisation hypothesis as they found that a high level of extra-cellular glycine, and subsequent increased binding of glycine, led to the internalisation of NMDA receptors through a clathrin-dependent mechanism. This reduction in the number of NMDA receptors led to a reduction in NMDA-mediated neurotransmission. One other possibility would be that some 'glycine-sensitive' NMDA receptors are also located on neurons that release inhibitory neurotransmitters, such as GABA or somatostatin. The addition of exogenous $glycine_B$ agonists would greatly increase the magnitude of the release of these inhibitory neurotransmitters in the presence of NMDA, thereby providing a greater negative modulation of dopamine release. This hypothesis is in keeping with the finding of Paudice et al. (1998) mentioned above, showing that the release of somatostatin from synaptosomes can be evoked by an NMDA receptor requiring glycine as a sole agonist. In addition, a recent study by Javitt et al. (2005a) has demonstrated that glycine and glycine uptake inhibitors produce a potentiation of the NMDA-evoked release of tritiated GABA in the striatum, while reducing the NMDA-evoked release of tritiated dopamine. However, other investigators have failed to observe any stimulatory effects of glycine on NMDA-evoked tritiated GABA release in the striatum (Harsing et al., 2001).

We found that haloperidol is capable of interfering with the function of the NMDA receptors that control the striatal dopamine release, possibly at the level of the $glycine_B$ site. Whether this occurs at a dose that correlates to a clinically relevant concentration is hard to say conclusively as there is significant variation in concentration measurements from patients for a variety of reasons (Tokunaga et al., 1997; Ohara et al., 2003; Ohnuma et al., 2003). However, because haloperidol can accumulate in the brain to a level 30 times higher than in the plasma (Sunderland and Cohen, 1987), one could approximate that haloperidol could be present at a concentration close to the low micromolar range, a concentration that was found to interact with the glycine-evoked release of dopamine in this study. The effect of haloperidol on the glycine/NMDA-evoked release is probably independent of its affinity for D_2 receptors, as the stimulatory effect of glycine was not blocked in the presence of the other, more specific, D_2 antagonist eticlopride. Haloperidol has been shown to attenuate NMDA-induced currents in hippocampal cell cultures when applied in the low micromolar range (Fletcher and MacDonald, 1993), and to interact with the NMDA receptor as a partial agonist of the $glycine_B$ site. The possibility that haloperidol itself may be able to act on the $glycine_B$ site of the NMDA receptor is supported by our data, showing that haloperidol attenuates the effects of glycine at a lower dose than that at which it interferes with the NMDA-induced release. However, other studies, involving electrical recordings from oocytes expressing different subunit combination (Ilyin et al., 1996), as well as studies performed in the rat brain (Coughenour and Cordon, 1997), have demonstrated that haloperidol interacts preferentially with NR1A/2B composed NMDA receptors probably at the same site as ifenprodil. Interestingly, in our study, glycine-mediated effects, compared to NMDA-mediated effects, were more sensitive to both ifenprodil and haloperidol, indicating that haloperidol may block the striatal dopamine efflux induced by $glycine_B$ agonists as a selective antagonist of NR1A/2B NMDA receptors. However, a direct interaction with $glycine_B$ sites, as described by Fletcher and MacDonald (1993) (but see also McCoy and Richfield, 1996), could not be ruled out.

In conclusion, our results demonstrate that $glycine_B$ agonists can control the release of endogenous dopamine in rat striatal slices in vitro. They interact with an NMDA receptor probably composed of NR1A/2B subunits, and whose activity is affected by the GlyT1 transporter (which probably maintains the concentration of glycine at levels low enough to prevent its activation). Interestingly, this subtype of NMDA receptor seems particularly sensitive to the typical antipsychotic haloperidol at a relatively low concentration. Whether this interaction can help to explain the beneficial effects of the adjunctive therapy in schizophrenia deserves to be examined in more detail. For example, are $glycine_B$ agonists correcting a deficit of the function of some NMDA/$glycine_B$ receptors that has been induced by the administration of some antipsychotics? This hypothesis could be investigated by examining the extent to which other typical and atypical antipsychotics alter the effects of $glycine_B$ agonists on dopamine release.

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