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Interaction between dopamine and glutamate receptors following treatment with NMDA receptor antagonists

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Abstract

Interactions between dopamine and glutamate neurotransmission have been reported to play an important role in a number of different systems. We were interested in examining the effects of sub-chronic treatment with NMDA receptor antagonists (dizocilpine [MK-801], and 3-carboxy-piperazin-propyl phosphonic acid [CPP]) on dopamine D_1-like, dopamine D_2-like, as well as glutamate receptors of the NMDA and AMPA receptor subtypes in the neostriatum and substantia nigra of rats that had received a massive dopamine denervation at 3 days of age. Using quantitative ligand binding autoradiography, we demonstrated that the two NMDA receptor antagonists did not have different profiles of action. Furthermore, while we found a significant negative relationship between NMDA receptors and dopamine receptors (both dopamine D_1-like and D_2-like receptor subtypes) in the neostriatum, AMPA receptors were positively correlated with dopamine D_1-like binding sites in all regions investigated. These findings suggest that the interrelationship between dopamine and glutamate receptors is highly controlled and that the nigrostriatal dopamine systems play an important role in this interaction.

Keywords: 6-Hydroxydopamine; Ligand binding autoradiography; Neostriatum; Substantia nigra

1. Introduction

The neostriatum receives a massive glutamatergic projection from the cerebral cortex as well as a dopaminergic innervation from the substantia nigra pars compacta (Wilson and Groves, 1980; Fonnum et al., 1981). These projections appear to converge on the same medium spiny neurons (Wilson and Groves, 1980), which are known to contain the neurotransmitter γ-aminobutyric acid (GABA).

Adult rats that have received a neonatal intracerebral injection of the neurotoxin 6-hydroxydopamine exhibit a profound irreversible loss of dopamine innervation particularly to the neostriatum (Stachowiak et al., 1984), with only a few dopamine fibres remaining in its paraventricular portion. A number of neurochemical changes have been noted in this model including increased dopamine synthesis and release, attenuation and elevation of dopamine D_1-like and D_2-like receptors (see Reader and Dewar, 1999), respectively, as well as substantial augmentation of neostriatal serotonin innervation and receptor number (Stachowiak et al., 1984; Radja et al., 1993). Moreover, following 6-hydroxydopamine lesions, the medium-sized spiny neurons have fewer dendritic spines in response to the loss of dopamine inputs. The majority of these seemingly establish symmetrical synapses in the neostriatum (Bouyer et al., 1984). More recently, it has also been shown that there is a comparable decrease in the number of asymmetrical synapses (Ingham et al., 1998), implying a secondary loss of non-dopamine afferents to the neostriatum. The majority of the presynaptic fibers, that are a component of asymmetrical synapses, are likely to be of cortical origin, but some may also arise from thalamic nuclei (Bouyer et al., 1984), and are probably of glutamatergic nature (Fonnum et al., 1981). Loss of nigrostriatal dopamine innervation in Parkinson's disease is known to result in an overactivity of excitatory glutamate neurons arising in the subthalamic nucleus and projecting to the substantia nigra.

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Functional interactions between neostriatal dopamine and glutamine containing nerve terminals have also been observed (Löscher et al., 1993; Dall'Olio et al., 1996). Rats which have received a unilateral nigrostriatal dopamine exhibit striking contralateral rotatory behaviour in response to low doses of apomorphine and NMDA receptor antagonists. AMPA receptor antagonists have also been shown to potentiate this stimulatory response (Loschmann et al., 1992; Wachtel et al., 1992). Furthermore, this interaction is dependent upon whether dopamine D_1-like or D_2-like receptors are stimulated. Contralateral turning behaviour induced by dopamine D_1-like receptor agonists is potentiated by the NMDA receptor antagonist dizocilpine (MK-801), while turning behaviour induced by dopamine D_2-like receptor agonists is decreased (Morelli et al., 1992). These data suggest a differential relationship between these sites. It has been reported that dopamine D_1 receptor agonists elicits Fos expression in the striatum of control and 6-hydroxydopamine lesioned rats (Johnson et al., 1992; Nakazato et al., 1998). Prior treatment with an NMDA receptor antagonist can block or significantly attenuate this production (Nakazato et al., 1998).

The existence of an anatomical and function interrelationship between dopamine and glutamate systems in the neostriatum of the rat suggest that activation and/or inhibition of the NMDA receptors would influence dopamine neuronal activity and consequently alter dopamine D_1-like and D_2-like receptor binding. In fact, the systemic administration of MK-801 enhances the firing rate of dopamine neurons (Overton and Clark, 1992) and increases dopamine release (Rosales et al., 1997). Since it is unclear whether competitive and non-competitive NMDA receptor antagonists differentially alter dopamine neuronal activity, we decided to compare the effects of the non-competitive NMDA receptor antagonists MK-801 as well as the competitive antagonist 3-carboxy-piperazin-propyl phosphonic acid (CPP) in our study. We hypothesize that there will be a relationship between glutamate and dopamine D_1-like and/or D_2-like receptors following sub-chronic treatment with NMDA receptor antagonists MK-801 and CPP. Furthermore, since dopamine synthesis, release and receptor binding are altered in 6-hydroxydopamine-treated rats, we hypothesize that this relationship will be different in 6-hydroxydopamine lesioned animals.

2. Material and methods

2.1. Neonatal 6-hydroxydopamine lesions and MK-801 and CPP injections

Female Sprague–Dawley rats (Charles River; Montreal, Canada) were purchased pregnant and individually housed under a fixed light/dark cycle with free access to food and water. Three days after delivery, the pups received a subcutaneous injection of desmethylimipramine (Sigma, 25 mg/kg) in order to protect noradrenaline neurons from the high affinity uptake and consequent cytotoxicity of 6-hydroxydopamine. Forty-five minutes after, they were anaesthetized over ice and given bilateral cerebroventricular injections of 6-hydroxdopamine (Sigma, 100 μg free base in 10 μl 0.9% NaCl containing 0.1% ascorbic acid) according to the procedure initially described by Stachowiak et al. (1984). Littermate controls received the vehicle solution alone.

Since data from our laboratory has shown that [^{3}H]MK-801, [^{3}H]SCH23390 and [^{3}H]nemonapride ([^{3}H]YM-09151-2) binding was at its maximum and did not differ significantly between 3 and 6 months (Dewar et al., unpublished observations), this survival period was chosen for the present study. Sham- or 6-hydroxydopamine-lesioned rats were treated for 8 days with MK-801 (RBI, Oakville, Canada, 0.1mg/kg), CPP (Tocris, Ballwin, MO, 1mg/kg) or the saline vehicle and sacrificed 24 h after the last injection. Their brains were quickly removed and frozen at −30°C in *N*-methylbutane cooled with liquid nitrogen. Transverse cryostat sections (20 μm) were thaw mounted onto gelatin-coated slides and kept at −80°C until processed for quantitative ligand binding autoradiography.

2.2. Ligand binding autoradiography

Dopamine D_1-like receptors were visualized with the selective dopamine D_1 receptor antagonist [^{3}H]SCH23390 according to well established procedures. In brief, sections were incubated with 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. Sections were then incubated in the same buffer containing 100 μM mianserin (RBI) to block binding to the 5-HT_2 receptor subtypes and in the presence of 1 nM [^{3}H]SCH23390 (RBI) for 60 min. Non-specific binding was determined in the presence of 30 μM (±)-SKF-38393.

Dopamine D_2-like receptors were labelled using the mixed dopamine D_2 receptor antagonist [^{3}H]YM-09151-2 (DuPont, 86 Ci/mmol). All incubations and washes were carried out in the dark. Sections were incubated at 25°C for 30 min in 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 5 mM MgCl2, 1.5 mM CaCl2, 1 mM EDTA, 10 μM pargyline (RBI) and 0.1% ascorbic acid. They were then incubated in the same buffer in the presence of 0.4 nM [^{3}H]YM-09151-2 for 60 min at room temperature. Non-specific binding was determined in adjacent section incubated with 300 μM (±)-sulpiride (RBI).

The phencyclidine (PCP) binding site of the NMDA receptor was labelled with the non-competitive NMDA receptor antagonist [^{3}H]MK-801 (DuPont, 22,0 Ci/mmol). Sections were incubated at 25°C for 30 min in 50 mM Tris–acetate buffer (pH 7.4), then for a further 120 min in 50 mM Tris–acetate buffer containing 2 nM [^{3}H]MK-801

in the presence of 30 μM glutamate and 10 μM glycine. Non-specific binding was determined with 10 μM ketamine (RBI).

The AMPA receptor was labelled with [^{3}H]AMPA (DuPont, 46 Ci/mmol). Sections were incubated at 25°C for 60 min in 50 mM Tris–acetate buffer (pH 7.4) containing 100 μM EGTA. Sections were then incubated at 0°C for 45 min with 50 mM Tris–acetate buffer (pH 7.4) containing 100 μM EGTA, 50 mM potassium thiocyanate and 50 nM [^{3}H]AMPA. Non-specific binding was determined in the presence of 50 mM quisqualate (RBI).

Following incubation, the slides were washed in ice-cold buffer (3×5 min for [^{3}H]SCH23390, [^{3}H]YM-09151-2 and [^{3}H]MK-801 or 3×10 s for [^{3}H]AMPA) and dried under a stream of cold air. Autoradiograms were generated by apposing slides to [^{3}H]-sensitive film ([^{3}H]Hyperfilm, Amersham, Missisauga, Canada) together with tritium standards (Microscales, Amersham). Autoradiographic exposures lasted 1–3 weeks ([^{3}H]AMPA, [^{3}H]MK-801, [^{3}H]SCH23390) or 2–4 months [^{3}H]YM-09151-2 at 4°C. The films were developed in D-19 (Kodak) for 4 min at 19°C.

2.3. Quantitative analysis of autoradiograms

Each ligand was examined in the 5–6 animals from the six experimental groups. The rostral and caudal neostriatum were defined as rostral and caudal to the decussation of the anterior commissure. The microcomputer-based image analysis system (MCID, Imaging Research, St. Catherines, Canada) was used for quantitative measurements. Standard curves generated from [^{3}H]microscales (Amersham) were used to convert density values into fmol/mg protein assuming a protein content of the tissue to be 30%. Multiple readings were made for each region (five to six sections for each brain region). Non-specific binding was determined in adjacent sections and specific binding was obtained by subtracting non-specific from total binding.

2.4. Statistical analysis

The differences in binding of ligands to the dopamine D_1-like, dopamine D_2-like, NMDA and AMPA receptors between the six parallel groups of rats were analysed for statistical significance using an analysis of variance (ANOVA) for a 2×3 factorial design. Sham- and 6-hydroxydopamine-lesions were compared in the presence or absence of MK-801 and CPP and possible drug × lesion interactions were assessed after orthogonalization of the comparisons (Draper and Smith, 1966). Since the binding of ligands to dopamine D_1-like and dopamine D_2-like receptors, as well as NMDA and AMPA glutamate receptors were carried out in adjacent sections from the same animal, the relationship in binding between NMDA or AMPA receptors and dopamine D_1-like and dopamine D_2-like receptors was assessed for each using a linear regression model (Draper and Smith, 1966). Heterogeneity of the regression slopes (or non-parallelism) between the six groups was tested. The critical level of significance to accept differences in binding data or regression slope heterogeneity was set at 5%.

3. Results

3.1. [^{3}H]MK-801 binding

[^{3}H]MK-801 bound with high affinity to the PCP site of the NMDA receptor throughout the brain. Binding was highest in the rostral and caudal aspects of the neostriatum and significantly lower in the substantia nigra ($F(1,30) = 109.45$, $P < 0.001$). In the rostral and caudal neostriatum [^{3}H]MK-801 binding did not differ among the treatment groups (i.e., saline, MK-801- or CPP-treated) either for sham or 6-hydroxydopamine-lesioned animals.

In the substantia nigra of saline-treated animals, a significant increase in the density of [^{3}H]MK-801 binding was observed in the 6-hydroxydopamine-lesioned group ($F(1,21) = 14.06$, $P < 0.01$) (Table 1). In contrast, a reduction in the [^{3}H] MK-801 binding was observed in 6-hydroxydopamine-lesioned rats subsequently treated with MK-801 or CPP leading to a significant drug by lesion interaction ($F(1,21) = 14.06$, $P < 0.01$). Both MK-801 and CPP significantly attenuated binding as compared to the saline group, ($F(1,21) = 21.18$, $P < 0.001$) (Table 1).

3.2. [^{3}H]AMPA binding

The density of [^{3}H]AMPA binding sites was consistent with previous studies (Cha et al., 1992). Within the neostriatum [^{3}H]AMPA binding was uniform in nature, the density of sites in the substantia nigra was significantly lower than in the neostriatum ($F(1,23) = 95.94$, $P < 0.001$). In rostral neostriatum ($F(1,23) = 0.03$), caudal neostriatum ($F(1,23) = 0.65$), or the substantia nigra ($F(1,23) = 3.51$) there was no significant difference between sham lesioned and 6-hydroxydopamine-treated rats (Table 1).

As compared to saline, [^{3}H]AMPA binding did not change with MK-801 or CPP treatment in the rostral ($F(1,23) = 1.99$) and the caudal ($F(1,23) = 2.50$) neostriatum. In the substantia nigra, however, a drug by lesion interaction was found ($F(1,23) = 5.13$, $P < 0.05$); in 6-hydroxydopamine lesioned but not sham-lesioned rats, MK-801 and CPP significantly increased [^{3}H]AMPA binding ($F(1,23) = 7.49$, $P < 0.02$) as compared to saline (Table 1). Furthermore, the augmentation in [^{3}H]AMPA binding with MK-801 was significantly different in 6-hydroxy-

Table 1
Effects of MK-801 and CPP on [^{3}H]MK-801 and [^{3}H]AMPA binding in sham- and 6-hydroxydopamine lesioned rats
Results are expressed as fmol/mg protein (mean ± S.E.M.).
Statistical difference between the six parallel groups of rats was assessed using analysis of variance (ANOVA) for a 2 × 3 factorial design.

		Saline	MK-801 (0.1 mg/kg)	CPP (1 mg/kg)
[^{3}H]MK-801				
Rostral neostriatum	sham	247 ± 14.6	311 ± 36.3	260 ± 23.3
	6-hydroxydopamine	276 ± 27.2	254 ± 34.8	229 ± 18.1
Caudal neostriatum	sham	261 ± 28.3	270 ± 37.8	263 ± 30.9
	6-hydroxydopamine	283 ± 30.0	224 ± 31.3	238 ± 16.1
Substantia nigra	sham	64 ± 9.5	74 ± 9.3	71 ± 7.2
	6-hydroxydopamine	104 ± 2.3[a]	58 ± 7.12[b]	67 ± 6.8[b]
[^{3}H]AMPA				
Rostral neostriatum	sham	264 ± 10.2	354 ± 110.4	373 ± 104.6
	6-hydroxydopamine	266 ± 41.5	267 ± 83.1	229 ± 18.1
Caudal neostriatum	sham	245 ± 9.50	252 ± 37.1	297 ± 69.0
	6-hydroxydopamine	229 ± 29.0	294 ± 60.3	369 ± 64.8
Substantia nigra	sham	88 ± 13.9	84 ± 6.4	75 ± 7.7
	6-hydroxydopamine	70 ± 13.7	136 ± 14.8[a,b]	111 ± 26.7[b]

[a] Significant difference between sham and 6-hydroxydopamine lesioned rats, $P < 0.05$.
[b] Significant difference between saline and MK-801 or CPP treatment, $P < 0.05$.

dopamine-lesioned animals as compared to sham-lesioned controls ($F(1,23) = 5.25$, $P < 0.05$).

3.3. [^{3}H]SCH23390 binding

The radioligand binding of [^{3}H]SCH23390 to dopamine D_1-like receptors was similar in sham- and 6-hydroxydopamine lesioned rats within the rostral ($F(1,23) = 2.46$) and caudal ($F(1,23) = 0.04$) neostriatum. In these areas, no differences in binding were observed between MK-801, CPP or saline-treated animals ($F(2,23) = 0.19$ and $F(2,23) = 0.63$ for rostral and caudal neostriatum, respectively). In contrast to the neostriatum, a lower density of [^{3}H]SCH23390 binding ($F(1,23) = 9.81$, $P < 0.01$) was observed in the substantia nigra of MK-801- or CPP-treated rats as compared to saline controls in the sham-lesioned rats ($F(1,23) = 5.73$, $P < 0.05$, Table 2).

3.4. [^{3}H]YM-09151-2 binding

[^{3}H]YM-09151-2 bound with high affinity to dopamine D_2-like receptors in the neostriatum and substantia nigra. In saline-treated animals there was a significant increase in the binding [^{3}H]YM-09151-2 to dopamine D_2-like receptors in the rostral ($F(1,22) = 4.27$, $P < 0.05$) but not the

Table 2
Effects of MK-801 and CPP on [^{3}H]SCH23390 and [^{3}H]YM-09151-2 binding in sham- and 6-hydroxydopamine lesioned rats
Results are expressed as fmol/mg protein (mean ± S.E.M.).
Statistical difference between the six parallel groups of rats was assessed using analysis of variance (ANOVA) for a 2 × 3 factorial design.

		Saline	MK-801 (0.1 mg/kg)	CPP (1 mg/kg)
[^{3}H]SCH23390				
Rostral neostriatum	sham	326 ± 27.1	373 ± 46.8	332 ± 66.3
	6-hydroxydopamine	280 ± 35.4	282 ± 17.1	284 ± 63.3
Caudal neostriatum	sham	262 ± 5.75	243 ± 25.7	223 ± 44.1
	6-hydroxydopamine	270 ± 34.0	230 ± 17.9	243 ± 44.3
Substantia nigra	sham	236 ± 18.3	158 ± 33.5[a]	174 ± 29.4[a]
	6-hydroxydopamine	208 ± 7.69	200 ± 13.1	162 ± 28.3
[^{3}H]YM-09151-2				
Rostral neostriatum	sham	344 ± 59.4	606 ± 62.6[a]	784 ± 98.4[a]
	6-hydroxydopamine	681 ± 144.4[b]	684 ± 171.4	785 ± 104.3
Caudal neostriatum	sham	366 ± 64.1	444 ± 67.4	601 ± 118.2
	6-hydroxydopamine	559 ± 121.4	590 ± 107.8	696 ± 88.3
Substantia nigra	sham	73 ± 4.4	58 ± 8.0	64 ± 5.2
	6-hydroxydopamine	34 ± 5.3[b]	36 ± 2.4[b]	34 ± 3.2[b]

[a] Significant difference between saline and MK-801 or CPP treatment, $P < 0.05$.
[b] Significant difference between sham and 6-hydroxydopamine lesioned rats, $P < 0.05$.

caudal neostriatum ($F(1,22) = 1.8$) of 6-hydroxydopamine denervated rats as compared to sham-lesioned controls (Table 2). Furthermore, in the rostral neostriatum, both MK-801 and CPP increased ($F(1,22) = 5.95$, $P < 0.05$) [^{3}H]YM-09151-2 binding over saline treatment in the sham-lesioned group. Within the substantia nigra [^{3}H]YM-09151-2 binding was significantly reduced in 6-hydroxydopamine lesioned animals ($F(1,23) = 49.97$, $P < 0.01$) as compared to sham-lesioned controls. However, binding was not significantly different between MK-801, CPP and saline treatment groups ($F(1,23) = 1.06$).

3.5. Relationship between dopamine receptors and NMDA or AMPA receptors

3.5.1. Dopamine D_1-like receptors

The relationship between the binding of [^{3}H]SCH23390 and that of [^{3}H]MK-801 or [^{3}H]AMPA was assessed using a linear regression model. In the rostral neostriatum, a significant negative correlation (Pearson's $r = -0.52$; $F(1,22) = 8.67$, $P < 0.01$) was observed between [^{3}H]SCH23390 and [^{3}H]MK-801 binding sites without heterogeneity in the regression slopes among the six treatment groups ($F(5,17) = 0.75$) (Fig. 1). In contrast a positive relationship ($r = 0.67$; $F(1,22) = 12.43$, $P < 0.01$) was found between [^{3}H]SCH23390 and [^{3}H]AMPA binding sites that was without heterogeneity among the six treatment groups ($F(5,17) = 0.79$) (Fig. 1).

In the caudal neostriatum, no significant correlation ($r = -0.25$; $F(1,22) = 1.56$) was found between dopamine D_1-like and NMDA receptors. A significant positive relationship ($r = 0.61$; $F(1,22) = 10.93$, $P < 0.01$) however, was observed between [^{3}H]SCH23390 and [^{3}H]AMPA binding sites. This relationship did not show heterogeneity between the six treatment groups ($F(5,17) = 0.39$) (Fig. 1).

Similarly, in the substantia nigra, the relationship between [^{3}H]SCH23390 binding and [^{3}H]MK-801 binding was not significant ($r = 0.36$; $F(1,22) = 2.83$) but its correlation with [^{3}H]AMPA binding was positive and significant ($r = 0.53$; $F(1,22) = 10.93$, $P < 0.01$). No differences in the regression slopes among the treatment groups ($F(5,17) = 0.09$) was observed (Fig. 1).

3.5.2. Dopamine D_2-like receptors

Using the same paradigm, the relationship between [^{3}H]YM-09151-2 binding to dopamine D_2-like receptors and [^{3}H]MK-801 binding to NMDA or [^{3}H]AMPA binding

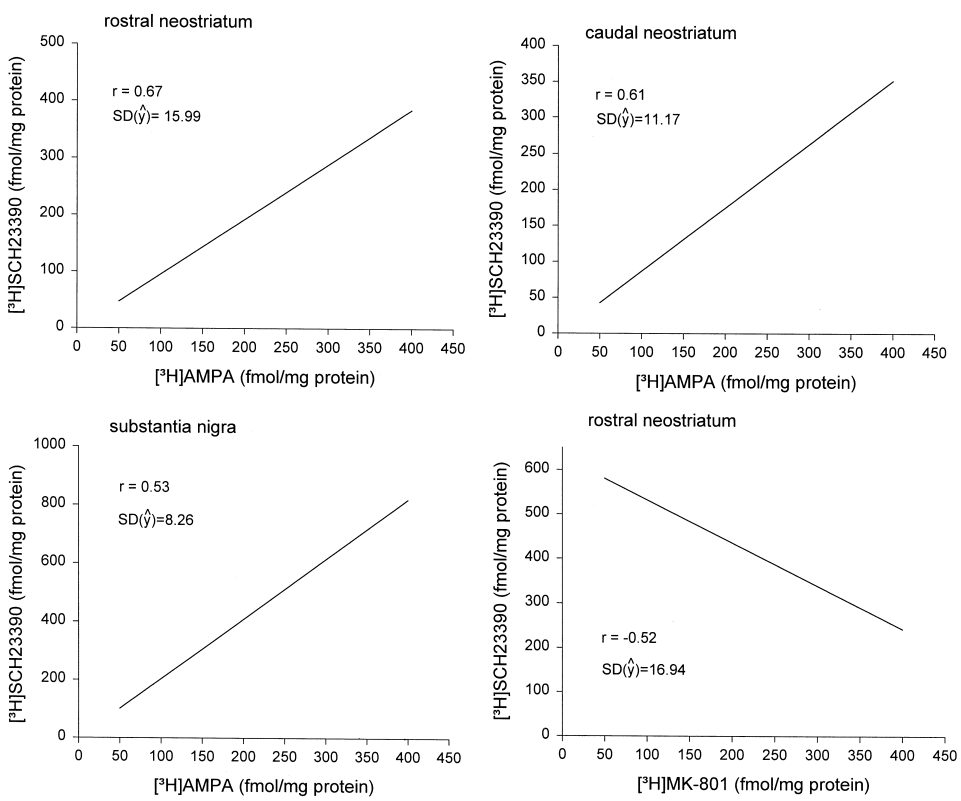

Fig. 1. Within group estimated linear relationship and mean standard deviation of the estimate (SD($\hat{y}$)) between [^{3}H]MK-801 or [^{3}H]AMPA binding and [^{3}H]SCH23390 binding to dopamine D1-like receptors in the rostral neostriatum, caudal neostriatum and substantia nigra. The slope (b) of the regression was calculated according to the method of least squares and was negative for the estimated [^{3}H]SCH23390 binding in the rostral neostriatum using [^{3}H]MK-801 as the predictor ($b = -0.967$, SD(b) = 0.3282). In contrast a positive relationship was found with [^{3}H]AMPA binding as the predictor in the rostral neostriatum ($b = 0.365$, SD(b) = 0.1035), the caudal neostriatum ($b = 0.383$, SD(b) = 0.1160), and the substantia nigra ($b = 0.915$, SD(b) = 0.2732).

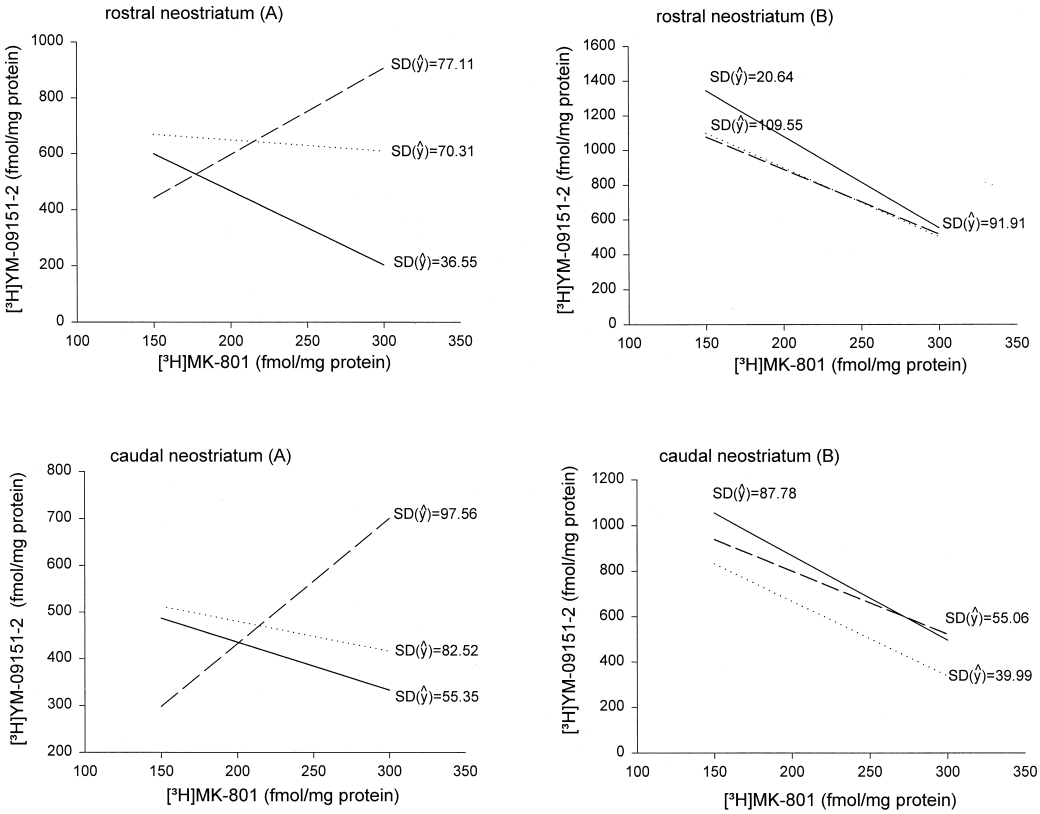

Fig. 2. Between group estimated linear relationship and mean standard deviation of the estimate (SD($\hat{y}$)) between the binding of [^{3}H]MK-801 and that of [^{3}H]YM-09151-2 in the rostral neostriatum and caudal neostriatum of sham-lesioned (A) and 6-hydroxydopamine-lesioned (B) rats. In sham-treated animals, the slope of the regression was positive in both A and B following CPP (---) treatment ($b = 3.101$, SD(b) = 1.6527 and $b = 2.677$, SD(b) = 1.5793, for rostral and caudal neostriatum, respectively). In contrast, a negative relationship was observed in (—) saline-treated animals ($b = -2.640$, SD(b) = 1.2674 and $b = -1.091$, SD(b) = 0.9755, for rostral and caudal neostriatum, respectively). The slopes of the regression were not significant following MK-801 treatment ($\cdots$) ($b = -0.400$, SD(b) = 0.9695 and $b = -0.567$, SD(b) = 0.9755, for rostral and caudal neostriatum, respectively). The slopes were negative in both the rostral and caudal neostriatum of 6-hydroxydopamine-lesioned animals following saline ($b = -5.277$, SD(b) = 01.6527 and $b = -3.990$, SD(b) = 2.0276), MK-801 ($b = -3.990$, SD(b) = 2.0276 and $b = -3.291$, SD(b) = 0.7395) or CPP treatment ($b = -3.741$, SD(b) = 2.5450 and $b = -2.779$, SD(b) = 2.7196).

to AMPA receptors was estimated in the rostral neostriatum, caudal neostriatum and substantia nigra. In the rostral neostriatum, a significant heterogeneity in the regression slopes among the six treatment groups ($F(5,16) = 4.37$, $P < 0.02$) was found in the relationship between dopamine D_2-like and NMDA receptors (Fig 2). The variation explained by the regression in each group was therefore used to calculate the overall correlation for the six groups (multiple regression) and was negative ($r = -0.91$; $F(6,16) = 5.41$, $P < 0.01$) in the rostral neostriatum. No significant relationship was observed between dopamine D_2-like and AMPA receptors ($r = 0.36$; $F(1,21) = 3.93$) in this region.

Similarly in the caudal neostriatum, differences in the regression slopes between [^{3}H]YM-09151-2 and [^{3}H]MK-801 sites among the six treatment groups were observed ($F(5,16) = 3.54$, $P < 0.05$) (Fig 2). The overall estimate of this relationship for the multiple regression indicated a negative correlation ($r = -0.89$; $F(6,16) = 3.61$, $P < 0.02$). No significant relationship was found between [^{3}H]YM-09151-2 and [^{3}H]AMPA binding in this area ($F(1,22) = 3.68$).

In the substantia nigra, no significant relationship was observed between [^{3}H]YM-09151-2 and either [^{3}H]MK-801 (Pearson's $r = 0.19$, $F(1,21) = 2.47$) or [^{3}H]AMPA (Pearson's $r = 0.01$, $F(1,21) = 0.007$) binding.

4. Discussion

The present study demonstrates a strong relationship between glutamate receptors (NMDA and AMPA subtypes) and dopamine D_1-like and D_2-like receptors in the striatum and substantia nigra following treatment with the non-competitive NMDA receptor antagonists MK-801 or the competitive antagonist CPP. The relationship between these sites was not similar and differed between intact and 6-hydroxydopamine-lesioned rats.

It has been reported that competitive and non-competitive NMDA receptor antagonists differentially alter dopamine neurotransmission (Keefe and Gerfen, 1996; Leslie et al., 1998). While others have suggested that these agents possess similar biochemical and behavioural profiles (Löscher et al., 1993; Dall'Olio et al., 1996). In the present study, MK-801 and CPP had slightly different profiles of action, although these differences may be quantitative in nature. In fact, competitive and non-competitive NMDA receptor antagonists are known to have a similar effect on dopamine systems when behaviorally equipotent doses are used (Löscher et al., 1993; Wedzony et al., 1996).

[^{3}H]MK-801 binding to NMDA receptors was increased in the substantia nigra of rats that had received a neonatal dopamine lesion, while MK-801 and CPP treatment reversed these effects. The alterations in [^{3}H]MK-801 binding found in the present study could be due to changes in either the affinity or the density of receptors; although previous studies using similar binding conditions suggest that the density of these sites are altered (Quirion et al., 1982; Mannallack et al., 1989). It is unclear, however, how [^{3}H]MK-801 binding may be increased in response to neonatal 6-hydroxydopamine treatment. It is possible that either the expression of NMDA receptors is under inhibitory control of dopamine acting through dopamine D_1-like and/or D_2-like receptors in the substantia nigra or, alternatively, that dopamine neuronal loss elicits compensatory change in glutamatergic activity and NMDA receptors in the substantia nigra.

If dopamine acting via dopamine D_1-like and/or D_2-like receptors inhibits the expression and production of NMDA receptors in the substantia nigra, it may be expected that treatment with dopamine D_1-like and/or D_2-like receptor antagonists would result in a similar increase in [^{3}H]MK-801 binding. Chronic blockade of dopamine D_1-like and/or D_2-like receptors does not, however, alter [^{3}H]MK-801 binding to NMDA receptor in the substantia nigra of the rat (Tarazi et al., 1996).

The degeneration of nigral dopamine neurons results in a glutamatergic overactivity in the neostriatum and globus pallidus as well as a disinhibition of the subthalamic nucleus (Murer et al., 1997). Since output neurons from the subthalamic nucleus to the substantia nigra and globus pallidus are known to use glutamate as their neurotransmitter, increased activity of subthalamic output neurons would increase glutamatergic activity in these regions (Rosales et al., 1997) and possibly increase [^{3}H]MK-801 binding. Furthermore, this increased activity is reversed by treatment with glutamate antagonists (Vila et al., 1999). Similarly, the blockade of NMDA receptors with MK-801 or CPP reversed the increased binding seen in the denervated rats. These findings suggest that neonatal dopamine denervation may alter nigral NMDA receptors indirectly via alterations in glutamatergic activity particularly in glutamate neurons arising from the subthalamic nucleus. This is supported by the findings that lesions of the subthalamic nucleus reduce [^{3}H]MK-801 binding in the substantia nigra pars reticulata (Blandini et al., 1995).

In contrast to NMDA receptors, MK-801 and CPP treatment increased nigral [^{3}H]AMPA binding as compared to saline in 6-hydroxydopamine lesioned rats. Whether these changes are related to a direct interaction between NMDA receptor antagonists and the AMPA receptor or indirectly through alterations in dopamine or glutamate neurotransmission has to be further explored.

In the adult unilaterally lesioned rat, both [^{3}H]AMPA binding (Porter et al., 1994; Wüllner et al., 1994) and cells immunopositive for the AMPA receptor subunit GluR1 were found to be decreased in the ipsilateral substantia nigra (He et al., 1998) signifying that at least some [^{3}H]AMPA sites are localized on dopamine neurons. We failed to find a significant difference in [^{3}H]AMPA binding in these regions of 6-hydroxydopamine-lesioned animals as compared to sham-lesioned controls. Since the survival period of our rats was 3–5 months after the lesion, it is possible that there was an initial loss of binding that was reversed by this time.

In accordance with the findings that NMDA receptor blockade did not alter either neostriatal dopamine D_1-like receptor binding (Dall'Olio et al., 1994) or D_1 mRNA levels (Qin et al., 1994), MK-801 and CPP failed to modify binding to dopamine D_1-like receptor and NMDA receptors in either sham- or 6-hydroxydopamine-lesioned rats. The negative relationship demonstrated between dopamine D_1-like receptor and NMDA receptors supports, however, the hypothesis of a functional interaction between these two sites. This interaction is in agreement with the effects of NMDA receptor antagonists on the behavioural effects (Morelli et al., 1992; Dall'Olio et al., 1996) and Fos expression (Johnson et al., 1992; Nakazato et al., 1998) of dopamine D_1-like receptor agonists. Dopamine, acting through dopamine D_1-like receptors, appears to regulate NMDA receptor sensitivity through modulation of the phosphorylation and dephosphorylation of NMDA receptor subunits (Snyder et al., 1998; Oh et al., 1999). Such a regulation may contribute to the interaction observed in the present study.

In addition, a positive relationship was observed between dopamine D_1-like receptors and AMPA receptors. This relationship is in agreement with the findings that the AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-quinoxaline (NBQX) potentiates contralateral rotations induced by the dopamine D_1-like receptor agonists or mixed dopamine D_1-like and/D_2-like receptor agonists in unilateral 6-hydroxydopamine lesioned rats (Loschmann et al., 1997). Furthermore, NBQX was found to significantly augment SKF 38393-induced c-fos expression in these animals (Fenu et al., 1995), further supporting an interaction between these two sites.

In the present study, MK-801 decreased [^{3}H]SCH23390 binding in the substantia nigra of sham- but not 6-hydroxy-

dopamine-lesioned rats. A similar decreased binding has been found 4 and 24 h after acute MK-801 (0.4 mg/kg) and CGP40116 (5 mg/kg) administration in adult rats (Wedzony et al., 1996). Since these agents failed to alter [^{3}H]SCH23390 binding in dopamine denervated animals, it can be postulated that MK-801 acts indirectly via dopamine neurons. In accordance with this hypothesis, it has been reported that the systemic administration of MK-801 enhanced the firing rate of dopamine neurons (Overton and Clark, 1992) and increased dopamine release (Rosales et al., 1994), suggesting that nigral dopamine D_1-like receptors may be desensitized in response to increased dopamine neuronal activity.

In accordance with some previous studies, NMDA receptor antagonists significantly increased [^{3}H]YM-09151-2 binding to dopamine D_2-like receptors in the neostriatum of sham- but not 6-hydroxydopamine-lesioned rats (Micheletti et al., 1992; Dall'Olio et al., 1994). In contrast, others have reported either no change (Wedzony et al., 1993) or decreased binding (Gandolfi and Dall'Olio, 1993; Qin et al., 1994). An increased behavioural responsiveness to the dopamine D_2-like receptor agonist LY17155 has also been reported following chronic MK-801 treatment (Mele et al., 1995). Since dopamine D_2 mRNA levels were increased in the dorsal and ventral neostriatum (Healy and Meador-Woodruff, 1996) following chronic MK-801 treatment, it has been suggested that NMDA receptor blockade increases dopamine D_2 receptor expression and dopamine D_2-like receptor number. Furthermore, MK-801-induced expression of dopamine D_2L receptors in human neuroblastoma SH-SY5Y cells was inhibited by phosphatase (Nair et al., 1996), leading to the suggestion that this mechanism involves the phosphorylation and/or dephosphorylation states of regulatory proteins.

In conclusion, we find a strong relationship between glutamate and dopamine receptors following sub-chronic treatment with both competitive and non-competitive NMDA receptor antagonists. While dopamine D_1-like receptors and NMDA receptors were negatively related in the striatum of both sham- and 6-hydroxydopamine-lesioned animals, there was a differential relationship between NMDA and dopamine D_2-like receptors in these two experimental groups. These findings suggest that the interaction between NMDA receptor and dopamine D_2-like, but not dopamine D_1-like, receptors is dependent upon an intact nigrostriatal dopamine system and further highlights the complexities of dopamine/glutamate interactions.

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