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Dopamine D_1 receptor-mediated control of striatal acetylcholine release by endogenous dopamine

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Abstract

The role of dopamine D_1 and D_2 receptors in the control of acetylcholine release in the dorsal striatum by endogenous dopamine was investigated by monitoring with microdialysis the effect of the separate or combined administration of the dopamine D_1 receptor antagonist, SCH 39166 {(−)-*trans*-6,7,7*a*,8,9,13*b*-exahydro-3-chloro-2-hydroxy-*N*-methyl-5*H*-benzo-[*d*]-naphto-[2,1*b*]-azepine hydrochloride} (50 μg/kg subcutaneous (s.c.)), of the dopamine D_2/D_3 receptor agonist, quinpirole (*trans*-(−)-4*aR*,4*a*,5,6,7,8,8*a*,9-octahydro-5-propyl-1*H*-pyrazolo-(3,4-*g*)-quinoline hydrochloride) (5 and 10 μg/kg s.c.), and of the D_3 receptor selective agonist, PD 128,907 [*S*(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol hydrochloride] (50 μg/kg s.c.), on in vivo dopamine and acetylcholine release. Microdialysis was performed with a Ringer containing low concentrations (0.01 μM) of the acetylcholinesterase inhibitor, neostigmine. Quinpirole (10 μg/kg s.c.) decreased striatal dopamine and acetylcholine release. Administration of PD 128,907 (50 μg/kg) decreased dopamine but failed to affect acetylcholine release. SCH 39166 (50 μg/kg s.c.) stimulated dopamine release and reduced acetylcholine release. Pretreatment with quinpirole reduced (5 μg/kg s.c.) or completely prevented (10 μg/kg s.c.) the stimulation of dopamine release elicited by SCH 39166 (50 μg/kg s.c.); on the other hand, pretreatment with quinpirole (5 and 10 μg/kg) potentiated the reduction of striatal acetylcholine release induced by SCH 39166 (50 μg/kg s.c.). Similarly, pretreatment with PD 128,907 (50 μg/kg) which prevented the increase of dopamine release induced by SCH 39166 (50 μg/kg), potentiated the reduction of striatal acetylcholine transmission elicited by SCH 39166. Thus, pretreatment with low doses of quinpirole or PD 128,907 influences in opposite manner the effect of SCH 39166 on striatal dopamine and acetylcholine release, counteracting the increase of dopamine release and potentiating the decrease in acetylcholine release. These results provide further evidence for the existence of a tonic stimulatory input of endogenous dopamine on striatal acetylcholine transmission mediated by dopamine D_1 receptors.

Keywords: Acetylcholine; Dopamine; Microdialysis; Quinpirole; PD 128,907; SCH 39166; Striatum

1. Introduction

Dopamine receptors modulate striatal acetylcholine function in an opposite manner depending on the dopamine receptor subtype that is taken into consideration. Thus, while dopamine D_2 receptor stimulation inhibits (Bertorelli and Consolo, 1990; Damsma et al., 1990a), D_1 receptor stimulation facilitates acetylcholine release (Consolo et al., 1987; Damsma et al., 1990b). Conversely, D_2 receptor blockade stimulates acetylcholine release (Bertorelli and Consolo, 1990; Damsma et al., 1991; Imperato et al., 1993), while D_1 receptor blockade reduces it (Consolo et al., 1987; Bertorelli and Consolo, 1990; Damsma et al., 1991; Imperato et al., 1994; Acquas and Fibiger, 1998). On this basis, it has been suggested that endogenous dopamine controls acetylcholine neurotransmission in a reciprocally symmetric manner through stimulatory D_1 and inhibitory D_2 receptors (Di Chiara et al., 1994). However, the interpretation of the role of dopamine receptor subtypes in the control of acetylcholine release by dopamine is complicated by the fact that both D_1 and D_2 receptor agonists and antagonists evoke major changes in endogenous dopamine transmission which might themselves mediate indirectly the changes in acetylcholine transmission. For example, feedback-induced release of dopamine onto stimulatory D_1 receptors might account for the stimulation of acetylcholine release elicited by D_2 receptor blockade (Damsma et al., 1991; Imperato et al., 1993; Russi et al., 1993). Similarly, reduction of dopamine release onto inhibitory D_2 receptors has been suggested to account for

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the increase of acetylcholine release following D_1 receptor stimulation (Acquas et al., 1997).

In a recent study by DeBoer et al. (1996) it was suggested that low doses of quinpirole (*trans*-(−)-4*aR*, 4*a*,5,6,7,8,8*a*,9-octahydro-5-propyl-1*H*-pyrazolo-(3,4-*g*)-quinoline hydrochloride), while reducing dopamine release, stimulate acetylcholine release. According to these authors these observations challenge the hypothesis of a double reciprocal D_1/D_2 receptor control of acetylcholine release by endogenous dopamine as they would indicate that such control is primarily if not exclusively mediated by D_2 receptors. It was also argued by these authors that the evidence obtained on the role of D_1 receptors in the control of acetylcholine release is an artifact of the use of high concentrations of the acetylcholine esterase inhibitor which would magnify the role of D_1 receptors on the control of acetylcholine release (DeBoer and Abercrombie, 1996).

In order to reevaluate the role of D_1 receptors in the control of acetylcholine function by endogenous dopamine we have reexamined the effects of D_1 receptor blockade on in vivo acetylcholine release under the conditions of DeBoer et al. (1996). To this end we studied the effect of low doses of the dopamine D_2/D_3 receptor agonist, quinpirole, and of the selective agonist of D_3 receptors, PD 128,907 [*S*(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol hydrochloride] (Sautel et al., 1995), on dopamine and acetylcholine release in the striatum either alone or in combination with the D_1 receptor antagonist, SCH 39166 {(−)-*trans*-6,7,7*a*,8,9,13*b*-exahydro-3-chloro-2-hydroxy-*N*-methyl-5*H*-benzo-[*d*]-naphto-[2,1*b*]-azepine hydrochloride} (McQuade et al., 1991). Administration of low doses of quinpirole or PD 128,907 would inhibit feedback stimulation of dopamine release evoked by D_1 receptor blockade. Thus, in order to investigate the role of the stimulation of dopamine release in the reduction of acetylcholine release by D_1 receptor blockade, we studied the effect of SCH 39166 on striatal acetylcholine release after administration of doses of quinpirole or PD 128,907 which would reduce the increase of striatal dopamine release elicited by SCH 39166. We reasoned that if stimulation of D_2/D_3 receptors by quinpirole or of D_3 receptors by PD 128,907 attenuates the increase of striatal dopamine release evoked by SCH 39166, but further decreases striatal acetylcholine transmission, one might conclude that endogenous dopamine controls acetylcholine release by acting on D_1 receptors.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (275–300 g) were housed in groups of two to three per cage for at least 3 days before use and were maintained on a 12:00/12:00 h light/dark cycle (lights on at 07:30 h) with food and water available ad libitum. After surgery the rats were housed individually in Plexiglas hemispherical bowls (50 cm in diameter) which also served as the experimental environment, where they recovered for 24–30 h prior to the microdialysis experiments. Experiments were carried out between 09:00 and 16:00 h. Animal care and experimental protocol were conducted in accordance with guidelines of national (D.L. 116, Suppl. G.U. 40, 18/2/1992 and circolare no. 8 G.U. 14/7/1994) and international (EEC Council Directive 86/609, OJ L 358/1, December 12, 1987) policies.

2.2. Surgery and microdialysis

Rats were anaesthetized with ketamine HCl (Ketalar, Parke Davis Spa, Italy) (100 mg/kg i.p.) and stereotaxically implanted with a vertical concentric microdialysis probe in the left striatum. The coordinates, measured from bregma, were AP = +0.7 mm, DV = −6.5 mm, ML = −3.0, according to Paxinos and Watson (1986). The membrane used was a polyacrylonitrile/sodium methallyl sulphonate copolymer (i.d. 0.22 mm, o.d. 0.31 mm; cutoff 40,000 Da, AN 69 filtral 8, Hospal Industrie, France).

The membrane was covered with epoxy glue along its whole length except for 3 mm corresponding to the area of dialysis. The day of the experiment rats were connected to a microperfusion pump (CMA 100, Carnegie Medicine, Sweden) by polyethylene tubing (PE-50, Portex, Kent, UK) (inlet) connected to a 2.5-ml glass syringe (Hamilton, USA) containing the perfusion solution. The perfusion flow was set at 1 μl/min. The first three dialysate samples were discarded. Samples were collected every 10 min (10 μl/sample) into a 20-μl sample loop (outlet) and subsequently injected in the high-pressure liquid chromatograph (HPLC) injector valve operated manually. The perfusion solution contained 147 mM NaCl, 4 mM KCl, 2.2 mM $CaCl_2$, in twice distilled water. To achieve consistently detectable amounts of acetylcholine in the dialysate, the reversible acetylcholine esterase inhibitor neostigmine bromide (0.01 μM) (Sigma, St. Louis, MO, USA) was added to the perfusion solution. acetylcholine was assayed by HPLC–electrochemical detection (ECD) in conjunction with an enzyme reactor (Damsma et al., 1987). In the experiments in which dopamine was measured, the composition of the perfusion solution was identical and in these experiments the acetylcholine esterase inhibitor, NEO 0.01 μM, was included in the perfusion solution.

Acetylcholine and choline were separated on a reverse phase Chromspher C_{18} 5 μm (Merck, Darmstad, FRG) column (75 × 2.1 mm) pretreated with lauryl sulphate. The mobile phase passed directly through the enzyme reactor (10 × 2.1 mm) containing acetylcholine esterase (ED 3.1.1.7; type VI-S — Sigma) and choline oxidase (EC 1.1.3.17; Sigma) covalently bound to glutaraldehyde-activated Lichrosorb 10-NH_2 (Merck); acetylcholine and choline were quantitatively converted into hydrogen peroxide which was detected electrochemically at a platinum

working electrode set at 500 mV vs. an Ag/AgCl reference electrode (LC-4B, BAS, Lafayette, IN, USA). The mobile phase was an aqueous potassium phosphate buffer (1.9 mM K_2HPO_4, 0.2 mM tetramethyl ammonium hydroxide, pH = 8) delivered at a constant flow of 0.4 ml/min by an HPLC pump (Bischoff HPLC Pump Mod. 2200, Bischoff, FRG). The chromatograms were recorded on a chart recorder. The detection limit of the assay was 50 fmol/sample. Injections of an acetylcholine standard (20 μl, 0.1 μM) were made every 60 to 90 min in order to monitor changes in electrode sensitivity and sample concentrations were corrected accordingly. Dopamine was assayed by HPLC–ECD. The mobile phase was delivered by an HPLC pump (Pharmacia LKB, HPLC pump 2150) at the constant flow of 1.20 ml/min and consisted of NaH_2PO_4 (50 mM), Na_2HPO_4 (5 mM), Na_2EDTA (0.1 mM), octanesulphonic acid (0.5 mM) (Eastman Kodak, NY), and methanol 15% v/v, pH = 5.5. Dopamine was separated by reverse phase liquid chromatography (150 × 4.6 mm, Supelco 5 μm $LC_{18}DB$). The electrochemical detector (Coulochem II, ESA, Bedford, MA, USA) was set as follows: oxidation electrode +75 mV, reduction electrode −125 mV. The sensitivity of the assay for dopamine was 2 fmol/sample. The chromatograms were recorded on a chart recorder.

2.3. Drugs

SCH 39166 (Schering-Plough, Milan, Italy) was dissolved in saline solution (NaCl 0.9%) and injected subcutaneously (s.c.) in a volume of 0.1 ml/100 g at a dose of 50 μg/kg. Quinpirole (RBI, Natick, MA, USA), was dissolved in saline solution and injected s.c. in a volume of 0.1 ml/100 g at the doses of 5 and 10 μg/kg. PD 128,907 (RBI) was dissolved in saline solution and injected s.c. in a volume of 0.1 ml/100 g at the dose of 50 μg/kg.

2.4. Statistics

Values are expressed as changes percent with respect to baseline (100%). Baseline was set as the average of the last six pretreatment samples (not differing more than 15%). One-, two- and three-way analyses of variance (ANOVAs), with time as the repeated measure, were used to analyze the treatment effects. Tukey's post-hoc analyses were applied for multiple comparisons, with the statistical significance set at $P < 0.05$.

3. Results

3.1. Basal striatal dopamine and acetylcholine output and effects of saline

Basal acetylcholine and dopamine (femtomoles per sample), were calculated and defined as the average ± S.E.M. of the six pretreatment samples for each experimental group. The overall mean ± S.E.M. baseline of dopamine was 106.5 ± 5.8 fmol/sample ($n = 40$). The overall mean ± S.E.M. baseline of acetylcholine in the dialysate was 351.9 ± 19 fmol/sample ($n = 45$). The administration of saline failed to affect dopamine and acetylcholine output ($F(6,30) = 1.2$, NS and $F(6,30) = 1.7$, NS, respectively).

3.2. Effect of SCH 39166, quinpirole and PD 128,907 on dopamine and acetylcholine output

Figs. 1–3 show the effects of quinpirole (5 or 10 μg/kg), PD 128,907 (50 μg/kg) and/or SCH 39166 (50 μg/kg) on dopamine and acetylcholine output. The D_1 receptor antagonist, SCH 39166 (50 μg/kg), increased dopamine ($F(6,30) = 9.57$, $P < 0.0001$; $P < 0.05$ post-

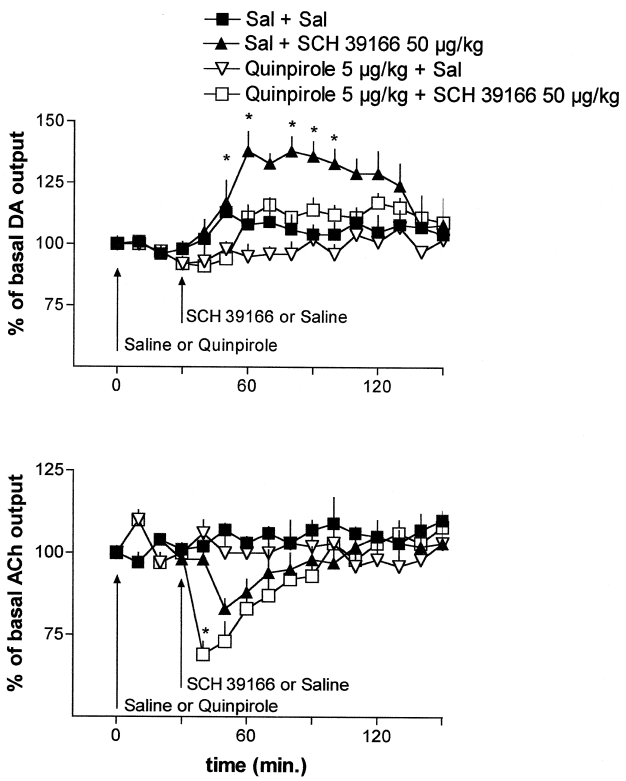

Fig. 1. *Top*: Effect of the administration of saline (1 ml/kg s.c.), followed by a second administration of saline (1 ml/kg s.c.) 30 min thereafter ($n = 6$) or SCH 39166 (50 μg/kg s.c.) ($n = 5$), on striatal dopamine release. Effect of quinpirole (5 μg/kg s.c.), followed by the administration of saline (1 ml/kg s.c.) 30 min later ($n = 6$) or SCH 39166 (50 μg/kg s.c.) ($n = 5$), on basal striatal dopamine release. *Bottom*: Effect of the administration of saline (1 ml/kg s.c.), followed by a second administration of saline (1 ml/kg) 30 min thereafter ($n = 6$) or by SCH 39166 (50 μg/kg s.c.) ($n = 6$), on striatal acetylcholine release. Effect of quinpirole (5 μg/kg s.c.), followed by the administration of saline (1 ml/kg s.c.) 30 min later ($n = 5$) or by SCH 39166 (50 μg/kg s.c.) ($n = 5$), on basal striatal acetylcholine release. Values are expressed as percentage baseline. Vertical bars represent S.E.M. Arrows indicate the last pretreatment sample. $^*P < 0.05$ with respect to the corresponding time point of the quinpirole (5 μg/kg s.c.) + SCH 39166 (50 μg/kg) group.

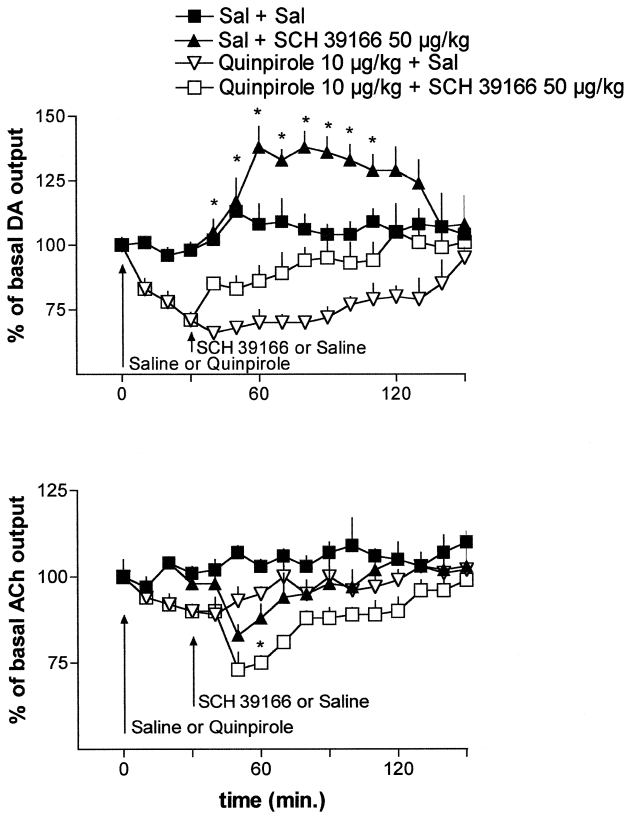

Fig. 2. *Top*: Effects of saline (1 m/kg s.c.) followed by a second administration of saline (1 ml/kg s.c.) ($n = 6$) or SCH 39166 (50 μg/kg s.c.) ($n = 5$), on striatal dopamine release. Effect of the administration of quinpirole (10 μg/kg s.c.), followed by the administration of saline ($n = 4$) or SCH 39166 (50 μg/kg s.c.) 30 min thereafter ($n = 5$), on striatal dopamine release. The effects of saline + saline and saline + SCH 39166 (50 μg/kg s.c.), from Fig. 1 are reported here for comparison. *Bottom*: Effects of saline (1 ml/kg s.c.) followed, 30 min later, by a second administration of saline (1 ml/kg) ($n = 6$) or SCH 39166 (50 μg/kg s.c.) ($n = 6$) on striatal acetylcholine release. Effect of the administration of quinpirole (10 μg/kg s.c.), followed by the administration of saline ($n = 6$) or SCH 39166 (50 μg/kg s.c.) ($n = 6$) 30 min thereafter, on striatal acetylcholine release. The effects of groups saline + saline and saline + SCH 39166 (50 μg/kg s.c.), from Fig. 1 (bottom), are reported here for comparison. Values are expressed as percentage baseline. Vertical bars represent S.E.M. Arrows indicate the last pretreatment sample. $^{*}P < 0.05$ with respect to the corresponding time point of the quinpirole (5 μg/kg s.c.) + SCH 39166 (50 μg/kg s.c.) group.

hoc) (Fig. 1, top) but decreased acetylcholine output ($F(6,30) = 4.46$, $P < 0.002$; $P < 0.05$ post-hoc) (Fig. 1, bottom). Two-way ANOVA of the results obtained after saline + saline and saline + SCH 39166 on acetylcholine release revealed a significant effect of group ($F(1,10) = 11.86$, $P < 0.006$) and Tukey's post-hoc test showed that SCH 39166 decreased acetylcholine output as compared to saline + saline.

The D_2/D_3 receptor agonist quinpirole, administered at 5 μg/kg (Fig. 1, top) and 10 μg/kg (Fig. 2, top), reduced dopamine release ($F(6,30) = 4.7$, $P < 0.04$ and $F(6,18) = 16.82$, $P < 0.0001$; $P < 0.05$ post-hoc); quinpirole reduced acetylcholine release at 10 μg/kg (Fig. 2) ($F(3,15) = 3.72$, $P < 0.03$; $P < 0.05$ post-hoc) but not at 5 μg/kg (Fig. 1) ($F(12,48) = 1.01$, NS). Two-way ANOVA of the results obtained after saline + saline, quinpirole 5 μg/kg + saline and quinpirole 10 μg/kg + saline revealed a significant effect of dose of quinpirole on dopamine output ($F(2,205) = 147$, $P < 0.0001$). Post-hoc analysis showed that quinpirole dose-dependently reduced dopamine output as compared to saline. Two-way ANOVA revealed a significant difference for the effects of saline + saline, quinpirole 5 μg/kg + saline and quinpirole 10 μg/kg + saline on striatal acetylcholine output ($F(2,218) = 15$, $P < 0.0001$); post-hoc analysis showed that quinpirole reduced acetylcholine output in a dose-dependent manner.

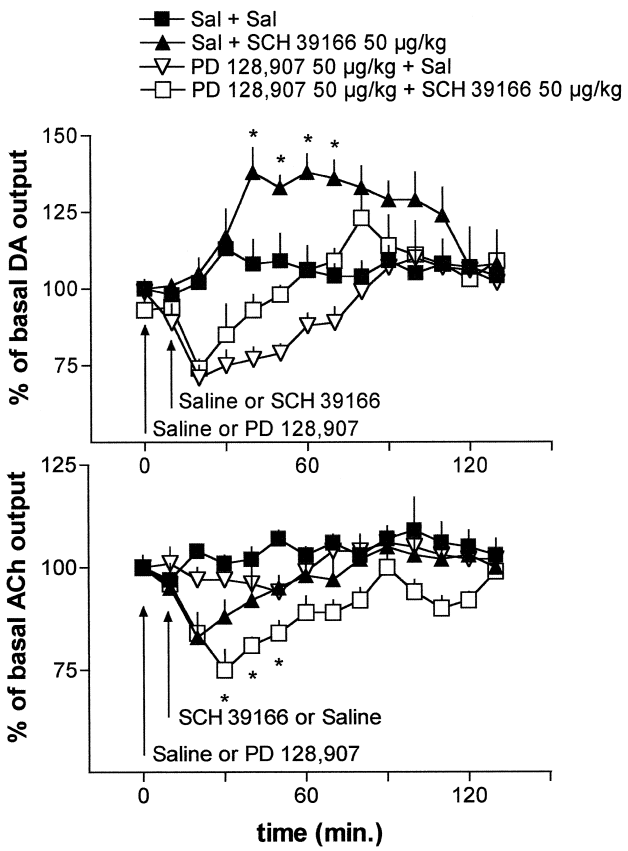

Fig. 3. *Top*: Effects of saline (1 m/kg s.c.) followed by a second administration of saline (1 ml/kg s.c.) ($n = 6$) or SCH 39166 (50 μg/kg s.c.) ($n = 5$), on striatal dopamine release. Effect of the administration of PD 128,907 (50 μg/kg s.c.), followed by the administration of saline ($n = 5$) or SCH 39166 (50 μg/kg s.c.) 10 min thereafter ($n = 4$), on striatal dopamine release. The effects of saline + saline and saline + SCH 39166 (50 μg/kg s.c.), from Fig. 1 are reported here for comparison. *Bottom*: Effects of saline (1 ml/kg s.c.) followed, 30 min later, by a second administration of saline (1 ml/kg) ($n = 6$) or SCH 39166 (50 μg/kg s.c.) ($n = 6$) on striatal acetylcholine release. Effect of the administration of PD 128,907 (50 μg/kg s.c.), followed by the administration of saline ($n = 5$) or SCH 39166 (50 μg/kg s.c.) ($n = 6$) 10 min thereafter, on striatal acetylcholine release. The effects of groups saline + saline and saline + SCH 39166 (50 μg/kg s.c.), from Fig. 1 (bottom), are reported here for comparison. Values are expressed as percentage baseline. Vertical bars represent S.E.M. Arrows indicate the last pretreatment sample. $^{*}P < 0.05$ with respect to the corresponding time point of the PD 128,907 (50 μg/kg s.c.) + SCH 39166 (50 μg/kg s.c.) group.

As shown in Fig. 3, PD 128,907 (50 μg/kg) decreased dopamine release ($F(5,20) = 13.2$, $P < 0.0001$), but failed to affect basal acetylcholine release ($F(6,24) = 1.1$, NS). Two-way ANOVA of the results obtained after saline + saline and PD 128,907 + saline revealed a significant effect of group ($F(1,9) = 21.1$, $P < 0.001$), and post-hoc analysis showed that PD 128,907 significantly decreased dopamine release ($P < 0.05$). In contrast, two-way ANOVA of the results of saline + saline and PD 128,907 + saline revealed that PD 128,907 failed to affect acetylcholine release ($F(1,9) = 2.78$, NS).

3.3. Interaction between quinpirole or PD 128,907 and SCH 39166 on dopamine and acetylcholine output

Fig. 1 shows the effects of saline + saline, quinpirole (5 μg/kg) + saline, saline + SCH 39166 (50 μg/kg) and quinpirole (5 μg/kg) + SCH 39166 (50 μg/kg) on dopamine and acetylcholine output. Three-way ANOVA of the changes in dopamine output revealed an effect of pretreatment ($F(1,18) = 11.47$, $P < 0.003$), treatment ($F(1,18) = 16.13$, $P < 0.0008$), time ($F(6,108) = 18.62$, $P < 0.00001$) and a time × treatment interaction ($F(6,108) = 13.53$, $P < 0.00001$). Post-hoc test demonstrated that quinpirole 5 μg/kg decreased dopamine output in comparison with saline + saline, and prevented the increase of dopamine output elicited by SCH 39166 ($P < 0.05$). Three-way ANOVA of the changes in acetylcholine output revealed an effect of pretreatment ($F(1,18) = 26.23$, $P < 0.00007$), treatment ($F(1,18) = 4.6$, $P < 0.045$), time ($F(5,90) = 4.7$, $P < 0.0007$) and a time × pretreatment × treatment interaction ($F(5,90) = 4.13$, $P < 0.001$). Tukey's post-hoc analysis revealed that pretreatment with quinpirole (5 μg/kg) before SCH 39166 (50 μg/kg) significantly potentiated the reduction of acetylcholine output elicited by the treatment saline + SCH 39166.

Fig. 2 shows the effects of saline + saline, quinpirole (10 μg/kg) + saline, quinpirole (10 μg/kg) + SCH 39166 and saline + SCH 39166 on striatal dopamine and acetylcholine output. Three-way ANOVA of the changes in dopamine output (Fig. 2, top) revealed a significant effect of pretreatment ($F(1,16) = 16.58$, $P < 0.0008$), treatment ($F(1,16) = 74.76$, $P < 0.00001$), time ($F(6,96) = 11.5$, $P < 0.00001$) and time × pretreatment × treatment interaction ($F(6,96) = 2.92$, $P < 0.011$). As shown in Fig. 2, quinpirole 10 μg/kg prevented the increase of dopamine output elicited by SCH 39166; post-hoc analysis demonstrated that the decrease of dopamine output after quinpirole (10 μg/kg) + saline was significantly lower than that after quinpirole (10 μg/kg) + SCH 39166. Three-way ANOVA of the changes in acetylcholine output revealed a significant effect of pretreatment ($F(1,20) = 28.76$, $P < 0.00003$), treatment ($F(1,20) = 19.07$, $P < 0.00002$) and time ($F(5,100) = 6.49$, $P < 0.00002$) and a significant pretreatment × treatment interaction ($F(1,10) = 14.2$, $P < 0.003$) but not a time × pretreatment × treatment interaction. Tukey's post-hoc test revealed that the differences between saline + SCH 39166 (50 μg/kg) and quinpirole (10 μg/kg) + SCH 39166 (50 μg/kg) were significant ($P < 0.05$).

Fig. 3 shows the effects of saline + saline, PD128,907 (50 μg/kg) + saline, PD 128,907 (50 μg/kg) + SCH 39166 and saline + SCH 39166 on striatal dopamine and acetylcholine output. Three-way ANOVA of the changes in dopamine output revealed a significant effect of pretreatment ($F(1,16) = 25.29$, $P < 0.0001$), treatment ($F(1,16) = 79.34$, $P < 0.00001$), time ($F(5,80) = 7.11$, $P < 0.00001$), time × pretreatment interaction ($F(5,80) = 3.12$, $P < 0.012$) and time × treatment interaction ($F(5,80) = 4.9$, $P < 0.0005$). As shown in Fig. 3, PD 128,907 prevented the increase of dopamine output elicited by SCH 39166; Tukey's post-hoc analysis demonstrated that the increase of dopamine output after saline + SCH 39166 was significantly higher than that after PD 128,907 + SCH 39166. Three-way ANOVA of the changes in acetylcholine output revealed a significant effect of pretreatment ($F(1,19) = 17.16$, $P < 0.005$), treatment ($F(1,19) = 68.54$, $P < 0.00001$), time ($F(3,57) = 7.8$, $P < 0.0001$) and a time × pretreatment × treatment interaction ($F(3,57) = 2.9$, $P < 0.04$). Saline + SCH 39166, but not PD 128,907 + saline reduced striatal acetylcholine output as compared to saline + saline ($P < 0.05$, Tukey's test). Furthermore, Tukey's test showed that pretreatment with PD 128,907 potentiated the decrease of acetylcholine output elicited by the D_1 receptor antagonist SCH 39166, the decrease of acetylcholine output after PD 128,907 + SCH 39166 being larger than that after PD 128,907 + saline and that after saline + SCH 39166.

4. Discussion

The present research was performed in order to further clarify how endogenous dopamine controls acetylcholine release in the striatum, and to specifically address the recent suggestion that endogenous dopamine preferentially, if not exclusively, controls acetylcholine release in the striatum via inhibitory dopamine D_2 receptors (DeBoer et al., 1996).

In the present study the dopamine D_1 receptor antagonist SCH 39166 at the dose of 50 μg/kg, reduced acetylcholine release and this effect was associated to an increase of dopamine release. According to the hypothesis that dopamine controls acetylcholine release primarily by inhibitory D_2 receptors (DeBoer et al., 1996), the reduction of acetylcholine release by SCH 39166 should be the result of the feedback stimulation of dopamine release by the D_1 receptor antagonist. However, if this were the case, prevention of SCH 39166-induced dopamine release stimulation should reverse the reduction of acetylcholine release by SCH 39166. Instead, prevention of SCH 39166-induced release of dopamine by doses of quinpirole (5 μg/kg) or

PD 128,907 (50 μg/kg) that by themselves do not affect acetylcholine release, actually potentiated the reduction of acetylcholine release induced by the D_1 receptor antagonist. From these observations therefore appears that endogenous dopamine released in response to D_1 receptor blockade tends to counteract the direct effect of D_1 receptor blockade on acetylcholine release, suggesting that endogenous dopamine, acting on D_1 receptors, controls in a facilitatory fashion acetylcholine release in the striatum independently from D_2 receptors. However, quinpirole has high affinity for D_2 and D_3 receptors and a role of D_2 receptors localized on acetylcholine neurons in the reduction of acetylcholine release cannot be excluded. It is known, that D_3 receptors are not expressed by cholinergic neurons (Yan et al., 1997). Therefore, a selective D_3 receptor agonist should be able to reduce dopamine release without affecting directly acetylcholine release. PD 128,907 is the most selective agonist of D_3 receptors available as indicated by a 210 ratio of K_i for D_2 and D_3 receptor binding of [^{125}I]iodosulpride and by a 53.7 ratio of EC_{50} for [^{3}H]thymidine incorporation in transfected cells (Sautel et al., 1995). PD 128,907, administered at doses that prevent the dopamine release by SCH 39166 and that by themselves do not affect basal acetylcholine, potentiated the decrease of acetylcholine release elicited by SCH 39166. These results therefore confirm those obtained with quinpirole and are consistent with the hypothesis that endogenous dopamine acting onto D_1 receptors exerts a facilitatory influence on striatal acetylcholine output (Consolo et al., 1987; Damsma et al., 1990a, 1991).

The interaction of SCH 39166 and quinpirole has been previously studied by Imperato et al. (1995) who reported that quinpirole, administered following SCH 39166, fails to further decrease acetylcholine release in the striatum. In the present study, instead, quinpirole pretreatment potentiated the reduction of acetylcholine release by SCH 39166. These differences can be accounted by different experimental conditions. Thus, Imperato et al. (1995) utilized doses of SCH 39166 and quinpirole that were, respectively, 10 and 20 times higher than those used in the present study. These doses of SCH 39166 are maximally effective in decreasing acetylcholine release. Moreover, the high dose of quinpirole utilized by Imperato et al. (1995) is likely to be able to stimulate, in addition to D_2 autoreceptors, also D_2 receptors on acetylcholine neurons. In contrast, the doses of SCH 39166 selected by us are threshold for decreasing acetylcholine release and the doses of quinpirole are sufficient to prevent the increase of dopamine release by SCH 39166 but not to fully reduce acetylcholine release.

The second issue addressed by our study is that of the role of neostigmine in the effects of D_1 receptor antagonists. The fact that the present evidence for a role of D_1 receptors in acetylcholine release by endogenous dopamine has been obtained under the same neostigmine concentrations utilized by DeBoer et al. (1996) contradicts the suggestion of these authors that the results obtained by previous studies on the role of D_1 receptors in the control of acetylcholine release are an artifact of the use of *unphysiologically* high concentrations of acetylcholine esterase inhibitors (DeBoer and Abercrombie, 1996).

Although the present data do not address the issue of the location (striatal or extrastriatal) of the dopamine D_1 receptors mediating these effects, the present results are consistent with the existence of a D_1-mediated influence of dopamine on acetylcholine release (Di Chiara et al., 1994). This hypothesis states that endogenous dopamine exerts a stimulatory tone on D_1 receptors located on acetylcholine neurons mediated by a facilitation of the transduction of information by glutamate acting on excitatory NMDA receptors. This hypothesis is consistent with the following evidence: (1) the high (70–90%) expression of the D_5 subtype of D_1 receptors in striatal acetylcholine neurons (Jongen-Rêlo et al., 1995; Yan et al., 1997); (2) the ability of NMDA receptor antagonists to reverse the D_1-dependent stimulation of acetylcholine release (Damsma et al., 1991; Consolo et al., 1996); (3) the similarity of the above action to that of the lesions of the parafascicular nucleus (Consolo et al., 1996), which provides an NMDA-dependent excitatory input onto acetylcholine neurons (Lapper and Bolam, 1992).

The present study could not confirm the observations of DeBoer et al. (1996) that quinpirole increases acetylcholine release. Different experimental conditions might account for these differences. However, close examination of the results of these authors shows that the increase of acetylcholine release following quinpirole was slight (max + 23%) and biphasic (at 15 min but not at 30 and 45 min and again at 60 min). Therefore, if dopamine D_2 receptors mediate an action of endogenous dopamine on acetylcholine release, this would account for only a fraction and not for the whole control of acetylcholine function by dopamine.

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