Nonstriatal Dopamine D1 Receptors Regulate Striatal Acetylcholine Release In Vivo

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ABSTRACT
The role of dopamine (DA) D1 receptors in the regulation of acetylcholine (ACh) release in the striatum was studied using in vivo microdialysis in freely moving rats. Systemic administration of the full D1 DA receptor agonist A-77636 (4 μmol/kg) increased striatal ACh release by 53% above the base line and decreased DA release by 33%. Local application of A-77636 (10 μM) or SCH 23390 (1.42 μmol/kg) or SCH 39166 (1.42 μmol/kg) blocked the stimulation of striatal ACh release produced by systemic A-77636 (4 μmol/kg). Local perfusion of either SCH 23390 or SCH 39166 did not decrease basal ACh release. Furthermore, when applied locally via the dialysis probe, SCH 23390 (12 μM) or SCH 39166 (50 μM) failed to attenuate the stimulation of striatal ACh release produced by systemic A-77636. Similarly, d-amphetamine (5.42 μmol/kg)-induced increases in striatal ACh release were not modified by simultaneous local perfusion with SCH 39166 (50 μM). These findings are consistent with the hypothesis that D1 receptor activation stimulates ACh release in the striatum. However, because local application of D1 receptor agonists and antagonists fail to influence ACh release, the relevant D1 receptors are not located in the striatum. The use of unphysiological dialysis conditions (high concentrations of acetylcholinesterase inhibitors, high Ca++ concentrations and an absence of Mg++ in the perfusion fluid) may account for some earlier suggestions that local D1 receptors regulate ACh release in the striatum.

The observations that dopamine receptor agonists and muscarinic receptor antagonists ameliorate symptoms of Parkinson’s disease has provided the basis for the hypothesis that DA and ACh have opposing actions on striatal function (Lehmann and Langer, 1983). Approximately 1% to 2% of striatal neurons are cholinergic (Fibiger, 1982; Satoh et al., 1983), and these neurons receive a direct input from dopaminergic neurons located primarily in the pars compacta of the substantia nigra (Kubota et al., 1987; Lehmann and Langer, 1983). Nearly all striatal cholinergic neurons express D2 receptor mRNA, whereas estimates of the extent to which these neurons also contain D1 receptor mRNA vary considerably (20%–70%) (Guennoun and Bloch, 1992; Jongen-Reilo et al., 1995; Le Moine et al., 1990; Le Moine et al., 1991).

In vitro studies using striatal slices have demonstrated that D2 receptor agonists decrease ACh release (Herrting et al., 1980; Stoof and Kebabian, 1982). Nonspecific DA receptor agonists decrease ACh turnover (Trabucchi et al., 1975) and increase tissue concentrations of ACh (McGeer et al., 1974), and this has been interpreted as indicating that these agents decrease ACh release. On the other hand, D2 agonists apparently enhance the activity of striatal cholinergic neurons, as reflected by increased ACh release (Stadler et al., 1973) and turnover (Trabucchi et al., 1975) and by reduced tissue concentrations (McGeer et al., 1974). In vivo microdialysis studies have confirmed that D2 receptor agonists reduce (Bertorelli and Consolo, 1990; Damsma et al., 1990a; De Boer et al., 1990), whereas D2 receptor antagonists increase, striatal ACh release (Bertorelli and Consolo, 1990; Damsma et al., 1990a). In contrast, systemic administration of D1 receptor agonists increases striatal ACh release (Damsma et al., 1990b), as does systemic administration of indirect dopaminergic agonists such as d-amphetamine, nomifensine (Consolo et al., 1992; Damsma et al., 1991; Florin et al., 1992) and cocaine (Imperato et al., 1993). Interestingly, no unequivocal evidence has yet been provided by in vitro studies for a role of striatal D1 receptors in the control of ACh release (Dolezal et al., 1992; Login et al., 1995; Scatton, 1982; Stoof and Kebabian, 1982; Stoof et al., 1992; Tedford et al., 1992).

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ABBREVIATIONS: ANOVA, analysis of variance; DA, dopamine; HPLC-ECD, high-performance liquid chromatography with electrochemical detection; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid.
The anatomical locus of the D1-mediated stimulant effect on striatal ACh neurotransmission in vivo is controversial. Evidence from some laboratories has pointed to a striatal location for these receptors (Ajima et al., 1990; Anderson et al., 1994; Consolo et al., 1992; Zocchi and Pert, 1993). In contrast, others have failed to confirm a striatal location (Damsma et al., 1991; De Boer et al., 1992; Johnson and Bruno, 1993) and have suggested that D1 receptors in other brain regions mediate these effects (Damsma et al., 1991; De Boer and Abercrombie, 1994; De Boer et al., 1993).

The present experiments were undertaken to address further the question of whether D1-mediated stimulation of striatal ACh release occurs by actions at D1 receptors located within the striatum and to ascertain whether the microdialysis conditions used to monitor striatal ACh release can influence, either qualitatively or quantitatively, the effects of dopaminergic drugs. To this end, we used the full DA D1 receptor agonist A-77636 (Keabian et al., 1992), the indirect dopaminergic agonist d-amphetamine and the two dopamine D1 receptor antagonists SCH 23390 (Iorio et al., 1983) and SCH 39166 (Chipkin et al., 1988). The first goal was to study the effects of the local application of SCH 23390 and SCH 39166 on stimulated striatal ACh release produced by systemic A-77636 or d-amphetamine. The second goal was to test the hypothesis that striatal D1 receptors regulate basal ACh release in this structure. This issue was addressed by determining the effects of locally applied D1 receptor agonists and antagonists on ACh release in the striatum. Finally, we examined the extent to which differing dialysis conditions may have contributed to earlier discrepant findings regarding the anatomical locus of D1 receptors that regulate striatal ACh release.

Materials and Methods

Animals. Male Wistar rats (275–300 g) were housed in groups of 2 to 3 per cage for at least 6 days before use and were maintained on a 12:00/12:00 h light/dark cycle (lights on at 7:30 A.M.) with food and water ad libitum. After surgery the rats were housed individually in Plexiglas cages (35 x 35 x 25 cm) that also served as the experimental chamber, where they recovered for 36 to 48 h before the microdialysis procedure. Experiments were carried out between 9:00 A.M. and 4:00 P.M.

Surgery and microdialysis. Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and stereotaxically implanted with a horizontal membrane passing through both striata (Imperato and Di Chiara, 1985; Damsma et al., 1988). The coordinates, measured from bregma, were AP = +1.5 mm, DV = −4.75 mm according to Paxinos and Watson (1986). The membrane used was a polycrlyl-onitrile/sodium methally sulfonate copolymer (I.D. 0.22 mm, O.D. 0.31 mm; cutoff 40,000 D, AN 69 filtral 8, Hospal Industrie, France).

The membrane was covered with epoxy glue along its whole length except for 6.4 mm corresponding to the area of dialysis (active surface 3.2 mm/striatum). Two days after surgery, rats were connected to a microperfusion pump (Harvard Apparatus Inc., South Natick, MA) by polyethylene tubing (PE-10, Becton Dickinson & Co., NJ), (volume 50 or 20 μl, I.D. 0.28 mm—INLET) connected to a 2.5-ml glass syringe containing the perfusion solution. The perfusion flow was set at 5 μl/min, except for one experiment where the flow rate was set at 2 μl/min.

The first three dialysate samples were discarded. Samples were collected every 10 min (50 or 20 μl/sample) into a sample loop of an HPLC injector valve electrically operated by a digital valve sequence programmer (model C10W, VICI, Valco Instruments Co., Houston, TX) connected to the rat by polyethylene tubing (volume 50 or 20 μl, I.D. 0.28 mm—OUTLET). The perfusion solution contained 125 mM NaCl, 3 mM KCl, 1.3 mM CaCl2, 1 mM MgCl2 and 23 mM NaHCO3 in aqueous potassium phosphate buffer (1 mM, pH = 7.4). To achieve consistently detectable amounts of ACh in the dialysate, the reversible AChE inhibitor neostigmine bromide (0.1 μM) (Sigma, St. Louis, MO), or in one experiment physostigmine sulfate (7 μM) (RBI, Natick, MA), was added to the perfusion solution. ACh was assayed by HPLC-ECD in conjunction with an enzyme reactor (Damsma et al., 1988). In the experiments in which DA and its metabolites DOPAC and HVA were measured, the composition of the perfusion solution was as follows: 148 mM NaCl, 3 mM KCl, 1.3 mM CaCl2 and 1 mM MgCl2 in aqueous potassium phosphate buffer (1.5 mM, pH = 7.4). In these experiments, an AChE inhibitor was not included in the perfusion solution.

ACh and choline were separated on a reverse-phase Chromspher C18 5-μm (Merck, Darmstadt, FRG) column (75 x 2.1 mm) pretreated with lauryl sulfate. The mobile phase passed directly through the enzyme reactor (10 x 2.1 mm) containing AChE (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-NH2 (Merck, Darmstadt, FRG). ACh 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). The mobile phase was an aqueous potassium phosphate buffer (1.9 mM K2HPO4, 0.2 mM tetramethyl ammonium hydroxide, pH = 8) delivered at a constant flow of 0.4 ml/min by an HPLC pump (Pharmacia LKB, HPLC pump 2150, Piscataway, NJ). The chromatograms were recorded on a chart recorder. The detection limit of the assay was about 50 fmol/sample. Injections of an ACh 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. DA, DOPAC and HVA were assayed by HPLC-ECD. The mobile phase was delivered by an HPLC pump (Pharmacia LKB, HPLC pump 2150, Piscataway, NJ) at the constant flow of 1.25 ml/min and consisted of 0.1 M sodium acetate adjusted to pH 4.1 with acetic acid, 0.5 mM octanesulfonic acid (Eastman Kodak Co., NY) 0.01 mM disodium EDTA and methanol 12% v/v. DA, DOPAC and HVA were separated by reverse-phase liquid chromatography (150 x 4.6 mm, Nucleosil 5 μm ODS C18). The electrochemical detector (Coulochem II, ESA Inc., Bedford, MA) was set as follows: guard cell +400 mV; oxidation electrode +400 mV and reduction electrode −300 mV. The chromatograms were recorded on a chart recorder. The detection limit of the assay was approximately 5 fmol/injection for DA and DOPAC and approximately 20 fmol/injection for HVA.

Drugs. A-77636 ([1R,3S]-3-(1-amantadyl-1-aminomethyl-3,4-di-hydro-5,6-dihydroxy-1H-2-benzopyran hydchlordro) was dissolved in distilled water and injected s.c. in a volume of 0.1 ml/100 g at a dose of 4 μmol/kg (1.46 mg/kg). When applied locally by reverse dialysis, A-77636 was dissolved in a small amount of distilled water and then diluted in the perfusion solution containing neostigmine bromide (0.1 μM) to 10 and 100 μM concentrations. d-Amphetamine sulfate (Sigma, St. Louis, MO), dissolved in water, was injected in a volume of 0.1 ml/100 g, and the dose of 5.42 μmol/kg (2 mg/kg) refers to the salt. SCH 39166 ([R+(-)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7ol]-maleate) (Scherimg-Plough, Bloomfield, NJ) or SCH 39166 ([R+(-)-8-trans-6,7,5,8,13b-exahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo-[d]-naphtho[2,1-b]-azepine hydrochloride) (Scherimg-Plough, Milan, Italy) was dissolved in distilled water and injected s.c. in a volume of 0.1 ml/100 g at a dose of 0.74 μmol/kg (0.3 mg/kg) or 1.42 μmol/kg (0.5 mg/kg), respectively. When applied locally through the dialysis membrane, SCH 39166 or SCH 393290 was dissolved in water and then diluted to 12, 24, 50 or 60 μM in perfusion solution containing neostigmine bromide (0.1 μM) or physostigmine sulfate (7 μM).

Statistics. One-way and two-way ANOVA, with time as the repeated measure, were used to analyze the treatment effects. Reported F values refer to the main group effect of the experimental
Results

In vitro recovery of A-77636. In order to determine the extent to which A-77636 diffuses across the dialysis membrane, in vitro recovery of A-77636 was measured using UV spectroscopy. This experiment was conducted in triplicate, and recovery of A-77636 was 35% ± 3% from a 100 μM solution of A-77636 when the flow rate was 5 μl/min.

Basal ACh, DA and DA-metabolite output. Basal ACh and DA (fmol/min), DOPAC and HVA (pmol/min) 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. base-line ACh in the dialysate was 94 ± 5 fmol/min (n = 69) in the 0.1 μM neostigmine condition. The overall mean ± S.E.M. base line was (n = 11) 29 ± 1 fmol/min for DA, 1.3 ± 0.05 pmol/min for DOPAC and 0.7 ± 0.04 pmol/min for HVA. For all of the group comparisons made below, there were no significant between-group differences in the base-line values of ACh.

Effects of SCH 39166 and SCH 23390 on A-77636-induced stimulation of striatal ACh release. At a dose of 4 μmol/kg, A-77636 produced long-lasting (>3 h) increases in striatal ACh release (fig. 1, top panel) [F(1,11) = 13.97; P < .004] compared with the vehicle group. The effect on ACh release was accompanied by behavioral stimulation characterized by locomotor activity and sniffing. The dose of A-77636 (4 μmol/kg) was selected because it has previously been demonstrated to be maximally effective on either neurochemical (Acquas et al., 1994) or behavioral measures (Kebabian et al., 1992). Systemic administration of SCH 23390 (0.74 μmol/kg) and that of SCH 39166 (1.42 μmol/kg) blocked the effects of A-77636 on ACh release [F(1,9) = 12.27; P < .008] for SCH 39166 (fig. 1, middle panel) and [F(1,10) = 14.66; P < .004] for SCH 39166 (fig. 1, bottom panel). Systemic administration of SCH 23390 or SCH 39166 also blocked the behavioral stimulation produced by A-77636 (not shown). In contrast, local application of SCH 23390 or SCH 39166 by reverse dialysis failed to influence the effects of A-77636. Thus the maximal stimulation of striatal ACh release produced by A-77636 in combination with local application of SCH 23390 (12 μM) did not differ significantly from that produced by A-77636 alone (fig. 2, top panel) [F(1,10) = 0.42; N.S.]. Similarly, local application of SCH 39166 (50 μM) [F(1,11) = 0.62; N.S.] (fig. 2, bottom panel) did not modify the effects of A-77636.

Effects of systemic A-77636 on striatal DA release. The effects of systemic administration of A-77636 (4 μmol/kg s.c.) on the release of DA, DOPAC and HVA are shown in figure 3. A-77636 significantly reduced striatal DA output to about 70% of baseline [F(G-D)(1.8, 7.2) = 33.32; P < .0001], and this effect lasted for more than 3 h. Striatal output of DOPAC and HVA was also slightly reduced by A-77636, and these effects were statistically significant for HVA [F(G-D)(1.8, 7.2) = 6.8; P < .05] but not for DOPAC [F(G-D)(1.2, 5.04) = 3.2; N.S.].

Effect of locally applied A-77636 on extracellular concentrations of ACh and DA. Local perfusion with A-77636 (10 and 100 μM) did not significantly influence ACh output [F(G-A)(3, 12) = 0.73; N.S.] (fig. 4, top panel). Similarly, local application of A-77636 (10 and 100 μM) did not significantly influence the output of DA [F(G-D)(2.8, 14) = 2.9; N.S.] and its metabolites DOPAC [F(G-D)(2.6, 13) = 2.5; N.S.] and HVA [F(G-D)(3.6, 18) = 0.6; N.S.] (fig. 4, bottom panel).


**Effects of locally applied SCH 23390 and SCH 39166.**

Figure 5 shows that SCH 23390 (12 μM) did not significantly modify ACh output \([F_{G,G}(2.3, 9.3) = 3.5; \text{N.S.}]\). Surprisingly, higher concentrations of SCH 23390 (24 μM and 60 μM) significantly increased striatal ACh release \([F_{G,G}(2.7, 10.8) = 4.2; P < .05]\) and \([F_{G,G}(2.7, 10.8) = 5.1; P < .05]\), respectively. In contrast, application of the more selective D1 antagonist SCH 39166 (50 μM), by reverse dialysis (fig. 5), did not significantly alter striatal ACh release \([F_{G,G}(1.8, 5.4) = 1.1; \text{N.S.}]\). ANOVA revealed that this effect was significantly different from that produced by SCH 23390 (60 μM), \([F(1,7) = 6.04; P < .04]\). In a result consistent with previous reports (Imperato and Di Chiara, 1988; Damsma et al., 1991), local application of SCH 23390 increased DA release in a concentration-dependent manner (not shown).

**Effect of local application of SCH 39166 on d-amphetamine-induced stimulation of striatal ACh release.** In agreement with previous reports (Damsma et al., 1991; Consolo et al., 1992; Florin et al., 1992), d-amphetamine sulfate significantly increased interstitial concentrations of ACh compared to vehicle injections \([F(1,11) = 17.25; P < .003]\) (fig. 6, top panel). Local application of SCH 39166 (50 μM) (fig. 6, bottom panel) failed to reduce d-amphetamine-induced stimulation of striatal ACh release \([F(1,10) = 0.04; \text{N.S.}]\).

**Effects of high AChE inhibition and high Ca\(^{++}\) concentrations.** In an attempt to replicate results reported by Consolo et al. (1992), we performed a series of experiments in which the dialysis conditions were modified so as to be as similar as possible to those used by these authors. The composition of the perfusion solution was as follows: 147 mM NaCl, 4 mM KCl and 2.2 mM CaCl\(_2\), dissolved in double distilled water. The AChE inhibitor physostigmine sulfate (7 μM) was added to the perfusion solution, and these experiments were carried out 1 day after surgery at a perfusion flow rate of 2 μl/min. As shown in figure 7, SCH 23390 (24 μM) failed to influence ACh output \([F_{G,G}(2.5, 12.6) = 1.2; \text{N.S.}]\); however, compared with the dialysis conditions used in the other experiments reported here, basal ACh levels were greatly increased to 1229 ± 18 fmol/min (n = 6). Analysis of variance comparing the effects of SCH 23390 in the presence of high [Ca\(^{++}\)] and high AChE inhibition (fig. 7) with those used in the other experiments (fig. 5) indicated that there were no significant differences between the effects of the D1 receptor antagonist under these two conditions \([F(1,9) = 1.26; \text{N.S.}]\).

**Discussion**

The present data confirm and extend previous reports indicating that systemically administered D1 receptor agonists increase ACh release in the striatum (Damsma et al., 1990b; Damsma et al., 1991; Imperato et al., 1994; Zocchi and Pert, 1993). Thus the selective D1 agonist A-77636 produced robust and long-lasting increases in ACh release that were readily blocked by pretreatment with systemically administered D1 receptor antagonists SCH 23390 or SCH 39166 (fig. 1). Although these data indicate that stimulation of D1 re-
ceptors enhances ACh release in the striatum, they provide no information about the anatomical locus of these receptors. Given the large number of D1 receptors in the striatum (Mansour et al., 1991; Yung et al., 1995), a striatal location might well be anticipated. However, the data reported here provide no support for such an organization. Thus, when the same D1 receptor antagonists were applied locally in the striatum, via reverse dialysis, the A-77636-induced increases in striatal ACh release were not affected (fig. 2). Furthermore, when A-77636 itself was delivered locally to the striatum via reverse dialysis, ACh release was not affected (fig. 4). The failure of locally delivered compounds to influence ACh release was not due to subthreshold quantities passing across the dialysis membrane. In the case of SCH 23390, it has been previously demonstrated that local application (10 μM) via reverse dialysis increases extracellular concentrations of DA (Damsma et al., 1991; Imperato and Di Chiara, 1988) but fails to affect interstitial concentrations of ACh (Damsma et al., 1991; De Boer et al., 1992; Johnson and Bruno, 1993). It is evident, therefore, that 12 μM SCH 23390 does produce significant neurochemical effects in the vicinity of the dialysis probe and that the lack of effects on striatal ACh release cannot be attributed to the failure of sufficient quantities of the compound to reach the local environment outside the membrane. Similarly, local application of the D1 agonist CY 208-243 (10 μM), though it fails to stimulate ACh release in the striatum (Damsma et al., 1991), has been shown to increase Fos immunoreactivity in the vicinity of the probe in 6-OHDA-denervated striatum (Robertson et al., 1992). Finally, we have recently observed that A-77636 (10 μM), when applied by reverse dialysis under the same conditions utilized in the present experiments, significantly increases extracellular concentrations of cyclic AMP in the striatum (Acquas and Fibiger, unpublished observations). This confirms that the concentration of A-77636 delivered locally to the striatum was sufficient to elicit a neurochemical response.

Local perfusion with SCH 23390 (12 μM) or SCH 39166 (50 μM) did not alter basal striatal ACh release, whereas SCH 23390 (24 and 60 μM) had a stimulant effect. The latter may have been due to nonspecific (i.e., non-D1 receptor-mediated) effects of SCH 23390, because this compound has weak antagonist actions at D2 receptors (Doelezal et al., 1992; Plantje et al., 1984). This finding stands in contrast to a previous report that local perfusion of SCH 23390 (20 μM) reduces basal ACh release in the striatum (Consolo et al., 1992). The microdialysis conditions used by Consolo et al. (1992) differed substantially from those used here (2.2 mM Ca++, no Mg++,...
7 μM physostigmine, 2 μl/min and 1 day postsurgery vs. 1.2 mM Ca\(^{++}\), 1.0 mM Mg\(^{++}\), 0.1 μM neostigmine, 5 μl/min and 2 days postsurgery). Because some of these variables can influence the nature of striatal ACh microdialysis results both qualitatively and quantitatively (Damsma et al., 1990a; De Boer et al., 1990), we attempted to replicate the results of Consolo et al. (1992) using their microdialysis conditions. As is evident in figure 7, in contrast to the results of Consolo et al. (1992), SCH 23390 failed to decrease ACh release in the striatum. At present we can offer no explanation for this failure to replicate, even though a slightly higher concentration (20%) of the D1 antagonist was used. However, the data in figure 7 are consistent with the results of the other experiments reported here and elsewhere: that local manipulations of the D1 receptors in the striatum fail to influence ACh release (Damsma et al., 1991; De Boer et al., 1992).

Despite the discrepant findings with locally applied D1 receptor agonists, there is general agreement that systemically administered D1 receptor antagonists block d-amphetamine-induced increases in striatal ACh release (Damsma et al., 1991; De Boer and Abercrombie, 1996; Imperato et al., 1993). However, the increases in striatal ACh release produced by d-amphetamine do not appear to be mediated by D1 receptors in the striatum, because local perfusion with the D1 antagonist SCH 39166 (50 μM) did not affect the d-amphetamine-induced increases. This finding stands in sharp contrast to a report that local perfusion of SCH 23390 (10 μM) blocks the stimulant effects of systemic d-amphetamine (2 mg/kg) and cocaine (10 mg/kg) on striatal ACh release (Consolo et al., 1992), and again the reasons for this discrepancy are not apparent. It is possible that differences in the microdialysis conditions contributed to these divergent results; however, this seems unlikely in view of the fact that, even when we used their dialysis conditions, we were unable to confirm the claim of Consolo et al. (1992) that SCH 23390 reduces basal ACh release in the striatum (fig. 7).

The results of the present study are not compatible with suggestions that D1 receptors in the striatum regulate ACh release in this structure (Ajima et al., 1990; Anderson et al., 1994; Consolo et al., 1992; Zocchi and Pert, 1993). However, our findings do not clarify the reasons for discrepancies that surround this issue. Among other variables, the concentration of the AChE inhibitor in the perfusion solution can markedly influence the effects of various DA agonists or antagonists on striatal ACh release (Acquas and Fibiger, 1995; De Boer and Abercrombie, 1996). It is noteworthy in this regard that the studies in which locally applied D1 agonists apparently had effects on ACh release used high concentrations of AChE inhibitors (Ajima et al., 1990; Anderson et al., 1994; Consolo et al., 1992; Sato et al., 1994; Zocchi and Pert, 1993) high, unphysiological concentrations of Ca\(^{++}\) and an absence of Mg\(^{++}\) (Ajima et al., 1990; Consolo et al., 1992; Sato et al., 1994; Zocchi and Pert, 1993) in the perfusion fluid. Our consistent inability to show any effects of locally applied D1 agonists or antagonists on striatal ACh release, while at the same time showing highly consistent effects of these agents when given systemically, points to a nonstriatal location of the relevant D1 receptors. This is supported by the observation that d-amphetamine-induced increases in striatal ACh release remain intact in animals with unilateral 6-hydroxydopamine lesions that abolish d-amphetamine-induced increases in DA release in the ipsilateral...
eral striatum (Herrera-Marschitz et al., 1994). These results indicate that \( d \)-amphetamine-induced increases in striatal ACh release that are mediated by D1 receptors (Damsma et al., 1991) are not dependent on local increases in striatal DA release. A nonstriatal location of the relevant D1 receptors is also supported by many in vitro studies that have failed to obtain evidence for D1-mediated regulation of cholinergic function in striatal slice preparations (Dolezal et al., 1992; Login et al., 1995; Scatton, 1982; Stoof and Kebabian, 1982; Stoof et al., 1992; Tedford et al., 1992). The location of the D1 receptors that regulate striatal ACh release therefore remains to be determined. Preliminary evidence suggests that the substantia nigra is one site at which such regulation may occur (De Boer and Abercrombie, 1994).

It is noteworthy that systemic administration of A-77636 decreased striatal DA release (fig. 3). This finding raises the possibility that systemic A-77636, by decreasing DA release in the striatum, stimulates ACh release at least in part by reducing inhibitory D2-mediated effects of endogenous DA on cholinergic neurons. According to this formulation, the failure of locally delivered A-77636 to influence striatal ACh release is consistent with its lack of effect on DA when applied in this manner (fig. 4). Finally, the fact that systemically applied, but not locally applied, A-77636 decreased stri-
Fig. 7. Effect of local application by reverse dialysis of SCH 23390 (24 μM) (n = 6) for 1 h of perfusion (indicated by the horizontal bar) on striatal ACh release. In this experiment, the flow rate and the composition of the perfusion solution were altered as indicated in “Materials and Methods,” and the AChE inhibitor physostigmine sulfate (7 μM) was added to the perfusion solution. Values are expressed as percent of baseline. Vertical bars represent S.E.M.

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References


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