Dopaminergic regulation of striatal cholinergic interneurons: an in vivo microdialysis study

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Summary. In vivo microdialysis was used to study the putative inhibitory effects of dopamine on cholinergic interneurons in the striatum of conscious rats. The dopamine receptor agonists apomorphine (0.3 and 3 mg/kg, s.c.) and (+)-N-0437 (1.4 mg/kg, s.c.) decreased interstitial concentrations of acetylcholine while increasing those of choline. In contrast, the dopamine receptor antagonists haloperidol (0.1 and 1 mg/kg, i.p.) and (+)-sulpiride (20 mg/kg, i.p.) enhanced striatal acetylcholine output but had little effect on choline. Previously, a lack of effect of these drugs on striatal acetylcholine was reported. The main methodological difference between these studies was that the calcium concentration of the microdialysis perfusion solution was 3.4 mM in the former study versus 1.2 mM in the present experiments. The results of this study reemphasize the importance of the calcium concentration in determining the effects of drugs on central neurotransmitter release, and confirm a role of dopamine in the regulation of striatal cholinergic interneurons.

Key words: Acetylcholine — Choline — Dopaminergic agents — Microdialysis — Rat — Striatum

Introduction

The observation that anticholinergic drugs can reverse neuroleptic induced extrapyramidal symptoms and that both L-DOPA and anticholinergic agents can ameliorate the symptoms of Parkinson's disease, gave rise in the early 1960s to the hypothesis that dopamine (DA) and acetylcholine (ACh) have opposing functions in the extrapyramidal system (McGeer et al. 1961; Barbeau 1962). In the 1970s, the hypothesis was refined: nigro-striatal dopaminergic fibres exert a direct inhibitory influence on striatal cholinergic interneurons; moreover it was proposed that cholinergic interneurons act as a essential "link" in expressing the information derived from dopamine terminals (for reviews, see Tarsy 1977; Lloyd 1978; but see Lehmann and Langer 1983).

A substantial literature in favour of the hypothesis that DA inhibits striatal cholinergic neurons has been generated by the findings that striatal tissue concentrations of ACh are increased by DA receptor agonists and decreased by DA receptor antagonists (e.g. Fibiger and Grewaal 1974; McGeer et al. 1974; Sethy and Van Woert 1974; Guyenet et al. 1975; Rommelspacher and Kuhar 1975). These results have been interpreted as being due to drug-induced decreases and increases in cholinergic transmission, respectively (Szerb et al. 1970; Pepeu 1973). Push-pull perfusion studies in gallamine immobilized cats have provided a second line of evidence for dopaminergic inhibition of striatal cholinergic function. For example, neuroleptic drugs have been shown to increase ACh release, whereas apomorphine and L-DOPA decrease the output of this neurotransmitter (Stadler et al. 1973, 1974). Finally, a third and impressive body of evidence has been provided by in vitro perfusion studies using striatal slice preparations. Numerous studies have indicated that the release of ACh from such slices is modulated in an inhibitory fashion by DA receptors, particularly the D-2 subtype (e.g. Stooft et al. 1979; Cubbedu et al. 1983; Chesselet 1984).

Although experimental support for an inhibitory action of DA on striatal cholinergic interneurons is extensive, the biochemical evidence is mainly indirect, being based on ex vivo tissue level and in vitro studies. Only a limited number of in vivo studies employing push-pull perfusion procedures in the caudate nucleus of gallamine immobilized cats have provided direct support for endogenous tonic inhibitory effects of DA in striatal cholinergic interneurons (Stadler et al. 1973, 1974). In an attempt to obtain further direct evidence, the microdialysis perfusion technique (Ungerstedt 1984) was used to study the effects of systemically applied dopaminergic

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drugs on striatal cholinergic function in awake, freely moving rats. The effects of local application of these compounds via the dialysis probe were also investigated and are presented in the previous report (De Boer et al. 1990).

Materials and methods

**Experimental protocol and drugs.** Experiments were performed on male Wistar rats (275 - 300 g) 2 days after the implantation of a microdialysis probe. The rats were housed individually in Plexiglas chambers (35 x 35 x 25 cm), had free access to food and water, and were maintained on a 12 h:12 h light:dark schedule. Microdialysis perfusion experiments were performed during the light phase. The effects of the following systemically administered dopaminergic drugs were studied with respect to their ability to affect the interstitial concentrations of striatal choline and acetylcholine: the classical D-1/D-2 receptor agonist apomorphine hydrochloride (0.3 and 3 mg/kg i.p.; Sigma), the potent and selective D-2 agonist (+)-sulpiride (20 mg/kg i.p.; Janssen, Beerse, Belgium), and the selective D-2 antagonist (+)-sulpiride (20 mg/kg i.p.; Sigma).

**Surgery and microdialysis.** Under anesthesia (pentobarbital, 50 - 60 mg/kg, i.p.) rats were stereotaxically implanted with a horizontal membrane passing both dorsol striata (Imperato and DiChiara 1984; Damsma et al. 1988). The coordinates measured from bregma were A: + 0.7 and V: -4.75 (Paxinos and Watson 1986). The membranes had 3.2 mm of active dialysis surface area in each striatum and consisted of a saponified cellulose fibre (ID = 0.22 mm, OD = 0.27 mm, molecular weight cut off < 10,000 Dalton; Cordis Dow, Rotterdam, The Netherlands). Upon completion of the perfusion experiment each rat was sacrificed and the brain was sectioned for histological verification of the probe location.

During the microdialysis experiments the probe inlet was connected to a 5 ml glass syringe (Hamilton) by polyethylene tubing (800 x 0.28 mm). The perfusion flow was controlled by a micro-perfusion pump (Carnegie, Stockholm, Sweden) and set at a flowrate (800 x 0.28 mm). The probe outlet was connected to the sample loop (100 µl) of the electrically actuated HPLC injector (Valco, no. C10W) by fused silica tubing (800 x 0.1 mm). Microdialysis samples (50 µl) were automatically injected at a 10 min frequency, the alternating load and inject positions of the injector valve being controlled by an adjustable timer (Valco, Houston, USA).

The composition of the perfusion solution was selected to match the estimated ionic composition of interstitial fluid in the brain (Hansen 1985) and contained NaCl (125 mM), KCl (3 mM), CaCl2 (1.3 mM), MgCl2 (1.0 mM), NaHCO3 (23 mM), and aqueous potassium phosphate buffer (1.5 mM; pH 7.3). To achieve detectable amounts of ACh in the dialysate, a cholinesterase inhibitor (neostigmine bromide, 0.1 µM; Sigma) was included in the perfusion solution.

**Assay of ACh.** ACh and choline were assayed by HPLC-ECD in conjunction with an enzyme reactor (Damsma et al. 1987a). Briefly, ACh and choline were separated on a reverse phase column (75 x 2.1 mm) pretreated with lauryl sulphate. The eluant was passed directly through the enzyme reactor where ACh and choline were converted quantitatively into hydrogen peroxide. The enzyme reactor (10 x 2.1 mm) contained acetylcholinesterase (EC 3.1.1.7; Sigma, type VI-S) and choline oxidase (1.1.3.17; Sigma), covalently bound to glutaraldehyde activated Lichrosorb 10 NH2 (Merck, Darmstadt, FRG). The hydrogen peroxide was electrochemically detected at a platinum working electrode set at + 500 mV versus an Ag/AgCl reference electrode (BAS, La Fayette, USA, no. LC4B). The mobile phase, 0.2 M aqueous potassium phosphate buffer pH 8.0, was delivered by an HPLC pump (LKB, Bromma, Sweden, no. 2150) at 0.45 ml/min. The detection limit of the assay was about 50 fmol/injection. The chromatograms were recorded on a chart recorder (Kipp, Delft, The Netherlands); the time required to complete a chromatogram was 4 - 5 min. The amounts of choline and ACh were calculated by comparing peak heights of these compounds in the samples with standards injected every 120 min.

**Data analysis and statistics.** Baseline dialysis output of ACh and choline varied from rat to rat. These values were normalized by defining the average amount of ACh or choline output of the samples collected between 40 and 10 min prior to the drug injection as 100%. All subsequent data were expressed relative to these basal values.

A mixed-design ANOVA was conducted on ACh and choline output values for each drug treatment versus saline and on repeated measures over time. In this analysis the last pre-injection sample (10 min) and the first 12 post-injection samples (120 min) were included. A one-way ANOVA with repeated measures over time was conducted on the ACh and choline output values of vehicle injected subjects.

**Results**

**Baseline values and saline administration**

There was no significant difference in the basal values of either ACh or choline between experimental groups (n = 4 - 6 subjects for each drug treatment). The overall mean ± SEM (n = 32) baseline value of ACh in the dialysates was 84 ± 2.6 fmol/min and of choline 3.2 ± 0.3 pmol/min.

Figure 1 shows that injection of saline (0.1 ml, s.c.; n = 6) did not have a significant effect on dialysate concentrations of ACh (ANOVA: F(18,90) = 0.60; p > 0.05). The decreasing trend in choline was also not statistically significant (ANOVA: F(18,72) = 1.24; p > 0.05).

**Dopamine receptor agonists**

Apomorphine and N-0437 decreased the dialysate concentrations of ACh and increased those of choline (Fig. 2). For ACh the main effect of saline versus drug