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Acetylcholine release in rat nucleus accumbens is regulated through dopamine D_2-receptors

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Summary. Experiments in slices of rat nucleus accumbens were carried out in order to investigate whether the release of acetylcholine in this tissue is modulated through dopamine receptors. The slices were preincubated with ^{3}H-choline and then superfused and stimulated electrically twice for 2 min each at a frequency of 3 Hz.

The electrically evoked overflow of tritium averaged 2.9–3.9% of the tritium content of the tissue in the various groups. The D_2-selective agonist quinpirole (0.01–1 μmol/l) reduced the evoked overflow of tritium by maximally 56%, an effect antagonized by the D_2-selective antagonist (−)-sulpiride (1 μmol/l). The D_1-selective agonist 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine (SKF 38393) caused a slight decrease only at the high concentration of 10 μmol/l. (−)-Sulpiride (0.1–10 μmol/l) moderately increased the evoked overflow of tritium when given alone. The dopamine uptake inhibitor nomifensine (10 μmol/l) caused a decrease, and in its presence the increase produced by (−)-sulpiride became much more marked, amounting to maximally 149%. (+)-Sulpiride (0.1–1 μmol/l) failed to change the evoked overflow of tritium in the presence of nomifensine. The dopamine-releasing agent (±)-amphetamine (1 μmol/l) also reduced the evoked overflow, an effect abolished by (−)-sulpiride. Finally, bretylium (1 mmol/l), which blocks the release of dopamine, increased the evoked overflow. (−)-Sulpiride (1 μmol/l) lost its facilitatory effect in slices treated with bretylium.

We conclude that the release of acetylcholine in rat nucleus accumbens, like its release in the nucleus caudatus-putamen, is modulated through dopamine D_2-receptors. The receptors are activated by endogenous dopamine under the conditions of these experiments.

Key words: Rat nucleus accumbens – Rat nucleus caudatus-putamen – Acetylcholine release – Dopamine receptors

Introduction

Both the nucleus caudatus-putamen (CP) and the nucleus accumbens (ACC) contain cholinergic interneurones but no cholinergic afferents (McGeer et al. 1982), and both nuclei are innervated by the mesostriatal dopaminergic system, specifically, its dorsal (CP) and ventral part (ACC), respectively (Björklund and Lindvall 1984). The cholinergic neurones of the CP are target cells (although not the only target cells) of the dopaminergic projection; dopamine and related agonists inhibit the cholinergic neurones, whereas dopamine receptor antagonists activate them by removing a tonic dopaminergic inhibition (see Lehmann and Langer 1983 for a critical review). The relationship between the cholinergic neurones of the ACC and the dopaminergic projection to this nucleus, on the other hand, is not clear. It has been reported that dopamine receptor antagonists such as haloperidol increase the in vivo turnover of acetylcholine in the CP as well as in the ACC of the rat, indicating "that the cholinergic neurons in (both) n. caudatus and n. accumbens are innervated by inhibitory dopaminergic synapses" (Mao et al. 1977). In another study in the rat, however, dopamine receptor antagonists reduced, and agonists increased, the concentration of acetylcholine only in the CP but not in the ACC, suggesting "that a dopaminergic-cholinergic link similar to that in the striatum is not present in the (nucleus accumbens)" (Consolo et al. 1977). The latter conclusion was also drawn from experiments in cats (see Stadler et al. 1975).

Part of the complexities of in vivo studies can be avoided by the use of isolated brain preparations (see Starke 1979). Experiments on slices of the CP have confirmed the inhibition of acetylcholine release by exogenous dopaminergic agonists as well as by endogenous dopamine (e.g., Stoof et al. 1979; Hertting et al. 1980; Cubeddu and Hoffmann 1983; Baud et al. 1985). In vitro studies on the ACC, however, have not led to a clarification. For instance, de Belleroche and Neal (1982) and de Belleroche and Gardiner (1983) examined the K^+-evoked release of ^{3}H-acetylcholine in slices of rat CP and ACC. The D_2-selective antagonist sulpiride increased the release of acetylcholine from ACC slices only at the high concentration of 10 μmol/l (de Belleroche and Gardiner 1983), and pretreatment of the animals with haloperidol or chlorpromazine before killing increased acetylcholine release only in CP but not in ACC slices (de Belleroche and Neal 1982). These findings might seem to support a lack of dopaminergic inhibition in the ACC. However, the release of acetylcholine was unusually resistant to dopaminergic modulation in the experiments of these authors even in the CP. Sulpiride 3 μmol/l was required for a significant increase, and 30 μmol/l of the dopaminergic agonist bromocriptine for a significant inhibition (de Belleroche and Gardiner 1983); these are 30 times and 3000 times, respectively, the concentrations found effective by others (Baud et al. 1985 for sulpiride; Hoffmann and Cubeddu 1984 for bromocriptine). Possibly the high concentration of K^+ (34 mmol/l) used by de Belleroche and Neal

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(1982) and de Belleroche and Gardiner (1983) made the release of acetylcholine insensitive to dopaminergic modulation in both the CP and the ACC.

We have re-investigated the question of a dopaminergic modulation of cholinergic neurones in the rat ACC using electrical stimulation to release neurotransmitters.

Methods

Male Wistar rats (250–300 g) were killed by decapitation and the brains quickly removed. A coronal cut was made at the anterior border of the pons, and the posterior portion of the brain was discarded. The lateral parts of the anterior portion were removed by two sagittal cuts, 4 mm to the right and to the left of the median plane. The block of tissue thus obtained was divided symmetrically by a sagittal cut in the midline, and frontal slices, approximately 350 µm thick, were prepared from each half by means of a McIlwain tissue chopper (Bachofer, Reutlingen, FRG). The slices were transferred into ice-cold medium where they were separated from one another by gentle shaking. Slices containing the ACC and corresponding approximately to planes A 8920 µm to A 9650 µm of the atlas of König and Klippel (1963) were selected using a magnifying glass. Finally, disks of the ACC were punched out with a sharp-edged steel tube 2 mm in diameter. In some experiments, disks of the CP were punched out from the same frontal slices.

Four to eight of the 2 mm diameter disks were incubated for 30 min at 37° C in 2 ml of medium containing 0.1 µmol/l of ^{3}H-choline (specific activity 80 Ci/mmol). They were then rinsed three times with 3 ml of medium. One slice was transferred to each of six glass superfusion chambers, where it was held by a polypropylene mesh between platinum electrodes 20 mm apart. The slices were superfused with medium prewarmed to 37° C at a rate of 1 ml/min by means of a roller pump. The tubings consisted of Teflon except in the pump where silicone tubings were used (Silikon C, Desaga, Heidelberg, FRG). After having passed through the chambers, the medium was either discarded or, from 50 min of superfusion onwards, collected by means of a fraction collector in 5-min samples. At the end of superfusion, slices were solubilized in 0.5 ml Soluene-100 (Packard Instrument, Frankfurt am Main, FRG). The tritium content of superfusate samples and solubilized slices was determined by liquid scintillation counting.

Slices were stimulated electrically twice for 2 min each, after 60 and 100 min (experiments with bretylium: 60 and 120 min) of superfusion. Rectangular pulses of 2 ms duration and a current strength of 24 mA were delivered at a frequency of 3 Hz. The voltage drop between the electrodes was 5 V/cm. Pulses were monitored on an oscilloscope.

Unless stated otherwise, the medium used for preincubation and superfusion contained (mmol/l): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, ascorbic acid 0.6, disodium EDTA 0.03, glucose 11. It was saturated with 5% CO_2 in O_2. The pH was adjusted to 7.4 with NaOH. Drugs were added to the medium either 15 min before S_2 or throughout superfusion, with the exception of bretylium which was added from 25 to 35 and again from 85 to 95 min of superfusion.

The outflow of tritium was expressed as fractional rate (min^{-1}), i.e., (nCi tritium outflow per 5 min)/5 · (nCi tritium in the slice at the onset of the 5-min collection period). The stimulation-evoked overflow was calculated as the difference between the total overflow during and 18 min after stimulation and the estimated basal outflow; the basal outflow was assumed to decline linearly from the 5-min interval before onset of stimulation to the interval 20 to 25 min after onset of stimulation; the difference (nCi) was expressed as a percentage of the tritium content (nCi) of the tissue at the onset of stimulation. For further evaluation of basal tritium efflux, ratios were calculated between the fractional rate of outflow in the 5-min period before S_2 over that in the 5 min before S_1 (b_2/b_1). For further evaluation of the electrically evoked overflow, ratios were calculated between the overflow elicited by S_2 and that elicited by S_1 (S_2/S_1).

Choline [methyl-^{3}H] chloride (NET-109), specific activity 80 Ci/mmol, was purchased from New England Nuclear (Dreieich, FRG), (±)-amphetamine sulfate from Merck (Darmstadt, FRG) and tetrodotoxin from Sigma (München, FRG). Bretylium tosylate was a gift from Burroughs Wellcome (London, UK), nomifensine hydrogene maleate from Höchst (Frankfurt am Main, FRG), quinpirole hydrochloride from Lilly (Bad Homburg, FRG), (−)- and (+)-sulpiride from Dr. P. Fresia (Ravizza, Milan, Italy), and 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrochloride (SKF 38393) from Smith, Kline and French (Philadelphia, PA, USA). Drugs were dissolved in a small amount of water or medium except tetrodotoxin (citrate buffer pH 4.8), sulpiride and SKF 38393 (tartaric acid 10 mmol/l). In control experiments, the respective solvents were added.

Results are expressed as arithmetic means ± SEM. Statistical comparisons were done with the Mann-Whitney test if Kruskal-Wallis analysis indicated a significant difference between group means. n is the number of experiments (= number of brain slices).

Results

In an initial series of experiments, the effects of two concentrations of the selective D_2-receptor agonist quinpirole were studied in parallel in slices of ACC and CP. As shown in Fig. 1, the basal outflow of tritium from either tissue was similar, whereas the response to electrical stimulation was slightly greater in the CP than in the ACC. The electrically evoked overflow of tritium from CP slices amounted to 4.39 ± 0.22% of the tritium content of the tissue in this initial series ($n = 25$), whereas the electrically evoked overflow from ACC slices averaged 3.15 ± 0.13% ($n = 25$; $p < 0.01$). In either tissue, the evoked overflow was abolished in Ca^{2+}-free medium as well as in the presence of tetrodotoxin 0.3 µmol/l (not shown). Figure 1 also demonstrates that quinpirole 1 µmol/l, when added 15 min before S_2, reduced the evoked overflow both in the CP and, although to a smaller extent, in the ACC. In the CP, quinpirole 0.1 and 1 µmol/l reduced the S_2/S_1 ratio from a control value of 0.75 ± 0.01 ($n = 11$) to 0.21 ± 0.04 (72% inhibition; $n = 8$) and 0.09 ± 0.01 (88% inhibition; $n = 6$), respectively. In the ACC, quinpirole 0.1 and 1 µmol/l reduced S_2/S_1 from a control value of 0.71 ± 0.02 ($n = 11$) to 0.41 ± 0.02 (42% inhibition; $n = 6$) and 0.32 ± 0.03 (55% inhibition; $n = 8$), respectively. All differences from controls were significant at $p < 0.01$. It should be noted that control S_2/S_1 values below 1 are often found in brain slices preincubated with ^{3}H-choline (Hertting et al. 1980; Helmreich et al.

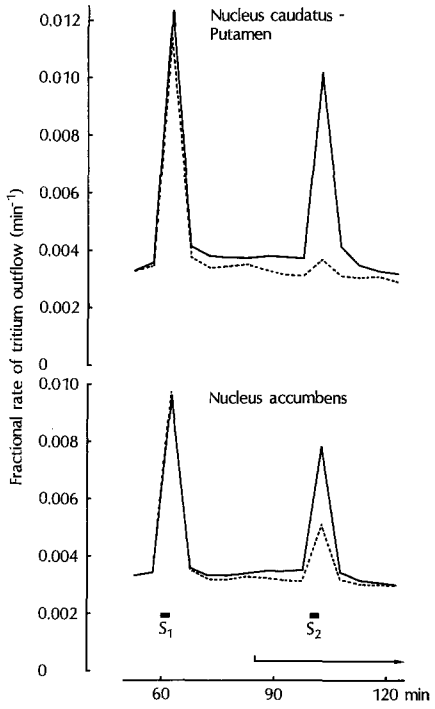

Fig. 1. Outflow of tritium from slices of rat CP and ACC. After preincubation with ^{3}H-choline, the slices were superfused with ^{3}H-free medium for 125 min. They were stimulated electrically after 60 and 100 min of superfusion (S_1,S_2; 3 Hz). Interrupted lines show experiments in which quinpirole 1 µmol/l was added 15 min before S_2 as indicated by the *horizontal arrow*; non-interrupted lines show control experiments. *Abscissa*, min of superfusion. *Ordinate*, fractional rate of tritium outflow (min^{-1}). Means of 6–11 experiments. Standard errors were 3–7% of corresponding means. The outflow of tritium in the 5 min before S_1, in absolute terms, averaged 8.1 ± 0.4 nCi/5 min for CP slices and 5.4 ± 0.3 nCi/5 min for ACC slices. The overflow of tritium evoked by S_1, in absolute terms, after subtraction of basal outflow, averaged 19.7 ± 1.5 nCi ($n = 17$) for CP slices, and 9.7 ± 0.5 nCi for ACC slices ($n = 19$)

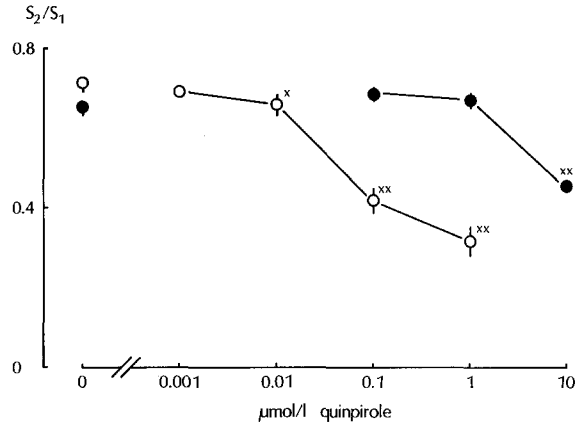

Fig. 2. Effect of quinpirole on electrically evoked overflow of tritium from ACC slices, and antagonism by (−)-sulpiride. After preincubation with ^{3}H-choline, slices were superfused either with sulpiride-free medium (○) or with medium containing (−)-sulpiride 1 µmol/l for the rest of the experiment (●). They were stimulated electrically after 60 and 100 min of superfusion (S_1,S_2). The overflow of tritium evoked by S_1 amounted to 2.93 ± 0.08% (sulpiride absent; $n = 63$) and 4.02 ± 0.18% (sulpiride present; $n = 29$; $p < 0.01$) of the tritium content of the tissue, respectively. Quinpirole was added 15 min before S_2. *Abscissa*, concentration of quinpirole; *ordinate*, S_2/S_1 ratios. Means ± SEM of 6 to 21 experiments. Significant differences from corresponding experiments without quinpirole: * $p < 0.05$; ** $p < 0.01$

1982; Starke et al. 1983; Hoffmann and Cubeddu 1984; Baud et al. 1985; Wichmann et al. 1987).

Since these experiments revealed no fundamental difference between the CP and the ACC, effects of dopamine receptor ligands in the ACC were studied in more detail. Figure 2 shows a full concentration-response curve of quinpirole. It agrees with the findings in the initial experiments; the maximal inhibition obtained amounted to 56%. When the preferential D_2-receptor antagonist (−)-sulpiride was present in the medium throughout superfusion, the overflow of tritium evoked by S_1 was significantly increased (legend to Fig. 2), and the concentration-response curve of quinpirole was shifted to the right approximately by a factor of 100 (Fig. 2). In contrast to quinpirole, the preferential D_1-receptor agonist SKF 38393, when added 15 min before S_2, did not change the evoked overflow of tritium (control S_2/S_1 ratio 0.73 ± 0.03; $n = 8$) at a concentration of 1 µmol/l (S_2/S_1 ratio 0.70 ± 0.02; $n = 8$) and reduced it only marginally at the high concentration of 10 µmol/l (S_2/S_1 0.64 ± 0.02; $n = 8$; $p < 0.05$).

The increase in tritium overflow at S_1 produced by (−)-sulpiride might be due to antagonism against an inhibitory effect of endogenous dopamine. In order to examine this possibility further, attempts were made to enhance the endogenous inhibition on the one hand, and to remove it on the other hand. Nomifensine, which inhibits the neuronal high affinity uptake of dopamine, and (±)-amphetamine, which releases dopamine into the extracellular space, were used for the first purpose. When nomifensine was added to the medium throughout superfusion at a concentration of 10 µmol/l, the overflow of tritium at S_1 was markedly reduced (Table 1). (−)-Sulpiride, which increased only slightly the evoked overflow of tritium when given alone 15 min before S_2 (by maximally 16% at 10 µmol/l), caused a pronounced increase in the presence of nomifensine (by 149% at 10 µmol/l). (+)-Sulpiride, in contrast to the (−)-enantiomer, failed to cause a significant change (Table 1). In separate experiments (no nomifensine), amphetamine 1 µmol/l was added 15 min before S_2. It reduced the ratio S_2/S_1 from 0.71 ± 0.02 (control; $n = 5$) to 0.44 ± 0.03 ($n = 6$; $p < 0.01$). (−)-Sulpiride 1 µmol/l, when present throughout superfusion, prevented the decrease (S_2/S_1 with (−)-sulpiride alone 0.67 ± 0.03, $n = 5$; S_2/S_1 for (−)-sulpiride plus amphetamine 0.66 ± 0.02, $n = 6$).

In an attempt to remove the endogenous dopaminergic inhibition, bretylium 1 mmol/l was added twice for 10 min each, before S_1 and between S_1 and S_2. Application of bretylium in this manner abolishes the electrically evoked release of dopamine and enhances the release of acetylcholine in rabbit caudate nucleus slices (Hertting et al. 1980; Helmreich et al. 1982). As shown in Table 2, exposure to bretylium increased the overflow of tritium evoked by S_1. (−)-Sulpiride, which increased the evoked overflow from slices not exposed to bretylium, failed to do so in bretylium-treated slices. The increase caused by (−)-sulpiride 1 µmol/l, given in the absence of other drugs, was more marked in this series (47%, Table 2) than in the previous one (12%; Table 1); the reason is not known.

Table 1. Interaction of nomifensine and (−)- or (+)-sulpiride on electrically evoked overflow of tritium from ACC slices. After preincubation with ^{3}H-choline, slices were superfused either with nomifensine-free medium or with medium containing nomifensine 10 μmol/l for the remainder of the experiment. They were stimulated electrically after 60 and 100 min of superfusion (S_1, S_2). Sulpiride was added 15 min before S_2. Indicated are the overflow of tritium evoked by S_1 (appropriate experiments pooled), the S_2/S_1 ratios, and the percentage increases caused by sulpiride. Means ± SEM of (*n*) experiments

Exposure to nomifensine	Overflow evoked by S_1 (% of tissue tritium)	Drug added before S_2	S_2/S_1	% increase caused by sulpiride
−	3.32 ± 0.18 (28)	−	0.71 ± 0.03 (10)	
		(−)-Sulpiride 0.1 μmol/l	0.75 ± 0.02 (6)	5
		(−)-Sulpiride 1 μmol/l	0.81 ± 0.03 (7)**	12
		(−)-Sulpiride 10 μmol/l	0.83 ± 0.04 (5)	16
+	1.01 ± 0.06 (46)*	−	0.66 ± 0.02 (15)	
		(−)-Sulpiride 0.01 μmol/l	0.74 ± 0.04 (6)**	11
		(−)-Sulpiride 0.1 μmol/l	1.28 ± 0.06 (8)***	92
		(−)-Sulpiride 1 μmol/l	1.58 ± 0.12 (9)***	137
		(−)-Sulpiride 10 μmol/l	1.65 ± 0.19 (8)***	149
+	0.96 ± 0.09 (18)*	−	0.77 ± 0.06 (6)	
		(+)-Sulpiride 0.1 μmol/l	0.76 ± 0.02 (6)	
		(+)-Sulpiride 1 μmol/l	0.89 ± 0.04 (6)	16

* Significant difference from slices not exposed to nomifensine ($p < 0.01$)
** Significant difference from corresponding experiments without sulpiride ($p < 0.05$)
*** Significant difference from corresponding experiments without sulpiride ($p < 0.01$)

Table 2. Interaction of bretylium and (−)-sulpiride on electrically evoked overflow of tritium from ACC slices. After preincubation with ^{3}H-choline, slices were superfused either with bretylium-free medium for the remainder of the experiment, or with medium containing bretylium 1 mmol/l from 25 to 35 and again from 85 to 95 min of superfusion. They were stimulated electrically after 60 and 120 min of superfusion (S_1, S_2). Sulpiride was added 15 min before S_2. Indicated are the overflow of tritium evoked by S_1 (appropriate experiments pooled), the S_2/S_1 ratios, and the percentage increases caused by sulpiride. Means ± SEM of (*n*) experiments

Exposure to bretylium	Overflow evoked by S_1 (% of tissue tritium)	Drug added before S_2	S_2/S_1	% increase caused by sulpiride
−	3.90 ± 0.20 (16)	−	0.72 ± 0.02 (8)	
		(−)-Sulpiride 1 μmol/l	1.06 ± 0.09 (8)**	47
+	6.26 ± 0.33 (14)*	−	0.79 ± 0.04 (6)	
		(−)-Sulpiride 1 μmol/l	0.80 ± 0.03 (8)	2

* Significant difference from slices not exposed to bretylium ($p < 0.01$)
** Significant difference from corresponding experiments without sulpiride ($p < 0.01$)

The basal outflow of tritium as reflected by the b_2/b_1 ratio was not changed significantly by the drugs used in this study.

Discussion

The electrically evoked overflow of tritium from brain slices preincubated with ^{3}H-choline reflects neuronal release of ^{3}H-acetylcholine (Stoof et al. 1979; Hertting et al. 1980; Cubeddu and Hoffmann 1983; Wichmann et al. 1987). Our finding that the evoked overflow of tritium was Ca^{2+}-dependent and sensitive to tetrodotoxin supports this view.

The D_2-selective agonist quinpirole inhibited the release of ^{3}H-acetylcholine in slices of the ACC as well as in those of the CP, and with a similar concentration dependence. For a given concentration, the degree of inhibition was less in the ACC than in the CP. The concentration-response curve of quinpirole in the ACC also agrees well with the curve describing the inhibition by the racemate of quinpirole (LY 141865) of K^+-evoked ^{3}H-acetylcholine release in slices of rat CP (Stoof and Kebabian 1982). The D_2-receptor-selective antagonist (−)-sulpiride shifted the concentration-response curve of quinpirole to the right. Although a full concentration-response curve of quinpirole in the presence of (−)-sulpiride was not obtained (Fig. 2), and although the presence of endogenous dopamine does not permit the calculation of exact antagonist-receptor dissociation constants (Starke et al. 1974; but see experiments by Helmreich et al. 1982 in which release of endogenous dopamine was prevented), the approximately 100 fold shift indicates a high affinity of (−)-sulpiride to the quinpirole receptor with a pA_2 value of about 8 (cf. the value of 7.71 in rabbit caudate nucleus obtained by Starke et al. 1983). The experiments demonstrate that the electrically evoked release of acetylcholine in the ACC, as in the CP, can be inhibited by activation of a dopamine D_2-receptor which may be located directly on the cholinergic neurones (see for the CP, Hertting et al. 1980; Joyce and Marshall 1987).

In contrast to these positive findings, there is no evidence for modulation of ^{3}H-acetylcholine release through a D_1-receptor in the rat ACC. The D_1-selective agonist SKF 38393 reduced the release of ^{3}H-acetylcholine only at the high concentration of 10 µmol/l. SKF 38393 activates adenylate cyclase in rat ACC and tuberculum olfactorium already at 1 µmol/l (Kelly and Nahorski 1987) and in rat CP, in which complete concentration-response curves are available, at less than 0.1 µmol/l (Setler et al. 1978). The small inhibition observed at 10 µmol/l may be a nonspecific effect. In vivo, the D_1-selective antagonist SCH 23390 (8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol) also failed to change the activity of cholinergic neurones in rat ACC (Consolo et al. 1987).

Under the conditions of our experiments, the D_2 receptor at the cholinergic neurones in the ACC seems to be activated by endogenous dopamine and, hence, to mediate an endogenous inhibition. In support of this view, (−)-sulpiride increased the release of ^{3}H-acetylcholine. Nomifensine and amphetamine, in contrast, caused a decrease, presumably because they reinforced the endogenous dopaminergic tone by blocking neuronal re-uptake or facilitating neuronal efflux of dopamine. (−)-Sulpiride antagonized the effect of amphetamine, and in the presence of nomifensine, the disinhibition by (−)-sulpiride became far more pronounced. (+)-Sulpiride failed to enhance the release of ^{3}H-acetylcholine in the presence of nomifensine, in agreement with the stereoselectivity of D_2-receptors (e.g., Dubocovich and Weiner 1981). Finally, bretylium increased ^{3}H-acetylcholine release, presumably by suppressing the release of dopamine (Hertting et al. 1980), and in fact, (−)-sulpiride lost its facilitatory effect in bretylium-treated slices. All these results are similar to those obtained previously in slices of the CP of rabbits (Hertting et al. 1980) and rats (Cantrill et al. 1983; Baud et al. 1985) and indicate that in the ACC, as in the CP, D_2-receptor-mediated inhibition of cholinergic neurones is a normally occurring regulatory mechanism.

Although activation of D_2-receptors reduces the formation of cyclic AMP in the CP, it has been suggested that the D_2-receptor-mediated inhibition of acetylcholine release does not operate through the adenylate cyclase pathway (Stoof and Kebabian 1982). The modulation of the release of acetylcholine in the ACC provides an additional argument for this view. In the ACC, in contrast to the CP, D_2-receptors may not be linked to adenylate cyclase inhibition at all (Stoof and Verheijden 1986; Kelly and Nahorski 1987), a possibility that, of course, requires a cyclase-independent transduction mechanism for the dopaminergic inhibition of acetylcholine release.

After this manuscript had been submitted for publication, we became aware of two recent similar studies in rat ACC slices. Apomorphine, bromocriptine (Gold and Bluth 1985) and quinpirole (Stoof et al. 1987) reduced the release of ^{3}H-acetylcholine elicited by high K^+ (Gold and Bluth 1985) or electrical stimulation (Stoof et al. 1987), an effect blocked by dopamine receptor antagonists. Our results confirm these findings and demonstrate, moreover, that the dopamine D_2-receptors involved are activated by endogenous dopamine under appropriate conditions. Dopamine in the ACC is important for various types of behaviour as well as for the effect of drugs of abuse (see Hernandez and Hoebel 1988). Dopaminergic inhibition of acetylcholine release, as shown here for amphetamine, may be one pathway through which these types of behaviour and these drug effects are expressed.

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References

Baud P, Arbilla S, Langer SZ (1985) Inhibition of the electrically evoked release of [^{3}H]acetylcholine in rat striatal slices: an experimental model for drugs that enhance dopaminergic neurotransmission. J Neurochem 44:331–337

Björklund A, Lindvall O (1984) Dopamine-containing systems in the CNS. In: Björklund A, Hökfelt T (eds) Handbook of chemical neuroanatomy, vol 2. Elsevier, Amsterdam, pp 55–122

Cantrill R, Arbilla S, Zivkovic B, Langer SZ (1983) Amphetamine enhances latent dopaminergic neurotransmission in the rat striatum. Effects on ^{3}H-acetylcholine release. Naunyn-Schmiedeberg's Arch Pharmacol 322:322–324

Consolo S, Ladinsky H, Bianchi S, Ghezzi D (1977) Apparent lack of a dopaminergic-cholinergic link in the rat nucleus accumbens septi-tuberculum olfactorium. Brain Res 135:255–263

Consolo S, Wu CF, Fusi R (1987) D-1 receptor-linked mechanism modulates cholinergic neurotransmission in rat striatum. J Pharmacol Exp Ther 242:300–305

Cubeddu LX, Hoffmann IS (1983) Frequency-dependent release of acetylcholine and dopamine from rabbit striatum: its modulation by dopaminergic receptors. J Neurochem 41:94–101

de Belleroche J, Gardiner IM (1983) Action of apomorphine, bromocriptine and lergotrile on γ-aminobutyric acid and acetylcholine release in nucleus accumbens and corpus striatum. J Neural Transm 58:153–168

de Belleroche JS, Neal MJ (1982) The contrasting effects of neuroleptics on transmitter release from the nucleus accumbens and corpus striatum. Neuropharmacology 21:529–537

Dubocovich ML, Weiner N (1981) Modulation of the stimulation-evoked release of [^{3}H]dopamine in the rabbit retina. J Pharmacol Exp Ther 219:701–707

Gold R, Bluth R (1985) Dopaminergic modulation of acetylcholine release from slices of rat nucleus accumbens. Biogenic Amines 2:211–218

Helmreich I, Reimann W, Hertting G, Starke K (1982) Are presynaptic dopamine autoreceptors and postsynaptic dopamine receptors in the rabbit caudate nucleus pharmacologically different? Neuroscience 7:1559–1566

Hernandez L, Hoebel BG (1988) Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Sci 42:1705–1712

Hertting G, Zumstein A, Jackisch R, Hoffmann I, Starke K (1980) Modulation by endogenous dopamine of the release of acetylcholine in the caudate nucleus of the rabbit. Naunyn-Schmiedeberg's Arch Pharmacol 315:111–117

Hoffmann IS, Cubeddu LX (1984) Differential effects of bromocriptine on dopamine and acetylcholine release modulatory receptors. J Neurochem 42:278–282

Joyce JN, Marshall JF (1987) Quantitative autoradiography of dopamine D_2 sites in rat caudate-putamen: localization to intrinsic neurons and not to neocortical afferents. Neuroscience 20:773–795

Kelly E, Nahorski SR (1987) Dopamine D-2 receptors inhibit D-1 stimulated cyclic AMP accumulation in striatum but not limbic forebrain. Naunyn-Schmiedeberg's Arch Pharmacol 335:508–512

König JFR, Klippel RA (1963) The rat brain. A stereotaxic atlas of the forebrain and lower parts of the brain stem. Krieger, Huntington, New York

Lehmann J, Langer SZ (1983) The striatal cholinergic interneuron: synaptic target of dopaminergic terminals? Neuroscience 10:1105–1120

Mao CC, Cheney DL, Marco E, Revuelta A, Costa E (1977) Turnover times of gamma-aminobutyric acid and acetylcholine in nucleus caudatus, nucleus accumbens, globus pallidus and substantia nigra: effects of repeated administration of haloperidol. Brain Res 132:375–379

McGeer PL, Kimura H, McGeer EG, Peng JH (1982) Cholinergic systems in the CNS. In: Bradford HF (ed) Neurotransmitter interaction and compartmentation. Plenum, New York, pp 253–289

Setler PE, Sarau HM, Zirkle CL, Saunders HL (1978) The central effects of a novel dopamine agonist. Eur J Pharmacol 50:419–430

Stadler H, Gadea Ciria M, Bartholini G (1975) "In vivo" release of endogenous neurotransmitters in cat limbic regions: effect of chlorpromazine and of electrical stimulation. Naunyn-Schmiedeberg's Arch Pharmacol 288:1–6

Starke K (1979) Presynaptic regulation of release in the central nervous system. In: Paton DM (ed) The release of catecholamines from adrenergic neurons. Pergamon, Oxford, pp 143–183

Starke K, Montel H, Gayk W, Merker R (1974) Comparison of the effects of clonidine on pre- and postsynaptic adrenoceptors in the rabbit pulmonary artery. Naunyn-Schmiedeberg's Arch Pharmacol 285:133–150

Starke K, Späth L, Lang JD, Adelung C (1983) Further functional in vitro comparison of pre- and postsynaptic dopamine receptors in the rabbit caudate nucleus. Naunyn-Schmiedeberg's Arch Pharmacol 323:298–306

Stoof JC, Kebabian JW (1982) Independent in vitro regulation by the D-2 dopamine receptor of dopamine-stimulated efflux of cyclic AMP and K^+-stimulated release of acetylcholine from rat neostriatum. Brain Res 250:263–270

Stoof JC, Verheijden PFHM (1986) D-2 receptor stimulation inhibits cyclic AMP formation brought about by D-1 receptor stimulation in rat neostriatum but not nucleus accumbens. Eur J Pharmacol 129:205–206

Stoof JC, Thieme RE, Vrijmoed-de Vries MC, Mulder AH (1979) In vitro acetylcholine release from rat caudate nucleus as a new model for testing drugs with dopamine-receptor activity. Naunyn-Schmiedeberg's Arch Pharmacol 309:119–124

Stoof JC, Verheijden PFHM, Leysen JE (1987) Stimulation of D_2-receptors in rat nucleus accumbens slices inhibits dopamine and acetylcholine release but not cyclic AMP formation. Brain Res 423:364–368

Wichmann T, Illing RB, Starke K (1987) Evidence for a neurotransmitter function of acetylcholine in rabbit superior colliculus. Neuroscience 23:991–1000

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