Cholinergic modulation of \[^3H\]dopamine release from dendrosomes of rat substantia nigra*

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Summary. Dendrosomes prepared from substantia nigra are able to take up and release \[^3H\]dopamine in a Ca\(^{2+}\)-dependent manner. The \(V_{\text{max}}\) values of \[^3H\]dopamine uptake in substantia nigra dendrosomes was about 5 times lower than that in caudate putamen synaptosomes. The pattern of the K\(^+\)-dependency of the \[^3H\]dopamine release in substantia nigra dendrosomes was significantly different from that found in caudate putamen synaptosomes. The release of \[^3H\]dopamine evoked by 15 mmol/l KCl from superfused dendrosomes was increased in a concentration-dependent manner by acetylcholine. The maximal potentiation produced by acetylcholine was about 40%. The potentiation of \[^3H\]dopamine release by 10 μmol/l acetylcholine was insensitive to mecamylamine but antagonized by atropine and by pirenzepine. The effects of acetylcholine on the release of \[^3H\]acetylcholine from substantia nigra nerve endings was also studied. Exogenous acetylcholine added to the superfusion medium decreased in a concentration-dependent manner the release of acetylcholine. This effect was not antagonized by mecamylamine or pirenzepine but fully antagonized by atropine. The data suggest the existence, in the substantia nigra of the rat, of two distinct muscarinic receptor subtypes regulating respectively dopamine release from dopamine dendrites and acetylcholine release from cholinergic nerve terminals.

Key words: Substantia nigra dendrosomes — \[^3H\]Dopamine release — \[^3H\]Acetylcholine release — Muscarinic receptor subtypes

Introduction

Among the multiple modulations of striatal dopamine release by several endogenous agents (for reviews see Chesselet 1984; Starke et al. 1989) the release of the catecholamine has been reported to be enhanced by acetylcholine. In particular it has been shown that cholinergic agents potentiate the release of \[^3H\]dopamine in the corpus striatum both from tissue slices and from isolated nerve endings (Giorguieff et al. 1977; Helmreich et al. 1982; Lehmann and Langer 1982; Raiteri et al. 1982). The potentiation of dopamine release occurs through the activation of muscarinic receptors located on striatal dopaminergic terminals and which belong to the M\(_1\) receptor subtype (Raiteri et al. 1984). It has been shown that dopamine nigral neurons are able to release dopamine not only at the level of their axon terminals in the caudate putamen but also from their dendrites in the substantia nigra (Nieouillon et al. 1977; Hattori et al. 1979; Chéramy et al. 1981; Nissbrandt et al. 1989). In particular it has been reported that homogenization of nigral tissue originates particles which are able to take up and release \[^3H\]dopamine and, which in analogy with synaptosomes, have been termed dendrosomes (Hefti and Liechtensteiger 1978a,b; Silbergeld and Walters 1979; see also Discussion).

Receptors of various types are present at the soma-dendritic level in the substantia nigra as well as on the caudate putamen dopaminergic terminals. Inasmuch as in other systems receptors, apparently subserving similar functions (for instance autoreceptors) are present both at the terminal level and at the soma-dendritic level but are pharmacologically different (De Montigny et al. 1984; Engel et al. 1986; Maura et al. 1989; Piercey and Lum 1990) it seemed of interest to investigate whether the dopamine nigral dendritic particles possess, similarly to the striatal dopamine nerve endings, release-regulating muscarinic receptors. The comparison between caudate putamen synaptosomes and substantia nigra dendrosomes has also been extended to other functional properties.

Materials and methods

Preparation of dendrosomes and synaptosomes

Adult male Wistar rats weighing 200–250 g were used. Rats were killed by decapitation, the brain was rapidly removed and the regions used

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(caudate-putamen or substantia nigra) were dissected out. Crude P2 fractions were prepared as previously described (Gray and Whittaker 1962; Raiteri et al. 1984). Briefly the tissue was homogenized in 40 volumes of 0.32 mol/l sucrose buffered at pH 7.4 with phosphate. The homogenate was centrifuged (5 min, 1000 g) to remove nuclei and debris and P2 fractions were isolated from the supernatant by centrifugation at 12000 g for 20 min. The P2 pellet was then resuspended in a physiological medium having the following composition (mmol/l): NaCl 125, KCl 3, CaCl2 1.2, MgSO4 1.2, NaH2PO4 1, NaHCO3 22, glucose 10 (aeration with 95% O2 and 5% CO2 at 37°C; pH 7.2-7.4). Protein was measured by a modification of the method of Lowry et al. (Petersen 1977).

**Uptake studies**

[^3H]dopamine uptake was studied in dendrosomes or synaptosomes from substantia nigra and caudate putamen or in tissue slices from the two areas.

Aliquots of dendrosomes or synaptosomes (500 µl; 100–200 µg/prot) were preincubated 10 min at 37°C in a rotary water-bath. Different concentrations (from 0.08 µmol/l to 1.28 µmol/l) of[^3H]dopamine were then added and incubation was continued for 2 min. At the end of the incubation period, aliquots (2 × 100 µl) of the dendrosomes or synaptosomes were transferred onto Whatman glass microfiber filters (GF/B). The particles were then washed twice with two 5-ml aliquots of medium. Each filter was removed, placed in a scintillation vial with 0.5 ml of 2 × 100 sodium dodecylsulfate and counted for radioactivity in a Packard liquid scintillation counter. Blank values were obtained by maintaining the samples at 0°C. In all experiments desipramine (1 µmol/l) and citalopram (0.3 µmol/l) were present in dendrosome or synaptosome suspensions from the beginning of the preincubation to prevent uptake of[^3H]dopamine into noradrenergic or serotonergic nerve terminals. The apparent Vmax and Km values were then calculated.

Substantia nigra slices (250 µm) were prepared by a McIlwain tissue chopper. The slices were transferred into vials and preincubated for 10 min at 37°C in a rotary water bath. The uptake of[^3H]dopamine in the slices was studied essentially with the same procedure used to study[^3H]dopamine uptake in synaptosomes and dendrosomes above described. Blank values were obtained by maintaining the slices at 0°C in presence of a high concentration of dopamine (10 µmol/l).

**Release studies**

**Depolarization-evoked release of[^3H]dopamine from dendrosomes and[^3H]acetylcholine from synaptosomes.** Substantia nigra dendrosomes were incubated with[^3H]dopamine (final concentration 0.01 µmol/l). Synaptosomes prepared with substantia nigra were prelabelled with[^3H]choline (final concentration 0.04 µmol/l) for 15 min at 37°C. As in the case of the uptake studies, the incubation with[^3H]dopamine was performed in presence of 1 µmol/l desipramine and 0.3 µmol/l citalopram (see above). Aliquots of the suspension were layered on 0.65-gin Millipore filters (0.20-0.50 mg of protein per filter) at the bottom of several parallel superfusion chambers (Raiteri et al. 1974). Superfusion was then started at a rate of 0.6 ml/min with standard medium aerated with O2/CO2. After 36 min to equilibrate the system fractions were collected according to the following scheme: two 3-min samples (basal outflow) one before and one after one 6-min sample (K+-evoked release). A 90-s period of depolarization was applied at the end of the first fraction collected by exposing the synaptosomes to high-K+. Unless otherwise stated the concentration of KCl used was 15 mmol/l (substituting an equimolar concentration of NaCl). Agonists were added concomitantly with high-K+ and antagonists 18 min before depolarization and also concomitantly with high-K+. Fractions collected and superfused filters were then counted for radioactivity. The amount of radioactivity released into each fraction was expressed as the percentage of the total synaptosomal tritium present on each filter at the start of the respective collection period. The K+-evoked overflow was estimated by subtracting from the evoked release the basal outflow. It has been already shown that the K+-evoked release of tritium in different experimental conditions was largely ac-

 counted for by unmetabolized[^3H]dopamine or[^3H]acetylcholine (Raiteri et al. 1984 and unpublished observations). In the case of the studies of the effect of GABA on basal release of[^3H]dopamine a separation between[^3H]dopamine and its metabolites was necessary and it has been performed.

**Basal release of[^3H]dopamine from substantia nigra dendrosomes.** Aliquots of dendrosomes from substantia nigra were incubated with[^3H]dopamine and layered on Millipore filters as described above. Superfusion was started with standard medium and after 36 min to equilibrate the system 8 separate 2-min fractions were collected. P2 particles were exposed to GABA, (-)baclofen or muscimol, at the end of the 2nd fraction collected. Bicuculline and SKF 89976A were added 8 min before GABA. Superfusate fractions were collected into vials containing 100 µl of a protective solution (1.5% EDTA, 1% ascorbic acid and 0.01% unlabeled dopamine) and the[^3H]dopamine present in each fraction and that remaining in the filters at the end of superfusion was separated from[^3H]deaminated metabolites on Bio Rex 70 (Bio Rad) columns according to the method of Smith et al. (1975). The release of[^3H]dopamine in the superfuse samples was calculated as the percentage of[^3H]dopamine content of dendrosomes at the onset of the respective collection period. The effects of the drug tested on[^3H]dopamine release were evaluated by obtaining the ratio between the percentage efflux in the fraction corresponding to the maximal effect of GABA (in general the 7th fraction collected) and that in the 2nd fraction. This ratio was compared to a corresponding ratio obtained under control conditions.

**Drugs**

[^3H]dopamine (spec. act. 49.6 Ci/mmol) and[^3H]Ch (spec. act. 76.0 Ci/mmol) were obtained from Amersham Radiochemical Centre (UK). GABA from Serva (Heidelberg, FRO), (+)bicuculline, atropine, oxotremorine, acetylcholine and mecamylamine from Sigma (St. Louis, MO, USA). The following drugs were generous gifts by the companies indicated: (-)baclofen (Ciba Geigy, Basel, Switzerland); muscimol (Zambon Farmaceutici, Milan, Italy), N-(4,4-diphenyl-3-buteryl)-nicotinic acid (SKF 89976A) (Smith Kline and French, Welwyn, UK), pirenzepine (De Angeli, Milan, Italy).

**Results**

Figure 1a reports the kinetics of[^3H]dopamine uptake in caudate putamen synaptosomes and substantia nigra dendrosomes. The Vmax values of[^3H]dopamine uptake in dendrosomes (66 picomol/mg prot/2 min) was almost 5 times lower than that in synaptosomes (235 picomol/mg prot/2 min). However the Km values of[^3H]dopamine uptake in the two preparations (36–57 µmol/l in dendrosomes and 12–34 µmol/l in synaptosomes) were in the same range. Figure 1b shows the data of[^3H]dopamine uptake in slices from substantia nigra or caudate putamen respectively. Similarly to what observed in dendrosomes or in synaptosomes,[^3H]dopamine uptake in substantia nigra dendrosomes was almost 5 times lower than in the slices of caudate putamen. Figure 1c shows the K+-dependency of the release of[^3H]dopamine from substantia nigra dendrosomes or caudate putamen synaptosomes previously labeled with the radioactive catecholamine. The percentage of the K+-dependency of the[^3H]dopamine released by the elevated K+ concentrations was smaller in substantia nigra dendrosomes than in caudate putamen synaptosomes, the latter particles being clearly more sensitive to the depolarizing stimulus. The pattern of the Ca2+-dependency of the K+-evoked re-