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UPTAKE OF RADIOCALCIUM BY NERVE ENDINGS ISOLATED FROM RAT BRAIN: PHARMACOLOGICAL STUDIES

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1 The uptake of radiocalcium by nerve-ending particles isolated from the striatum of rat brain was studied using lanthanum as a quenching agent.

2 High potassium-induced calcium uptake occurred in two phases: an initial rapid phase and a late slow phase. Following preincubation with $CaCl_2$ 2.2 mmol/l for 1 h, dopamine at 1 to 2 $\times 10^{-4}$ mol/l reduced the high potassium-induced calcium uptake which occurred during the initial rapid phase by 66 and 25% at 2 and 4 s of incubation, respectively, but had no effect on the late slow uptake phase.

3 Haloperidol at 1×10^{-6} mol/l abolished the inhibitory effect of dopamine on the initial rapid phase of the high potassium-induced calcium uptake. Haloperidol *per se* had no effect on the calcium uptake.

4 Dibutyryl cyclic adenosine monophosphate at 2.5×10^{-3} mol/l or prostaglandin E_1 (PGE_1) at 1×10^{-5} mol/l had no effect on the initial rapid phase of the high potassium-induced calcium uptake by striatal synaptosomes. Neither of these agents affect calcium uptake by whole brain synaptosomes.

5 It appears that in the striatum, dopamine regulates the depolarization-induced influx of calcium in presynaptic nerve endings. This mechanism could constitute a feed-back inhibition for transmitter release in the striatum.

Introduction

Noradrenaline probably regulates its own release by means of negative feed-back mediated through presynaptic α-receptors at peripheral adrenergic synapses (see Häggendal, 1974; Westfall, 1977 and refs. cited). The mechanism of this inhibition of transmitter release probably involves a restriction of the availability of calcium ions essential for the release process (see Stjärne, 1973; Westfall & Leighton, 1976). Studies conducted by Kehr, Carlsson, Lindqvist, Magnusson & Atack (1972), Farnebo & Hamberger (1971) and Di Chiara, Porceddu, Spano & Gessa (1977) indicate that a feed-back inhibition mediated through a presynaptic D_2-receptor regulation of tyrosine hydroxylase activity (for review see Kebabian & Calne, 1979 and Roth, Salzman & Nowycky, 1978), may be operative in striatal dopaminergic nerve endings. However, in the central nervous system the possible existence of physiological regulatory mechanisms of calcium fluxes has not been investigated. This is in part due to the fact that the study of calcium fluxes is methodologically complicated. In the preceding paper we have shown that the uptake of calcium by nerve endings occurs in two phases; an initial fast phase and a late slow phase. With this insight, in the present paper we attempt to identify and characterize a mechanism for regulation of calcium fluxes by studying the effect of dopamine and of a dopamine antagonist, haloperidol, on the uptake of radiocalcium by synaptosomes from the striatum of rat brain. In addition, the effect of prostaglandin E_1 (PGE_1) and dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP), agents known to modulate transmitter release probably by regulating calcium influx (for PGE_1 see Hedqvist 1973, Bergström, Farnebo & Fuxe, 1973, Westfall, 1977 and for cyclic AMP see Miyamoto & Breckenridge, 1974; Standaert, Dretchen, Skirboll & Morgenroth, 1976a, b), on the uptake of radiocalcium by striatal and whole brain synaptosomes was studied.

Methods

Isolation of synaptosomes

The striata were dissected from the brains of adult male rats of the Sprague–Dawley strain according to Glowinski & Iversen (1966) and whole brains were removed as previously described (Gripenberg, Heino-

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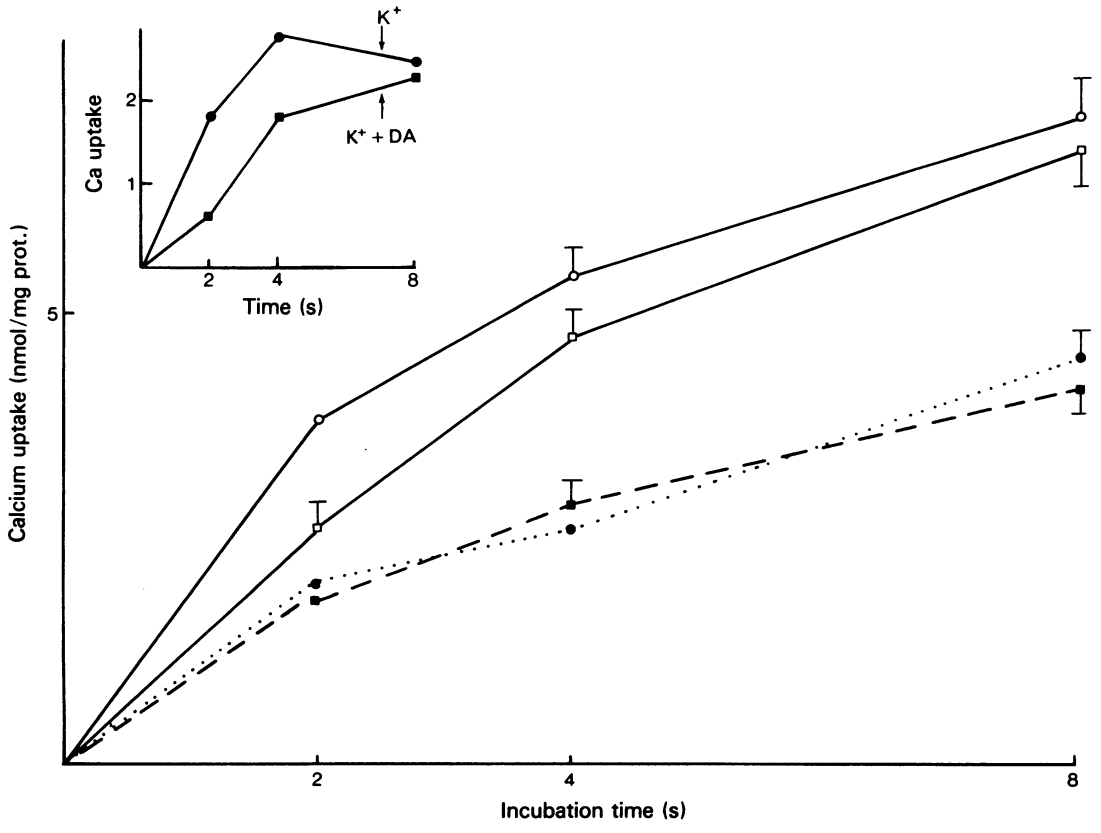

Figure 1 Inhibition of calcium uptake by dopamine in K^+-depolarized synaptosomes. The calcium uptake values are expressed as mean from 6 experiments, vertical lines show s.e. mean: (●) control medium without dopamine; (■) control with dopamine 1 to 2 × 10^{-4} mol/l. The K^+-stimulated calcium uptake values with (□) and without dopamine (○) showed highly significant differences at the 0.5% level ($P < 0.005$) at 2 s, and almost significant differences at the 5% level ($P < 0.05$) at 4 s (matched-pair t test). In the inset the K^+-induced uptake is obtained by subtracting the pooled mean of controls at each time point from the corresponding pooled mean of the K^+-stimulated uptake values in 6 paired experiments. Preincubation with dopamine continued for 1 min before radiocalcium was added.

nen & Jansson, 1980). Synaptosomes were isolated from homogenates by differential and density gradient centrifugation essentially according to Gray & Whittaker (1962). A more detailed description of the isolation procedure was presented in the preceding paper (Gripenberg *et al.*, 1980).

Solutions and chemicals

The millimolar composition of the electrolyte solutions used for incubations has been given earlier (Gripenberg *et al.*, 1980). The concentration of Na^+ was decreased when that of K^+ was increased in order to retain iso-osmolarity. ^{45}Ca, specific activity 10.5 mCi/mg Ca^{2+}, was obtained from the Radiochemical Centre, Amersham, N^6,$O^{2'}$-dibutyryl adenosine 3',5'-cyclic monophosphoric acid (db cyclic AMP) from the Sigma Chemical Co., St. Louis, Missouri, U.S.A., prostaglandin E_1 from the Upjohn Co., Michigan, U.S.A., dopamine hydrochloride from Fluka AG., Switzerland and haloperidol from Orion Oy., Finland.

Radiocalcium uptake studies

Synaptosomes were resuspended in incubation medium at a protein concentration of 1.5 to 3.0 mg/ml and exposed to $CaCl_2$ 2.2 mmol/l for 1 h at 0°C before starting the experiments. Following preincubation for 10 min at 37°C, synaptosomes were loaded with radiocalcium for time periods ranging from 2 s to 2 min. The uptake of ^{45}Ca was initiated by rapidly mixing 100 µl of synaptosome suspension and 100 µl of incubation medium supplemented with

Table 1 Effect of dopamine on potassium-induced calcium uptake in striatal synaptosomes

	Calcium uptake		
Incubation time (s)	8	15	60
Control	2.85 ± 0.72 (5)	2.91 ± 0.32 (8)	5.72 ± 0.73 (7)
Dopamine	3.85 ± 0.89 (5)	3.09 ± 0.56 (8)	6.17 ± 0.57 (7)

K^+-induced calcium uptake was obtained by subtracting the uptake value in the control medium, 5.6 mmol/l K^+, from the uptake value in the 40 mmol/l K^+ medium with and without dopamine in paired experiments. Preincubation with dopamine for 1 min before radiocalcium was added. Incubation with radiocalcium for 8, 15 and 60 s, respectively. A matched-pair *t* test showed no significant difference between control and dopamine. The uptake values are given as nmol Ca/mg prot. Means ± s.e. mean. Number of experiments in parentheses.

radiocalcium at a specific activity of 0.1 mCi $mmol^{-1}$ l^{-1} whereafter the uptake was stopped by diluting the samples with 3 ml of ice-cold incubation medium, without added calcium or phosphate ions, but supplemented with 0.5 mmol/l $LaCl_3$ (van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973). Methods for collection of synaptomes and determination of radioactivity and synaptosomal protein are as in the preceding paper (Gripenberg *et al.*, 1980).

Results

Inhibition of calcium uptake by dopamine in K^+-depolarized synaptosomes

In control medium the calcium uptake was 1.98 ± 0.46, 2.59 ± 0.52 and 4.51 ± 0.60 nmol Ca/mg prot. at 2, 4 and 8 s respectively, dopamine at 1 to 2×10^{-4} mol/l had no effect on this uptake as shown in Figure 1. In a medium containing 40 mmol/l K^+, the calcium uptake increased and was 1.6 to 2.1 times the control values. At 2 s, 4 s and 8 s respectively dopamine decreased this uptake to 68%, 87% and 94% of that occurring in 40 mmol/l K^+ medium without dopamine (Figure 1). Taking into account only the high K^+-induced calcium uptake (i.e. the uptake in control medium without dopamine subtracted from the uptake in 40 mmol/l K^+ medium), the uptake values in the presence of dopamine were reduced to 34% at 2 s, 75% at 4 s and 84% at 8 s (Figure 1, inset). The inhibition by dopamine was statistically significant only during the initial seconds ($P < 0.005$ and 0.05 at 2 and 4 s, respectively). When the incubation time was prolonged, no effect was obtained even at high dopamine concentrations (5×10^{-4} to 1×10^{-3} mol/l, Table 1). At a lower calcium concentration of 1.1 mmol/l (preincubation for 1 h at 1.1 mmol/l $CaCl_2$ at 0°C) dopamine at 5×10^{-4} mol/l had no effect on high potassium-induced calcium uptake (results from 6 experiments) although a potentiating effect due to decreased extracellular Ca^{2+} concentration on the negative feed-back inhibition of transmitter release at peripheral adrenergic synapses has been reported (for ref. see Westfall 1977).

Haloperidol reverses the inhibitory effect of dopamine on K^+-induced calcium uptake

Haloperidol at 1×10^{-6} mol/l (preincubation for 10 min at 37°C) blocked the inhibitory effect of dopamine at 5×10^{-4} mol/l on the high potassium-induced calcium uptake during the first 4 s. At the concentration used, haloperidol alone had no effect (Table 2) on the uptake of calcium.

Table 2 Effect of dopamine and haloperidol on potassium-induced calcium uptake in striatal synaptosomes

	Calcium uptake		
Incubation time (s)	2	4	8
Control	0.61 ± 0.15 (7)	0.85 ± 0.17 (8)	0.90 ± 0.12 (6)
Haloperidol	0.94 ± 0.52 (7)	0.70 ± 0.11 (8)	1.01 ± 0.19 (6)
Dopamine + haloperidol	0.64 ± 0.14 (7)	1.60 ± 0.38 (8)	1.31 ± 0.21 (6)

A matched-pair *t* test showed no significant difference from the control. K^+-induced uptake in nmol Ca/mg prot. (obtained as in Table 1). Means ± s.e. mean. Number of experiments in parentheses.

Db cyclic AMP, prostaglandin E_1 and uptake of calcium into synaptosomes

When present for 1 min before the addition of radiocalcium at 2.2 mmol/l, db cyclic AMP at 2.5×10^{-3} mol/l had no effect on the high potassium-induced calcium uptake into striatal synaptosomes (Table 3). This lack of effect was confirmed on whole brain synaptosome preparations. Without equilibration of the synaptosomes with $CaCl_2$ before the addition of ^{45}Ca, the high potassium-induced calcium uptake was 1.7 ± 0.31, 2.7 ± 0.38 and 3.5 ± 0.19 nmol/mg prot. at 2, 4 and 8 s respectively. With db cyclic AMP at 1 to 3×10^{-3} mol/l, the corresponding uptake values did not differ significantly from the controls (2.2 ± 0.27, 2.8 ± 0.40 and 3.8 ± 0.57 nmol/mg prot. respectively; means ± s.e. mean of 3 to 5 experiments). Pre-equilibration with $CaCl_2$ (2.2 mmol/l) lowered the high potassium-induced calcium uptake by about 28% but not even together with theophylline at 3 mmol/l (added 30 min before the uptake experiment was started) did db cyclic AMP affect calcium uptake during the first 8 s. Prolongation of the incubation time to include the late slow calcium uptake phase was not undertaken.

The effect of PGE_1 was studied using striatal as well as whole brain synaptosomes. With PGE_1 at 5×10^{-6} mol/l the high potassium-induced fast initial uptake into striatal synaptosomes did not differ significantly from the control values, which were 1.78 ± 0.45, 1.86 ± 0.46 and 3.33 ± 0.20 nmol Ca^{2+}/mg prot. at 2, 4 and 8 s, respectively (means ± s.e. mean from 3 experiments). With whole brain synaptosomes and 7, 15, 60 and 120 s incubation times the uptake in the control medium was 2.19 ± 0.19 and 5.96 ± 0.32 nmol Ca^{2+}/mg prot. at 7 and 120 s, respectively and with PGE_1 at 1×10^{-5} mol/l the uptake values in four paired experiments were within 83 to 107% of the control values. In high-potassium medium the uptake was 6.19 ± 0.49 and 12.5 ± 0.72 nmol Ca^{2+}/mg prot. at 7 and 120 s, respectively and with PGE_1 at 1×10^{-5} mol/l the corresponding values in four paired experiments were within 98 to 110% of the controls. None of the uptake values in the presence of PGE_1 differed in a statistically significant manner from the control values.

Discussion

Morphological evidence suggests that in the striatum, there is a rich supply of cholinergic and dopaminergic nerve terminals and that some 25% of all the nerve endings are of the dopaminergic type (Hattori, McGeer & McGeer, 1976). Further, the nerve terminals of corticostriatal neurones (see Hedreen, 1977) bind dopamine, indicating the presence of dopamine (D_2) receptors in these nerve endings (for review, see Kebabian & Calne 1979). Striatal synaptosomes are thus suitable for the study of the effects of dopamine on calcium uptake by nerve endings.

The main finding of the present study was the clear-cut inhibitory effect of dopamine on the high potassium-induced calcium uptake during the very early uptake phase. The results indicate that dopamine regulates calcium influx into striatal nerve endings and thus it possibly modulates transmitter release. Further, part of the possible feed-back inhibition of transmitter release in dopaminergic nerve terminals may occur by means of restriction of calcium influx during the depolarization-secretion coupling process. According to Roth *et al.* (1978), dopamine receptor agonists cause a significant decrease and antagonists an increase in the activation of striatal tyrosine hydroxylase activity induced by electrical stimulation, indicating a feed-back inhibition of transmitter synthesis mediated via presynaptic D_2-receptors. On the other hand, the increased activity of striatal tyrosine hydroxylase seen after removal of Ca^{2+}-ions from the incubation medium (Goldstein, Backstrom, Dhi & Frankel, 1970) and after addition of EGTA (Morgenroth, Boadle-Biber & Roth, 1976, for review see Roth *et al.*, 1978) indicates that in the striatum a decrease in extracellular calcium paradoxically increases transmitter synthesis in dopaminergic neurones.

Although haloperidol is probably not the most specific antagonist of presynaptic D_2-receptors (Kebabian & Calne 1979), a major part of haloperidol bind-

Table 3 Effect of dibutyryl cyclic AMP on potassium-induced calcium influx in striatal synaptosomes

	Calcium uptake		
Incubation time (s)	2	4	8
Control	0.74 ± 0.11 (4)	0.80 ± 0.26 (5)	1.50 ± 0.53 (5)
db cyclic AMP	0.81 ± 0.10 (4)	0.63 ± 0.08 (5)	1.14 ± 0.02 (5)

No significant difference was found between controls and db cyclic AMP using a matched-pair *t* test. K^+-induced uptake in nmol Ca/mg prot. (obtained as in Table 1). Means ± s.e. mean. Number of experiments in parentheses.

ing in the striatum occurs at the nerve terminals projected from other brain regions, chiefly the cortex (Schwarz, Creese, Coyle & Snyder 1978). Hence the observation that haloperidol abolishes the inhibition by dopamine of high potassium-induced calcium uptake can be taken to suggest that this inhibition occurs through stimulation of striatal D_2-receptors. D_2-receptors are not linked to adenylyl cyclase (Kebabian & Calne, 1979), which could explain why db cyclic AMP in our hands had no effect on calcium uptake into striatal synaptosomes. It has been shown that db cyclic AMP and depolarizing agents e.g. veratridine and ouabain increase tyrosine hydroxylase activity in striatal synaptosomes (Goldstein, Bronaugh, Ebstein & Roberge 1976) and that the effects of cyclic AMP and potassium-depolarization are additive (Roth *et al.*, 1978). In the present study db cyclic AMP had no effect on the high potassium-induced calcium uptake into striatal synaptosomes, which may indicate that the cyclic AMP-mediated increase of tyrosine hydroxylase activity does not involve changes in calcium influx into the nerve terminals.

Calcium has been found to displace neuroleptics, including haloperidol, from synaptosomal membranes (Seeman, Staiman & Chau-Wong. 1974) and neuroleptics (chlorpromazine) are thought to suppress depolarization-sensitive Ca^{2+} influx and thus inhibit transmitter release at the mammalian neuromuscular junction (Quastel, Hackett & Okamoto 1971). In spite of these possible interactions, haloperidol seemed to have no clear-cut effect on high potassium-induced calcium uptake in our striatal preparations.

In slices from striatum of rat brain the prostaglandin derivative E_2 seems to modulate stimulation-induced dopamine release (Bergström *et al.*, 1973). Together with the observations on peripheral adrenergic synapses, this modulation might be taken to reflect a restriction of calcium influx needed in depolarization-secretion coupling of transmitter release (see Hedqvist, 1973 and review by Westfall, 1977). However, the present results, do not support the theory that the modulatory effects of PGE derivatives on transmitter release in the central nervous system are mediated via regulation of calcium fluxes, as in our hands PGE_1 did not influence calcium uptake in either whole brain or in striatal synaptosomes. In interpreting the negative results with PGE_1 and db cyclic AMP on whole brain synaptosomes, it must be kept in mind that the preparation is heterogeneous and represents a mixture of various kinds of chemical synapses. Further, in accordance with the well-documented postsynaptic membrane effects of cyclic AMP (Nathanson & Greengard, 1977), any hypothetical regulatory effect on the presynaptic side might require minutes rather than seconds to be operative.

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