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THE EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS ON Na^+,K^+-ATPase AND Mg^{2+}-ATPase ACTIVITIES OF SYNAPTOSOMES

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Abstract—The effects of dopamine agonists and antagonists on the Na^+,K^+-ATPase and Mg^{2+}-ATPase activities of rat cerebral cortex synaptosomes have been determined. Dopamine, ADTN, apomorphine and S-584, but not piribedil, stimulated the activities of the enzymes. The stimulatory effect of dopamine was not antagonised by dopamine antagonists and apparently the catechol group is responsible for the enzyme stimulation.

Our previous results suggested that the Na^+,K^+-ATPase and Mg^{2+}-ATPase activities of rat cerebral cortex synaptosomes were stimulated, through a mechanism of de-inhibition, by dopamine, L-DOPA, noradrenaline and isoprenaline. The stimulation caused by noradrenaline and isoprenaline was not antagonised by α- or β-adrenergic antagonists [1], and non-catecholamines such as phenylephrine, ephedrine and clonidine failed to stimulate the enzymes. It is therefore likely that the catechol group was responsible for the stimulation observed in that earlier work. It is well established that the two free hydroxyl groups on C_3 and C_4 are important for a dopaminergic effect and the monohydroxylated aminotetralin is five times less potent than an aminotetralin containing hydroxyl groups at both C_3 and C_4 [2]. Akagawa and Tsukada [3] reported that the stimulatory effect of dopamine on Na^+,K^+-ATPase activity of rat striatum was not antagonised by phentolamine or propranolol but it was antagonised by the dopamine antagonist, fluphenazine. The possibility that dopamine and L-DOPA exert their action on the Na^+,K^+-ATPase and Mg^{2+}-ATPase activities by a mechanism similar to that of noradrenaline and isoprenaline, a mechanism depending upon the presence in the molecules of the catechol nucleus, has been investigated further in the present work.

MATERIALS AND METHODS

Cerebral cortices from male Sprague–Dawley rats (200–300 g) were used for the preparation of synaptosomes as previously mentioned [4]. ATPase activities were determined by measuring the hydrolysis of ATP (4 mM) in buffered media containing 0.07–0.12 mg protein of enzyme preparation. Na^+,K^+-ATPase activity was determined by subtracting the inorganic phosphate released in a medium containing (final concentration) ATP (4 mM, Vanadium free), NaCl (150 mM) and $MgCl_2$ (5 mM) in 50 mM imidazole/HCl buffer pH 7.4 from phosphate released in an identical medium which contained, in addition, KCl (10 mM). For Mg^{2+}-ATPase activity, the medium contained ATP (4 mM) and $MgCl_2$ (5 mM) in 50 mM imidazole/HCl buffer pH 7.4. The volume of each medium was 0.9 ml and it was made up to 1 ml in each case by the addition of 0.1 ml Tris–ATP when the reactions were started. Sodium dodecyl sulphate (DDS, 1 ml 0.8%) was used to stop the reactions after 10 min incubation at 37°. The phosphate contents of clear solutions were determined by the method of Bonting *et al.* [5]. Protein contents of solutions were determined by the method of Lowry *et al.* [6]. When used drugs were usually made up in 50 mM imidazole/HCl buffer pH 7.4 containing 25 mM $MgCl_2$ and added to the other components for the pre-incubation period of 10 min in the absence of ATP prior to starting the reaction. Controls in which DDS was added at the same time as the ATP were carried out simultaneously. Dopamine antagonists were added to the pre-incubation media 5 min before adding dopamine. Fresh solutions of the following drugs were prepared daily for each experiment: dopamine–HCl (Sigma, London, U.K.), sulpiride (Chemitechna, Petersfield, U.K.), metoclopramide (Lundbeck, Copenhagen, Denmark), domperidone, spiperone (Janssen Pharmaceutical, Beerse, Belgium), ADTN (2-amino-5,6-dihydroxytetrahydronaphthalene (Burroughs Wellcome, Raleigh, NC), piribedil (1-3,4 methylenedioxybenzyl-4-[2-pyrimidyl] piperazine) and S-584 (1-3,4-dihydroxybenzyl-4-[2-pyrimidyl] piperazine (Servier, Greenford, U.K.), apomorphine–HCl (Sigma). Haloperidol was used as a commercially available solution (Searle, High Wycombe, U.K.).

RESULTS

Effects of drugs on ATPase activities

Effects of dopamine. The Na^+,K^+-ATPase and Mg^{2+}-ATPase activities were increased by dopamine in a dose-dependent manner (Fig. 1a). The sodium, potassium-activated enzyme was markedly affected, the maximum increase in activity being some 54%

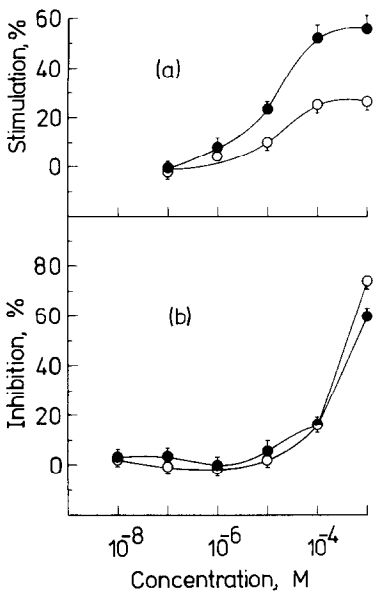

FIg. 1. Effects of dopamine (a) and haloperidol (b) on Na^+,K^+-ATPase (●) and Mg^{2+}-ATPase (○) activities of synaptosomes.

whereas the Mg^{2+}-ATPase was stimulated some 26% by 1×10^{-3} M dopamine.

Effects of dopamine antagonists. Haloperidol decreased the Na^+,K^+-ATPase and Mg^{2+}-ATPase activities in a dose-dependent manner (Fig. 1b). The maximum degrees of inhibition were 59 and 75%, respectively. Sulpiride did not significantly alter the activity of either enzyme at the concentrations of 10^{-7}, 10^{-6}, 5×10^{-4} (results not shown), 10^{-5} and 10^{-4} M (Table 1). Metoclopramide, domperidone and spiperone at a concentration of 10^{-5} M did not significantly affect the enzymic activities (Table 1).

Effects of dopamine antagonists on dopamine induced increases in the ATPase activities. Sulpiride at concentrations of 10^{-4} and 10^{-5} M and metoclopramide, haloperidol, domperidone and spiperone at a concentration of 10^{-5} M did not antagonise the stimulatory effects of dopamine (10^{-4} M) on the Na^+,K^+-ATPase and Mg^{2+}-ATPase activities (Table 1).

Effects of dopamine agonists. Table 2 shows that ADTN, S-584 and apomorphine at a concentration of 10^{-4} M significantly stimulated the Na^+,K^+-ATPase activity. Piribedil did not significantly alter the Na^+,K^+-ATPase activity. Mg^{2+}-ATPase activity was significantly stimulated by S-584, ADTN and to a smaller but significant extent by apomorphine. However, piribedil did not significantly alter the Mg^{2+}-ATPase activity (Table 2).

DISCUSSION

The present results are consistent with our previous findings [1] which have demonstrated the importance of catechol nucleus in the stimulation of Na^+,K^+-ATPase and Mg^{2+}-ATPase activities of synaptosomes.

Dopamine significantly stimulated the Na^+,K^+-ATPase and Mg^{2+}-ATPase activities in a dose-dependent manner and the dose–response curves were similar to those found for noradrenaline (see for example [4]). The dopamine effects were not antagonised by dopamine antagonists such as domperidone, metoclopramide, haloperidol or spiperone at a concentration of 10^{-5} M or by sulpiride at concentrations of 10^{-5} and 10^{-4} M. At these concentra-

Table 1. Effects of drugs on dopamine-stimulated ATPase activities of synaptosomes

Drug concentration (M)		Relative ATPase activities (control = 100 in each case) Na^+,K^+-ATPase	Mg^{2+}-ATPase	No. of experiments
Dopamine	10^{-4}	174.3 ± 4.0*	135.2 ± 0.9*	13
Domperidone	10^{-5}	101.3 ± 4.5	95.5 ± 1.2†	4
Domperidone + dopamine	10^{-5} 10^{-4}	159.0 ± 5.2*	132.0 ± 2.9*	4
Haloperidol	10^{-5}	102.8 ± 1.1	98.0 ± 2.5	4
Haloperidol + dopamine	10^{-5} 10^{-4}	161.5 ± 2.2*	128.0 ± 1.7*	4
Metoclopramide	10^{-5}	102.0 ± 2.6	100.0 ± 1.1	4
Metoclopramide + dopamine	10^{-5} 10^{-4}	171.0 ± 6.8*	132.3 ± 1.5*	4
Sulpiride	10^{-5}	101.0 ± 2.7	100.5 ± 1.8	4
Sulpiride + dopamine	10^{-5} 10^{-4}	173.0 ± 5.8*	129.3 ± 0.5*	4
Sulpiride	10^{-4}	99.0 ± 2.9	101.4 ± 1.0	5
Sulpiride + dopamine	10^{-4} 10^{-4}	171.6 ± 9.2*	137.4 ± 2.7*	5
Spiperone	10^{-5}	98.8 ± 2.8	99.8 ± 0.9	4
Spiperone + dopamine	10^{-5} 10^{-4}	175.0 ± 9.0*	134.5 ± 1.7*	4

Values represent mean ± S.E.M.

* $P < 0.005$, † $P < 0.05$, significantly different from control (Student's *t*-test). Typical absolute values for Na^+,K^+- and Mg^{2+}-ATPase activities of controls 19.6 ± 0.8 (31) and 18.1 ± 0.7 (31) μmole Pi/mg protein per hr, respectively.

Table 2. Relative ATPase activities*

Drug	concentration (M)	Na^+,K^+-ATPase	Mg^{2+}-ATPase	No. of experiments
Dopamine	10^{-4}	175.8 ± 3.3†	140.8 ± 3.0†	5
ADTN	10^{-4}	161.2 ± 6.3†	137.0 ± 3.7†	5
Apomorphine	10^{-4}	184.8 ± 7.1†	107.8 ± 2.1†	4
S-584	10^{-4}	189.8 ± 1.8†	144.6 ± 2.7†	5
Piribedil	10^{-4}	97.5 ± 3.7	93.2 ± 5.4	6

Values represent mean ± S.E.M.

* Control = 100, in each case.

† $P < 0.005$, significantly different from control (Student's *t*-test). Typical absolute values for Na^+,K^+- and Mg^{2+}-ATPase activities of controls 19.6 ± 0.8 (31) and 18.1 ± 0.7 (31) μmole Pi/mg protein per hr, respectively.

tions, the antagonists themselves had no significant effect on the enzymes although at higher concentrations haloperidol, for instance, significantly inhibited the enzyme activities. It is well established that the concentrations of antagonists tested, which do not themselves inhibit the enzymes, are sufficiently high to antagonise many pharmacological effects of dopamine on the striatum and substantia nigra [7, 8]. In agreement with our results, Van Der Krogt and Belfroid [9] reported that haloperidol and flupenthixol did not inhibit DA-stimulation of striatal Na^+,K^+-ATPase activity. Other dopamine agonists such as apomorphine, S-584 and ADTN which is a potent and long acting dopamine agonist [10, 11] markedly stimulated the Na^+,K^+-ATPase activity and L-DOPA has also been shown to exert this effect [1] but piribedil did not. Similarly, ADTN, S-584 and L-DOPA [1] markedly stimulated the Mg^{2+}-ATPase activity while apomorphine, slightly but significantly, also stimulated the enzyme. The lack of effect of piribedil is in agreement with the work of Van Der Krogt and Belfroid [9] who studied homogenates of rat striatum. Their work also showed that bromocriptine did not stimulate striatal Na^+,K^+-ATPase activity. The degrees of stimulation of the enzymes by all but one of the dopamine agonists and the lack of antagonism of the effects by the antagonists tested are similar to the situation observed for noradrenaline or isoprenaline and their antagonists.

The apparent antagonism of agonist action by fluphenazine (10^{-4} M) observed by Akagawa and Tskuda [3] might be due rather to direct inhibition of Na^+,K^+-ATPase [1]. 10^{-5} M Fluphenazine in their experiments did not alter the DA-stimulated Na^+,K^+-ATPase activity.

Apomorphine, ADTN and S-584 have a catechol nucleus in their chemical structure whereas piribedil and bromocriptine lack this nucleus. These results are consistent with observations of Wu and Phillis [12] that resorcinol, hydroquinone and gentisic acid (2,5-dihydroxyl-benzoic acid) failed to stimulate the Na^+,K^+-ATPase of rat brain homogenate whereas protocatechuic acid (3,4-dihydroxyl-benzoic acid) was active in stimulating the enzyme. Catechol containing molecules such as noradrenaline, isoprenaline, L-DOPA and catechol itself significantly stimulated the Na^+,K^+-ATPase and Mg^{2+}-ATPase activities of synaptosomes while non-catechol drugs such as ephedrine, phenylephrine and clonidine were inactive, and salbutamol at a concentration of 10^{-3} M was only weakly active in stimulating the Na^+,K^+-ATPase activity of synaptosomes [1]. Consequently, the stimulatory effect of dopamine and dopamine agonists are consistent with our previous finding that the catechol group specifically is required for the effect which is likely not to be one of direct stimulation of the two synaptosomal enzymes studied here. As in previous work we have to consider the possibility that the apparent stimulation is really due to an effect of the catechol nucleus of opposing the inhibitory action of cytoplasmic factor(s) on the activities of the enzymes [1, 4].

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