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

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Abstract—The effects of adrenergic agonists, antagonists and other drugs on the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of rat cerebral cortex synaptosomes have been determined. Noradrenaline, isoprenaline, dopamine, l-DOPA and catechol stimulated the activities of the enzymes. The stimulatory effects were not prevented by low concentrations of adrenergic antagonists and apparently depend upon the presence in the molecules of a catechol moiety.

The effects of various drugs on the adenosine triphosphatase (ATPase) activities of a number of tissues have been extensively studied. Our own work [1] and that of others [2–10] has strongly suggested that the Na^+, K^+-ATPase activity of nerve terminals isolated from the central nervous system (synaptosomes) is stimulated by catecholamines. The mechanism by which these compounds exert their effect on the enzyme is not clear. Gilbert *et al.* [6] reported that in two experiments performed the adrenoceptor antagonist phentolamine inhibited the stimulation of Na^+, K^+-ATPase activity brought about by noradrenaline. Iwangoff *et al.* [4] suggested that both α- and β-adrenergic antagonists reduced the stimulation of Na^+, K^+-ATPase activity caused by noradrenaline, and experiments supporting this observation were subsequently reported [11]. However, it should be noted that Iwangoff *et al.* [4] also reported that both phentolamine and propranolol themselves inhibited the Na^+, K^+-ATPase of cat brain in the absence of an adrenergic agonist. Prior to this, Roufogalis and Belleau [12] had shown that alkylating agents of the dibenamine family inhibited the activity of the enzyme and that this inhibition could not be completely reversed by noradrenaline even when it was present at 5 times the concentration of the antagonists. In the present work, we have investigated in greater depth the relationship between adrenergic receptors and ATPases by determining the effects of α- and β-adrenergic agonists and antagonists on the Na^+, K^+- and Mg^{2+}-ATPase activities of synaptosomes. The studies also led us to investigate the effects of catechol-containing and other molecules.

METHODS

Synaptosomes were prepared from male Sprague–Dawley rat cerebral cortices by a modification of the method of Gray and Whittaker [13] as described by Gilbert and Wyllie [14]. Six rats were usually used for any one preparation. There was minimal contamination of the synaptosomes by material from other subcellular fractions as judged by succinic acid dehydrogenase, acetylcholine esterase and Na^+, K^+-ATPase activities and by electron microscopy.

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) NaCl (150 mM) and $MgCl_2$ (5 mM) in 50 mM imidazole–HCl buffer, pH 7.4, from the 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 reaction mixture volume of 0.9 ml was made up to 1 ml by the addition of 0.1 ml Tris–ATP when the reaction was started. Sodium dodecyl sulphate (1 ml 0.8%) was used to stop the reaction after 10-min incubation at 37°.

The phosphate contents of clear solutions were determined by the method of Bonting *et al.* [15]. Protein contents of solutions were determined by the method of Lowry *et al.* [16]. When used, drugs were usually made up in 50 mM imidazole–HCl (pH 7.4) containing 25 mM $MgCl_2$ and added to the other components for a pre-incubation period of 10 min in the absence of ATP prior to starting the reaction. Controls were carried out simultaneously. α- and β-antagonists were added to the pre-incubation media 5 min before adding the α- or β-agonists. Fresh solutions of the following drugs were prepared daily for each experiment: noradrenaline bitartarate (Koch-Light), isoprenaline sulphate (Macarthy's), dopamine (Sigma), phenylephrine (Koch-Light), ephedrine sulphate (T & H Smith) clonidine hydrochloride (Boehringer Sohn)–oxprenolol–HCl (CIBA), phentolamine mesylate (CIBA), practolol (ICI), propranolol-HCl (ICI), L-DOPA (Koch-Light), D,L-3-methoxy-4 hydroxymandelic acid

Table 1. Relative ATPase activities (control = 100 in each case)

Drug	Concentration (M)	Na^+, K^+-ATPase	Mg^{2+}-ATPase	Expts
Noradrenaline	10^{-4}	139.0 ± 4.5†	115.0 ± 4.3†	6
Isoprenaline	10^{-4}	143.0 ± 4.8*	125.0 ± 4.1*	4
Dopamine	10^{-4}	160.8 ± 13.0*	127.0 ± 7.4†	4
L-DOPA	10^{-4}	142.0 ± 5.2*	120.0 ± 3.8†	4
Catechol	10^{-3}	151.6 ± 6.7*	107.9 ± 3.0†	6

The values represent mean ± S.E.M.
* $P < 0.01$ and † $P < 0.05$ significantly different from control (Student t-test).
Typical absolute values for Na^+, K^+- and Mg^{2+}-ATPase activities of controls 18.1 ± 1.1 [6] and 15.9 ± 0.7 [6] μmol Pi/mg protein per hr, respectively.

(Koch-Light), salbutamol (Allen & Hanbury's) and catechol (pyrocatechol, Sigma).

RESULTS

Effects of drugs on ATPase activities

1. *Effects of catechol containing molecules.* Noradrenaline, isoprenaline, dopamine, L-DOPA and catechol all significantly stimulated both the Na^+, K^+-ATPase and the Mg^{2+}-ATPase activities of synaptosomes (Table 1). Typical dose–response effects for noradrenaline on Na^+, K^+-ATPase activity have been given elsewhere [1]. The degrees of stimulation of the Mg^{2+}-ATPase by the drugs were much smaller than their effects on the Na^+, K^+-ATPase activities.

2. *Effects of non-catecholamines.* Phenylephrine, clonidine, ephedrine and D,L-3-methoxy-4-hydroxymandelic acid did not significantly alter the Na^+, K^+-ATPase or the Mg^{2+}ATPase activities of synaptosomes at the concentrations shown (Table 2) or at other concentrations tested. Salbutamol at a concentration of 10^{-3} M caused a small but significant stimulation of the Na^+, K^+-ATPase while at a concentration of 10^{-4} M the drug did not significantly influence either enzyme.

3. *Effects of adrenergic antagonists.* The Na^+, K^+-ATPase activities of synaptosomes were inhibited by both phentolamine and propranolol as shown in Fig. 1(a). The degree of inhibition of the enzyme increased steeply at drug concentrations above 10^{-3} M. The Mg^{2+}-ATPase activities were also inhibited by the antagonists (Fig. 1b) and in both cases the enzyme inhibition tended to become much more pronounced at concentrations above 10^{-4} M.

4. *Effects of adrenergic antagonists on noradrenaline induced increases in the ATPase activities of synaptosomes.* The stimulatory effect of noradrenaline (10^{-4} M) on the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities, was not antagonized by either phentolamine or propranolol at a concentration of 10^{-5} M (Table 3).

5. *Effects of adrenergic antagonists on isoprenaline induced increases in the ATPase activities of synaptosomes.* Propranolol, phentolamine, practolol and oxprenolol at a concentration of 10^{-5} M did not antagonize the stimulatory effect of isoprenaline on the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of synaptosomes (Table 4).

DISCUSSION

The results indicate that noradrenaline, isoprenaline and dopamine stimulated the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of synaptosomes. In view of these and other results which have possible implications for feedback control of neurotransmitter release through effects of noradrenaline on presynaptic receptors [6, 9, 11, 17], it was of interest to determine if the effects of noradrenaline on the ATPase activities could be antagonized by α- or β-

Table 2. Relative ATPase activities (control = 100 in each case)

Drug	Concentration (M)	Na^+, K^+-ATPase	Mg^{2+}-ATPase	Expts
D,L-3-Methoxy-4 hydroxy-mandelic acid	10^{-3}	99.0 ± 1.7	102.0 ± 1.2	4
Salbutamol	10^{-3}	123.0 ± 9.5*	102.0 ± 2.3	5
	10^{-4}	107.0 ± 5.0	104.0 ± 3.5	5
Ephedrine	10^{-3}	107.0 ± 4.4	103.6 ± 1.9	5
	10^{-5}	90.0 ± 6.0	100.0 ± 2.0	5
Phenylephrine	10^{-3}	103.4 ± 4.2	102.0 ± 2.0	8
	10^{-4}	98.8 ± 3.7	99.0 ± 3.2	8
Clonidine	10^{-4}	96.0 ± 8.3	95.5 ± 5.5	4
	10^{-5}	101.0 ± 4.0	99.5 ± 17.5	4

The values represent mean ± S.E.M.
* $P < 0.05$ significantly different from control (Student t-test).
Typical absolute values for Na^+, K^+- and Mg^{2+}-ATPase activities of controls 18.1 ± 1.1 [6] and 15.9 ± 0.7 [6] μmol Pi/mg protein per hr, respectively.

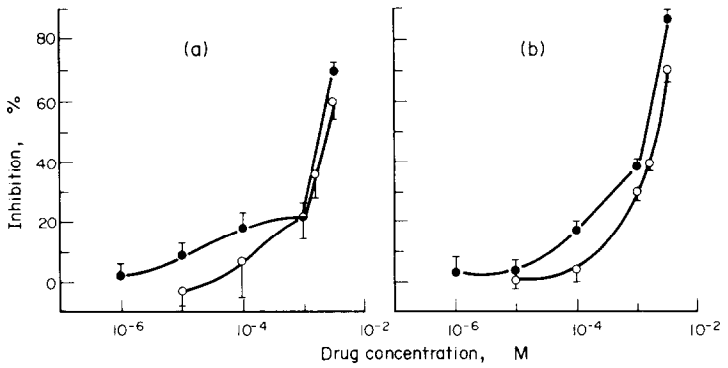

Fig. 1. Effects of propranolol (●) and phentolamine (○) on Na^+, K^+-ATPase activity (a) and Mg^{2+}-ATPase activity (b) of synaptosomes.

adrenergic antagonists. The stimulatory effects of noradrenaline and isoprenaline on the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities were not prevented by the antagonists tested at a concentration of 10^{-5} M. At this concentration, the antagonists themselves had no significant effect on the enzymes and it is well established that 10^{-5} M is a sufficiently high concentration to antagonize many pharmacological effects of noradrenaline (10^{-4} M). It is interesting that high concentrations (10^{-3} M or above) of phentolamine and propranolol did inhibit the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of synaptosomes and also inhibited the stimulatory effects of noradrenaline or isoprenaline on the enzymes [18]. Schaefer *et al.* [19] have found that phentolamine is a rather weak inhibitor of the effect of noradrenaline on the Na^+, K^+-ATPase activity of synaptic membranes.

Wu and Phillis [20] have found that both phentolamine and propranolol (10^{-5} M) abolished the stimulation of Na^+, K^+-ATPase caused by 10^{-6} M noradrenaline, but this action of the antagonists could be reversed by increasing the noradrenaline concentration to 10^{-5} M, some 10-fold lower than the concentration tested in our experiments. Since the stimulatory effects of noradrenaline and isoprenaline on the enzymes were not antagonized by adrenergic antagonists at concentrations which generally antagonize α- or β-adrenergic effects, it was felt that the situation might be clarified by testing the effects of other adrenergic agonists. The results indicate that phenylephrine, clonidine and ephedrine have no significant effect on either of the enzymes studied. Wu and Phillis [11] have also reported that phenylephrine has no effect on the Na^+, K^+-ATPase activity of synaptosomes. It appears therefore, that the effects of the drugs tested on the Na^+, K^+-ATPase are generally paralleled by the effects of the drugs on the Mg^{2+}-ATPase activity. This suggests that there is a close relation between the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities. This perhaps is not surprising in view of the very close structural association of the two enzymes [21, 22].

These results involving adrenergic agonists and antagonists strongly suggest that the stimulation of the enzymes by catecholamines is not an α- or β-adrenergic receptor mediated phenomenon. Consequently, it might be an effect dependent upon the catechol nucleus moiety of the agonists tested.

Noradrenaline, isoprenaline, dopamine, L-DOPA and catechol have two free phenolic groups attached to the phenyl ring at positions 3 and 4 and all these compounds significantly stimulated both the Na^+, K^+-ATPase and the Mg^{2+}-ATPase activities of the

Table 3. Effects of adrenergic antagonists on noradrenaline-stimulated ATPase activities of synaptosomes
Relative ATPase activities (control = 100 in each case)

Drug concentration (M)	Na^+, K^+-ATPase	Mg^{2+}-ATPase	Expts
Noradrenaline (10^{-4})	136.5 ± 3.1*	119.6 ± 2.1*	5
Phentolamine (10^{-5})	102.9 ± 1.5	101.5 ± 0.8	5
Phentolamine (10^{-5}) + Noradrenaline (10^{-4})	135.0 ± 3.5*	118.5 ± 2.0*	5
Noradrenaline (10^{-4})	122.0 ± 7.3†	109.0 ± 2.4*	8
Propranolol (10^{-5})	96.0 ± 5.2	101.0 ± 1.8	8
Propranolol (10^{-5}) + Noradrenaline (10^{-4})	116.0 ± 4.0†	105.0 ± 1.8†	8

The values represent mean ± S.E.M.
* $P < 0.01$ and † $P < 0.05$ significantly different from control (Student t-test).

Table 4. Effects of adrenergic antagonists on isoprenaline-stimulated ATPase activities of synaptosomes
Relative ATPase activities (control = 100 in each case)

Drug concentration (M)	Na^+, K^+-ATPase	Mg^{2+}-ATPase	Expts
Isoprenaline (5×10^{-5})	126.3 ± 3.3*	110.0 ± 1.7†	8
Propranolol (10^{-5})	96.0 ± 5.2	101.0 ± 1.8	8
Propranolol (10^{-5}) + Isoprenaline (5×10^{-5})	124.0 ± 6.9†	109.0 ± 2.5*	8
Isoprenaline (5×10^{-5})	138.0 ± 8.0*	118.0 ± 3.3*	6
Phentolamine (10^{-5})	106.0 ± 9.6	105.0 ± 2.3	6
Phentolamine (10^{-5}) + Isoprenaline (5×10^{-5})	122.5 ± 3.3†	115.3 ± 2.9*	6
Isoprenaline (5×10^{-5})	129.2 ± 2.6*	116.0 ± 1.5*	5
Practolol (10^{-5})	102.0 ± 3.1	100.0 ± 1.5	5
Practolol (10^{-5}) + Isoprenaline (5×10^{-5})	116.0 ± 5.5†	113.0 ± 3.1†	5
Isoprenaline (10^{-4})	143.0 ± 4.8*	125.0 ± 4.1*	4
Oxprenolol (10^{-5})	93.0 ± 10.0	106.0 ± 4.4	4
Oxprenolol (10^{-5}) + Isoprenaline (10^{-4})	141.0 ± 1.3*	122.0 ± 3.5*	4

The values represent mean ± S.E.M.
* $P < 0.01$ and † $P < 0.05$ significantly different from control (Student t-test).

synaptosomes. Adrenaline also stimulates the Na^+, K^+-ATPase activity of synaptosomes [11]. The replacement of one phenolic group by a methoxy group as in 3-methoxy-4 hydroxymandelic acid abolished the stimulatory effect. This suggests that both phenolic hydroxyl groups of the molecule have to be free in order to bring about stimulation of the enzymes. Salbutamol has two free hydroxyl groups at positions 3 and 4 on the phenyl ring, but one of them is attached to the phenyl ring as an alcoholic group and only one as a phenolic group. Salbutamol was only weakly effective in stimulating the Na^+, K^+-ATPase, whilst not influencing the Mg^{2+}-ATPase, at a concentration of 10^{-3} M, and the drug exerted no significant effect on either enzyme at a lower concentration (10^{-4} M). This suggests again that both hydroxyl groups have to be phenolic to affect both enzymes. In fact, phenols are more strongly acidic in properties than alcohols and this difference might therefore be responsible for the comparatively weak effect of salbutamol on the Na^+, K^+-ATPase activity of the synaptosomes.

Monohydroxylated compounds such as phenylephrine which differs chemically from adrenaline in lacking only an hydroxyl group in the position 4 on the benzene ring, failed to stimulate the enzymes at the concentrations tested. Tyrosine failed to stimulate Na^+, K^+-ATPase activity of beef brain microsomal preparations and tyramine apparently stimulates the enzymes only weakly [23]. Ephedrine, which lacks the two hydroxyl groups, failed to stimulate the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of the synaptosomes. Clonidine which has a 2, 6-dichloro structure instead of a 3,4-dihydroxy structure also failed to stimulate the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of synaptosomes. Taken together then, these observations suggest that the presence of the two free phenolic groups is essential for optimal stimulation of the Na^+, K^+-ATPase and Mg^{2+}ATPase activities of synaptosomes. It appears also that the substituted ethyl side chain of noradrenaline has no marked effect on the Na^+, K^+-ATPase activity, since catechol, which lacks the ethyl side chain, stimulated the Na^+, K^+-ATPase to the same extent as noradrenaline at the same concentrations [1]. Overall, it appears that the catechol group specifically is required for the stimulation of the two synaptosomal enzymes studied here. Interestingly, hydroquinone which has hydroxyl groups in the para positions is inhibitory in character for the Na^+, K^+-ATPase activity [23]. Our work has been specifically concerned with tissue derived from the central nervous system. Cheng *et al.* [24] reported that 1,2-dihydroxybenzene (catechol) represented the minimum structural requirement for Na^+, K^+-ATPase stimulation in the rat skeletal muscle membrane.

Gilbert *et al.* [1] reported that the apparent stimulation by noradrenaline of the Na^+, K^+-ATPase activity of synaptosomes really represents suppression of the inhibitory action on the enzyme of cytoplasmic factor(s). The present results might indicate that two phenolic hydroxyl groups play an essential role in opposing the activity of this factor. In fact, Colburn and Maas [25] reported that catechol and catecholamine can act as chelating agents and this property might be particularly pertinent to the effects observed.

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