Biochemical Pharmacology, Vol. 21, pp. 2289–2294. Pergamon Press, 1972. Printed in Great Britain.

THE EFFECTS OF A SOLUBLE FACTOR AND OF CATECHOLAMINES ON THE ACTIVITY OF ADENOSINE TRIPHOSPHATASE IN SUBCELLULAR FRACTIONS OF RAT BRAIN

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(*Received* 22 *December* 1971; *accepted* 18 *April* 1972)

Abstract—The ATPase activity in the presence of K^+, Na^+, Mg^{2+}, total ATPase activity of several subcellular fractions of rat brain was studied. The particle bound ATPase activity is lower in the presence of the soluble fraction. Noradrenaline, dopamine and their α-methylated analogues, adrenaline, isoprenalin and DOPA stimulate ATPase activity. The presence of the soluble fraction is essential for the enzyme stimulating effect. The ATPase stimulating action seems to depend on the catecholamine structure. The soluble fraction inhibits and the catecholamines stimulate both the ouabain sensitive and insensitive parts of the total ATPase activity. A heatstable dialysable factor is responsible both for inhibition and stimulation of the enzymes. The stimulating effect of catecholamines may occur as a reaction to the inhibition.

THE EFFECTS of various drugs on the ATPase (ATP phosphohydrolase EC 3.6.1.3.) bound to the brain, particular fractions have been extensively studied. [Na^+, K^+]-ATPase[1] is inhibited by a number of drugs acting on the central nervous system.[2–11] Some of these substances have a slight inhibitory effect on the Mg^{2+}-ATPase.[5,8,9] On the other hand the ATPase activity of hypothalamic vesical fraction determined in the presence of Mg^{2+} or Ca^{2+} is inhibited by drugs, which inhibit the ATP-Mg^{2+} dependent noradrenaline uptake into the vesicles.[12,13]

There is no data, however, on the physiological mediator substances and whether they have any influence on the ATPase activity. In the experiments published so far the particular fraction was suspended in buffer solution, without the presence of the soluble fraction. Our experiments on the other hand show that in the presence of the soluble fraction catecholamines stimulate the ATPase activity in several subcellular fractions of rat brain.

MATERIALS AND METHODS

White rats of the Wistar strain weighing 120–150 g were used. The subcellular fractions from the brain were prepared in 0·32 M sucrose according to De Robertis *et al.*[14–16] The mitochondrial, microsomal and supernatant primary fractions and after hypotonic treatment of the mitochondrial fraction, the M_1, M_2 and M_3 submitochondrial fractions were made. The whole procedure was carried out at a temperature of 4°.

The particular fractions were suspended in 0·32 M sucrose or in the adequate soluble fraction, so that the final suspension contained 1·0–1·5 mg particular protein per ml.

Total ATPase activity was estimated in the reaction mixtures containing 100 mM Tris–buffer pH 7·4, 2 mM $MgCl_2$, 100 mM NaCl, 30 mM KCl, 3 mM Tris–ATP, various amounts of investigated substances and 0·2 ml of the suspensions in a total volume of 2 ml. Ouabain insensitive ATPase activity was determined in identical reaction mixtures containing 10^{-4} M ouabain. Incubation was carried out for 15 min in a water bath at 37°. The mixture containing the enzyme was preincubated at 37° for 5 min before starting the reaction with ATP. The reaction was stopped by adding cold trichloroacetic acid to a final concentration of 5 per cent. Activity was estimated by measuring the production of inorganic phosphate using the method of Fiske and Subbarow.[17] The enzyme activity was expressed as μM phosphate produced per mg of protein in 15 min.

All experiments were performed at least four times. The results are presented as mean $\pm$S.E. Statistical significance was calculated with the Student's *t*-test. Proteins were determined according to Lowry *et al.*[18] Bovine serum albumin was used as a standard, its nitrogen content was previously controlled by a Coleman nitrogen-analyser.

ATP disodium salt was obtained from Reanal, Budapest. Ouabain (G-strophanthine) was a product of BDH Chemicals Ltd., Poole. The following drugs were used: dopamine hydrochloride; Sigma Chemical Company, St. Louis; tyramine hydrochloride, 3,4-dihydroxyphenyl acetic acid: Fluka, AG. Chemische Fabrik, Buchs; D-amphetamine sulphate; Koch–Light Laboratories Ltd., Colnbrook, Bucks; L-metaraminol; Merck Sharp and Dohme Research Lab., Rahway, N.J.; DL-noradrenaline bitartarate, DL-adrenaline; Rhone-Poulenc, Paris; L-α-methyl-noradrenaline hydrochloride; Farbwerke Hoechst, Frankfurt; DL-α-methyl dopamine hydrobromide; C.H. Boehringer Sohn, Ingelheim; DL-α-methyl-tyramine hydrochloride; Smith Kline & French Labs., Philadelphia; DL-isoprenalin hydrochloride, L-DOPA: E.GY.T., Budapest; β-phenyl-ethylamine bitartarate was made in our laboratory.

RESULTS AND DISCUSSION

Table 1 displays the ATPase activity in the presence of K^+, Na^+ and Mg^{2+} (i.e. total ATPase activity) in the subcellular fractions. All the particular fractions have a considerable enzyme activity, while the activity in the soluble fractions is very low. No experiments were performed with the nuclear fraction, which is too dense for ATPase assay.

It is noteworthy, that the activity is significantly higher both in the primary (microsomal) and submitochondrial (M_1, M_2) fractions if the incubation has been carried out without the soluble fraction. This indicates that the soluble fraction decreases the particulate bound ATPase activity.

Table 1 shows also that dopamine (DA) stimulates the ATPase activity of the particular fractions suspended in the adequate soluble fraction. DA does not influence the activity of these particular fractions suspended in sucrose and the low activities of the soluble fraction. The effect of DA seems to be a counteraction of the inhibition in the presence of the soluble fraction.

DA has a slight stimulating effect on the primary mitochondrial fraction suspended in sucrose. This fraction contains axoplasm within the nerve endings, that may explain this effect.

TABLE 1. THE EFFECT OF THE SOLUBLE FRACTION AND DOPAMINE ON THE TOTAL ATPASE ACTIVITY IN SUBCELLULAR FRACTIONS OF RAT BRAIN

Fractions	Ultrastructure*	Protein (%)	μM Pi/mg protein/15 min mean ±S.E. (n) control	with 5×10^{-5} M DA
Primary fractions				
Mitochondrial	Myelin, mitochondria, nerve endings	35·0	6·41 ± 0·25 (4)	7·29 ± 0·07 (4)‡
Microsomal	Microsomes	14·6	7·85 ± 0·04 (4)	8·08 ± 0·08 (4)
Supernatant	Soluble	15·6	1·04 ± 0·04 (4)	1·11 ± 0·16 (4)
Microsomal + supernatant	Microsomes, soluble		4·97 ± 0·18 (4)§	7·81 ± 0·41 (4)†
Submitochondrial fractions				
M_1	Myelin, mitochondria, nerve endings, ghosts	79·6	8·21 ± 0·09 (4)	8·48 ± 0·03 (4)
M_2	Synaptic vesicles, membranes	12·6	11·23 ± 0·20 (4)	11·14 ± 0·22 (4)
M_3	Soluble	7·7	0·89 ± 0·10 (4)	0·80 ± 0·17 (4)
$M_1 + M_3$	Myelin, mitochondria, nerve endings, ghosts, soluble		5·76 ± 0·29 (4)§	7·99 ± 0·05 (4)†
$M_2 + M_3$	Synaptic vesicles, membranes, soluble		8·26 ± 0·09 (4)§	11·32 ± 0·27 (4)†

* According to De Robertis *et al.*[14–16]

† The particles were suspended in the adequate soluble fraction. Specific activity was calculated for the particular protein content, inorganic phosphate liberated by the soluble fraction was subtracted. Significant difference from the control ($P < 0·01$).

‡ Significant difference from the control ($P < 0·05$).

§ Significant difference from the value measured without the soluble fraction ($P < 0·01$).

TABLE 2. THE EFFECT OF PHENYLALKYLAMINE DERIVATIVES ON THE TOTAL ATPASE ACTIVITY OF $M_2 + M_3$ FRACTIONS*

Substances 2×10^{-5} M	μM Pi/mg protein/15 min mean ± S.E. (n)	Activation (%)
None	8·12 ± 0·18 (14)	
Noradrenaline	10·79 ± 0·26 (7)†	32·9
Dopamine	10·96 ± 0·24 (6)†	34·9
Adrenaline	10·88 ± 0·44 (5)†	33·9
α-Methyl–noradrenaline	10·87 ± 0·27 (8)†	33·8
α-Methyl–dopamine	10·92 ± 0·31 (6)†	34·5
Isoprenalin	10·98 ± 0·42 (7)†	35·2
Tyramine	8·28 ± 0·32 (11)	2·0
α-Methyl–tyramine	8·30 ± 0·46 (5)	2·2
Metaraminol	8·26 ± 0·18 (8)	1·7
Amphetamine	8·10 ± 0·39 (12)	−0·2
Phenylethylamine	8·28 ± 0·35 (7)	2·0
DOPA	11·05 ± 0·45 (6)†	36·0
3,4-Dihydroxyphenyl acetic acid	8·92 ± 0·15 (5)	9·8

* M_2 particles were suspended in the adequate soluble fraction (M_3). Specific activity was calculated for the M_2 protein content, inorganic phosphate liberated by the soluble M_3 fraction was subtracted.

† Significant difference from the controls (none) $P < 0·01$.

Table 2 shows that not only DA stimulates the particle bound total ATPase in the presence of the soluble fraction, but other phenylalkylamine derivatives. These experiments were made on the $M_2 + M_3$ submitochondrial fractions. The results indicate that substances which contain hydroxyl groups in the 3,4-positions stimulate the enzyme activity. If the compound has only one hydroxyl group or none the stimulating effect fails to come about. On the other hand, the amino acid DOPA stimulates the enzyme activity as well as noradrenaline, dopamine, adrenaline etc., do. The intensity of the stimulation is about the same. The absence of effect of 3,4-dihydroxy-phenyl acetic acid indicates that for the stimulation the catecholamine structure is responsible.

We also studied the effect of some catecholamines in the presence of 10^{-4} M ouabain. As ouabain is a specific inhibitor of the [Na^+, K^+]-ATPase[1] within the so called total ATPase activity, the ouabain insensitive part can be separated. The ouabain insensitive part contains mostly Mg^{2+}-ATPase. As Table 3 demonstrates, the catecholamines stimulate the ouabain insensitive ATPase activity at about the same rate as the total activity (Table 2). This indicates that the catecholamines stimulate the ouabain sensitive part of the total activity.

TABLE 3. THE EFFECT OF CATECHOLAMINES ON THE OUABAIN INSENSITIVE ATPASE ACTIVITY OF $M_2 + M_3$ FRACTIONS*

Substances	μM Pi/mg protein/15 min mean ± S.E. (n)	Activation (%)
None	4·84 ± 0·04 (4)	
Noradrenaline	6·59 ± 0·11 (4)†	36·2
Dopamine	6·44 ± 0·13 (4)†	33·1
α-Methyl-noradrenaline	6·70 ± 0·18 (4)†	38·4
α-Methyl-dopamine	6·79 ± 0·18 (4)†	40·3
Isoprenalin	6·69 ± 0·09 (4)†	38·4

* The incubation mixture contained 10^{-4} M ouabain. Calculation of specific activity as in Table 2.
† Significant difference from controls $P < 0·01$.

On Table 4 we compare the total, ouabain insensitive and the calculated ouabain sensitive ATPase activity of the same fraction suspended in sucrose or in the adequate soluble fraction. The M_2 fraction contained about 60 per cent ouabain insensitive activity of the total activity. Germain and Proulx[19] report similar results using the same technique[16] for preparation of this fraction. The soluble fraction inhibits both the ouabain insensitive and sensitive parts of the total activity.

On the base of these experiments it can be assumed, that the inhibition produced by the soluble fraction is connected with the stimulating effect of catecholamines. Figure 1 demonstrates that both for the inhibition and activation by catecholamines, a heatstable dialysable factor in the soluble fraction is responsible. The total ATPase activity of the M_2 fraction suspended in thermodenatured or in the untreated soluble fraction is lower than when suspended in sucrose. DA and noradrenaline (NA) stimulate the ATPase activity to the same degree in the thermodenatured as in the

TABLE 4. THE EFFECT OF THE SOLUBLE FRACTION (M_3) ON THE TOTAL OUABAIN INSENSITIVE AND OUABAIN SENSITIVE ATPASE ACTIVITY OF THE M_2 FRACTION*

Preparatum	Total activity	Ouabain insensitive activity†	Ouabain sensitive activity‡
M_2	11·25 ± 0·13	6·53 ± 0·09	4·72
M_2 + M_3§	8·17 ± 0·07	4·70 ± 0·11	3·47

* Values are expressed as μM Pi/mg protein/15 min mean ±S.E. n = 4.

† Ouabain insensitive activity was measured with 10^{-4} M ouabain in the incubation mixture.

‡ Difference of means of the total and ouabain insensitive activities.

§ M_2 particles were suspended in the adequate soluble fraction (M_3). Specific activity was calculated for the M_2 protein content, inorganic phosphate liberated by the soluble fraction was subtracted.

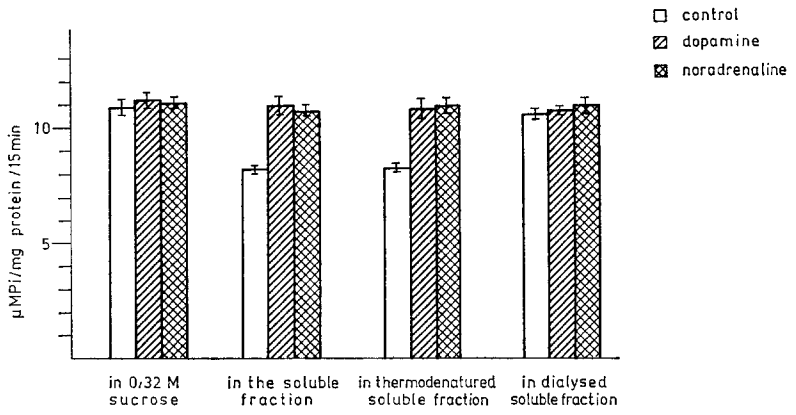

FIG. 1. The total ATPase activity of the M_2 fraction suspended in sucrose, or in the untreated, thermodenatured, dialysed soluble fraction. Effect of 2×10^{-5} M dopamine and noradrenaline. The soluble fraction was thermodenatured by boiling for 5 min. Dialysis was carried out again 0·32 M sucrose. Activity was calculated by the M_2 protein content and inorganic phosphate liberated by the soluble fraction was subtracted. Means of at least six experiments ± S.E.

untreated soluble fraction. If the particles are suspended in the dialysed soluble fraction the ATPase activity is the same as suspended in sucrose. DA and NA do not stimulate this enzyme activity. It seems that DA and NA prevent the inhibition of the particle bound ATPase activity by a heatstable, dialysable factor.

To sum up it can be stated that the soluble fractions contain a heatstable, dialysable factor which inhibits the particle bound total ATPase activity, both the ouabain sensitive and insensitive parts. The stimulating effect of catecholamines on the same ATPase activities in the presence of the soluble fraction may be explained by a counteraction of this inhibition. It may be supposed that this inhibitory factor and the catecholamines have an opposite role in the regulation of particle bound activity of ATPase enzymes.

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