Biochemical Pharmacology, Vol. 22, pp. 2375–2379. Pergamon Press, 1973. Printed in Great Britain.

EFFECTS OF TETRABENAZINE AND OF CHLORPROMAZINE ON SUBMITOCHONDRIAL ADENOSINE TRIPHOSPHATASES OF RAT BRAIN IN THE PRESENCE OF THE SOLUBLE FRACTION

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(*Received* 4 *September* 1972; *accepted* 2 *March* 1973)

Abstract—The effects of tetrabenazine and of chlorpromazine on Na^+-K^+-ATPase and Mg^{2+}-ATPase in two submitochondrial fractions prepared from rat brain were studied. The soluble fraction inhibits the ATPases in the two particulate fractions. Tetrabenazine, like the catecholamines, counteracts the inhibition by the soluble fraction. Chlorpromazine inhibits differently the ATPases in the two fractions. In the presence of the soluble fraction chlorpromazine can reduce both the inhibition by the soluble fraction and the stimulation by dopamine or tetrabenazine. It is suggested that an endogenous inhibitor and the catecholamines regulate the ATPase activity of certain membrane structures of the rat brain. Drugs like tetrabenazine and chlorpromazine might affect this regulation.

ADENOSINE TRIPHOSPHATASE (ATPase) activity associated with the subcellular fractions of brain has been the subject of intensive study. One type of the ATPase the so-called Na^+-K^+-ATPase is presumably involved in nervous tissue, as in other tissues in the transport of cations through the cell membrane.[1–3] The role of the other type of ATPase activated by the divalent cations (Mg^{2+} or Ca^{2+}) has not yet clarified. An actomyosin like protein (neurostenin) has been isolated from the synaptosomal fraction of bovine and rat brains.[4] The Mg^{2+}- and Ca^{2+}-ATPase activities of other fractions (mitochondrial, microsomal, myelin and supernatant), however, do not possess an actomyosin-like character.[4,5]

The factors regulating the activity of ATPases are also unknown. In a previous work[6] we reported that the soluble fraction of the rat brain contains a heat-stable dialysable factor inhibiting the ATPase activity of the particulate fractions. This inhibition was abolished by catecholamines. This paper summarizes the effects of tetrabenazine and chlorpromazine on the system.

MATERIALS AND METHODS

White CFE rats of both sexes weighing 130–180 g were used in this investigation. Fractionation of brain cells was carried out in 0·32 M sucrose according to De Robertis *et al.*[7,8] Because Ca^{2+} inactivates Na^+-K^+-ATPase[9,10] this cation was omitted from solutions used in the fractionation procedure. The mitochondrial fraction was isolated and after hypotonic treatment it was separated to three submitochondrial fractions by differential centrifugation, i.e. M_1 fraction, 11,500 *g* particles, M_2 fraction, 11,500–100,000 *g* particles, M_3 fraction, soluble.[7,8] The M_1 fraction contained 58–66 per cent

and the M_2 fraction 15–19 per cent of the protein of the primary mitochondrial fraction. Fractions M_1 and M_2 were resuspended in 0·32 M sucrose or in the same amount of the soluble M_3 fraction so that the final suspension contained 1·0–1·5 mg particulate protein/ml. The suspensions were kept at 4° and were used for ATPase assay within 24 hr.

Total ATPase activity was estimated in a reaction mixture containing 50 mM Tris-buffer pH 7·4, 3 mM $MgCl_2$, 100 mM NaCl, 30 mM KCl, 3 mM Tris–ATP, various amounts of chlorpromazine, tetrabenazine or dopamine and 0·2 ml of the suspension in a total volume of 2 ml. Mg^{2+}-ATPase activity was measured in a similar reaction mixture but with the omission of NaCl and KCl and containing 0·1 mM ouabain. The mixture containing the enzyme was preincubated at 37° for 5 min before starting the reaction with ATP. Incubation was carried out for 15 min in a water bath at 37°. The reaction was stopped by adding cold trichloroacetic acid to a final concentration of 5 per cent. ATP hydrolysis was estimated by measuring the production of inorganic phosphate using the method of Fiske and Subbarow.[11] All values were corrected for the Pi content found in zero-time incubation and for spontaneous ATP hydrolysis. The enzyme activity was expressed as μmoles inorganic phosphate released/mg protein/15 min. The Na^+-K^+-ATPase activity was calculated by subtracting Mg^{2+}-stimulated activity from total ATPase activity. All experiments were performed at least five times and samples were assayed in duplicate.

Protein was determined by the method of Lowry *et al.*[12] Statistical significance was calculated by an analysis of variance and Dunnett-contrast and by the Student *t*-test.

The following substances were used: ATP disodium, Reanal, Budapest (Tris–ATP was made by treating disodium ATP with an exchange resin[13]); ouabain (G-strophanthine), BDH, Poole; tetrabenazine (Nitoman ampoullas) Hoffman–La Roche, Basle (controls contained methansulphonic acid to the corresponding concentration); chlorpromazine hydrochloride, E.GY.T. Budapest; dopamine hydrochloride, Sigma, St. Louis.

RESULTS

Table 1 shows the effect of 10^{-4} M tetrabenazine (TBZ) on the ATPase activity in two submitochondrial fractions (M_1, M_2).[7,8] The control values were lower in the presence of the soluble fraction (M_3) containing an endogenous inhibitor.[6] This inhibition was significant in both Mg^{2+}-ATPase and Na^+-K^+-ATPase, although the Mg^{2+}-ATPase activity of fraction M_1 was inhibited only slightly. TBZ increased the reduced activity observed in the presence of the soluble fraction, while it had no effect on M_1 and M_2 particles suspended in sucrose. This effect is similar to that of the catecholamines reported previously.[6] This prompted us to compare the effect of TBZ to that of a catecholamine, dopamine (DA). $2{\cdot}5 \times 10^{-5}$ M DA affected the total ATPase activity to the same extent as 10^{-4} M TBZ. Mg^{2+}-ATPase activity was somewhat less, and Na^+-K^+-ATPase somewhat more affected by TBZ than by DA, but the difference was not significant. The stimulation by both compounds seems to depend on the extent of the inhibition observed in the presence of the soluble fraction.

Table 2 demonstrates the effect of 10^{-4} M chlorpromazine (CPZ) on ATPase activity in both fractions in the presence and absence of the soluble fraction. CPZ inhibited both the Mg^{2+}- and Na^+-K^+-ATPase. The Mg^{2+}-ATPase was more

TABLE 1. THE EFFECT OF TETRABENAZINE (10^{-4} M) AND DOPAMINE ($2{\cdot}5 \times 10^{-5}$ M) ON THE ATPASES OF SUBMITOCHONDRIAL FRACTIONS*

Fractions†	Substances	Total-ATPase	Mg^{2+}-ATPase	Na^+-K^+-ATPase
M_1 in sucrose	Control	12·34 ± 0·20‡	5·36 ± 0·14§	6·98 ± 0·18‡
	TBZ	12·26 ± 0·12‡	—	—
M_1 in the soluble fraction	Control	9·49 ± 0·13	4·71 ± 0·16	4·78 ± 0·25
	TBZ	12·19 ± 0·20‡	5·28 ± 0·15¶	6·92 ± 0·24‡
	DA	12·08 ± 0·19‡	5·53 ± 0·22‡	6·55 ± 0·19‡
M_2 in sucrose	Control	17·78 ± 0·15‡	7·49 ± 0·13‡	10·30 ± 0·16‡
	TBZ	17·77 ± 0·13‡	—	—
M_2 in the soluble fraction	Control	14·15 ± 0·20	6·02 ± 0·15	8·13 ± 0·23
	TBZ	17·72 ± 0·28‡	6·78 ± 0·16‡	10·94 ± 0·30‡
	DA	17·67 ± 0·21‡	7·25 ± 0·13‡	10·41 ± 0·22‡

* Values are expressed as μmoles Pi released/mg protein/15 min. Means of at least five experiments in duplicate ± S.E. Specific activity was calculated for the particulate protein content, inorganic phosphate liberated by the soluble fraction was subtracted.

† M_1: Myelin, mitochondria, nerve endings, ghosts. M_2: Synaptic vesicles, membranes—according to DE ROBERTIS *et al.*[7,8] The particles were suspended in 0·32 M sucrose or in the same amount of the soluble fraction.

‡ $P < 0{\cdot}01$ compared to control in the presence of the soluble fraction.

§ $P < 0{\cdot}05$, ¶ $P \leq 0{\cdot}05$ compared to control in the presence of the soluble fraction.

affected by CPZ than the Na^+-K^+-ATPase, mainly in fraction M_1, where CPZ had only a very slight effect on the Na^+-K^+-ATPase.

TABLE 2. EFFECT OF CHLORPROMAZINE (10^{-4} M) ON THE ATPASES OF SUBMITOCHONDRIAL FRACTIONS*

Fractions	Substance	Total-ATPase	Mg^{2+}-ATPase	Na^+-K^+-ATPase
M_1 in sucrose	Control	12·34 ± 0·20	5·36 ± 0·14	6·98 ± 0·18
	CPZ	9·98 ± 0·24†	3·57 ± 0·16†	6·41 ± 0·17‡
M_1 in the soluble fraction	Control	9·49 ± 0·13	4·71 ± 0·16	4·78 ± 0·25
	CPZ	9·95 ± 0·21	3·67 ± 0·19†	6·28 ± 0·31†
M_2 in sucrose	Control	17·78 ± 0·15	7·49 ± 0·13	10·30 ± 0·16
	CPZ	14·44 ± 0·16†	5·66 ± 0·12†	8·78 ± 0·15†
M_2 in the soluble fraction	Control	14·15 ± 0·20	6·02 ± 0·15	8·13 ± 0·23
	CPZ	14·31 ± 0·17	5·78 ± 0·15	8·53 ± 0·30

* For details see Table 1.

† $P < 0{\cdot}01$ compared to the corresponding control.

‡ $P < 0{\cdot}05$ compared to the corresponding control.

The effect of CPZ was similar in the presence and absence of the soluble fraction. This indicates a decrease in Mg^{2+}-ATPase and an increase in Na^+-K^+-ATPase in fraction M_1 in the presence of the soluble fraction compared to the control. The inhibitory effect of CPZ was not affected by the presence of the soluble fraction, while CPZ abolished the inhibition by the soluble fraction. CPZ did not induce any change in fraction M_2 in the presence of the soluble fraction compared to the control, because

its inhibitory effect on this fraction did not differ significantly from the inhibition by the soluble fraction.

To see if the stimulating effect of DA and TBZ was affected by CPZ, experiments were carried out with fraction M_2, where CPZ did not influence the basal activity in the presence of the soluble fraction. Results are shown in Table 3. It can be concluded that CPZ abolished the DA and TBZ effect on Mg^{2+}-ATPase and decreased their effect on the Na^+-K^+-ATPase.

TABLE 3. INTERACTION OF CHLORPROMAZINE WITH DOPAMINE AND TETRABENAZINE EFFECTS ON THE ATPASES OF THE FRACTION M_2 IN THE PRESENCE OF THE SOLUBLE FRACTION*

Substances	Total-ATPase	Mg^{2+}-ATPase	Na^+-K^+-ATPase
Control	14·15 ± 0·20	6·02 ± 0·15	8·13 ± 0·23
CPZ	14·31 ± 0·17	5·78 ± 0·15	8·53 ± 0·30
DA	17·67 ± 0·20	7·25 ± 0·13	10·41 ± 0·22
TBZ	17·72 ± 0·28	6·78 ± 0·15	10·94 ± 0·30
CPZ + DA	15·70 ± 0·19†	6·05 ± 0·11†	9·65 ± 0·26‡
CPZ + TBZ	15·35 ± 0·24†	5·77 ± 0·10†	9·58 ± 0·31†

* For details see Table 1.
† $P < 0·01$ compared to the corresponding sample without CPZ.
‡ $P < 0·05$ compared to the corresponding sample without CPZ.

DISCUSSION

The results show that TBZ has a catecholamine-like effect on the ATPase activity of the two submitochondrial fractions, namely it counteracts the inhibition of the ATPase activity observed in the presence of the soluble fraction.

The sensitivity of the ATPases in the two fractions to the inhibitory effect of the soluble fraction was different. Twelve per cent of the Mg^{2+}-ATPase and 32 per cent of the Na^+-K^+-ATPase activity was inhibited by the soluble fraction in fraction M_1, while 20 per cent of the Mg^{2+}-ATPase and 21 per cent of the Na^+-K^+-ATPase activity was inhibited in fraction M_2. This indicates that the Mg^{2+}-ATPase and Na^+-K^+-ATPase activities, sensitive to the inhibitory effect of the soluble fraction, may be structurally separated. The sensitivity of the Mg^{2+}-ATPase is greater in fraction M_2, while the sensitivity of the Na^+-K^+-ATPase is greater in fraction M_1. Considering the ultrastructure of the fractions[7,8] and the localization of ATPase enzymes in submitochondrial fractions[14–16] it can be suggested that the Na^+-K^+-ATPase of the nerve ending membranes might predominate in fraction M_1, while the Mg^{2+}-ATPase of the synaptic vesicles might predominate in fraction M_2. However, this evidence is not sufficient to conclude that the enzyme activity sensitive to the endogenous inhibitor is bound to the above particles. Further experiments are needed to validate such conclusions on the association of subdivisions of ATPase activity with various subcellular structures.

Several authors have reported that the Na^+-K^+-ATPase activity of the brain microsomes was inhibited by CPZ.[3,9,17,18] The Mg^{2+}-stimulated enzyme was not affected by CPZ, except after deoxycholate treatment of the microsomes.[17] In our experiments on the submitochondrial fractions CPZ strongly inhibited the Mg^{2+}-ATPase activity of both fractions, although it inhibited the Na^+-K^+-ATPase of fraction M_1 only very

slightly. This suggests that in the various subcellular fractions there exist Na^+-K^+- and Mg^{2+}-ATPases differing in their sensitivity to the inhibitory effect of CPZ. A similar phenomenon has recently been discussed in connection with the effects of diphenylhydantoin.[19]

A correlation was found between the inhibitory effect of phenothiazine derivatives on the microsomal Na^+-K^+-ATPase and some of their effects on the central nervous system.[18] However, our experiments suggest that CPZ not only inhibits ATPase enzymes but may influence their function by another mechanism, too. Namely, the Na^+-K^+-ATPase of fraction M_1 is only slightly inhibited by CPZ, which also protected the enzyme against the inhibitor in the soluble fraction. It seems interesting that CPZ affects this ATPase activity like DA and TBZ. A certain protective effect against the stimulation by DA and TBZ could be demonstrated on the ATPases of fraction M_2. Hence CPZ seems to reduce the sensitivity of ATPase enzymes to the endogenous inhibitor and the studied compounds, independent of the extent of inhibition of the basal ATPase activity. This action of CPZ is in accord with former suggestions explaining its effects and the unifying hypothesis of Guth and Spirtes[20] that phenothiazines act by altering membrane properties as "membrane stabilizers".

We have suggested that the endogenous inhibitor in the soluble fraction and the catecholamines have a role in the regulation of ATPase activity of certain membrane structures or membrane properties connected with the ATPase activity.[6] The present results show that drugs such as TBZ and CPZ might affect such a regulation. Recently we have found that L-ascorbic acid inhibits the ATPases of rat brain particles as the soluble fraction does and this inhibition can be also antagonized by catecholamines and by TBZ or reserpine.*

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* Manuscript in preparation.