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INHIBITION OF CELLULAR ATP-HYDROLYZING ACTIVITY BY TRICYCLIC ANTIDEPRESSANTS AND PHENOTHIAZINE TRANQUILIZERS

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SUMMARY

In a variety of cellular and subcellular preparations, total ATP-hydrolyzing activity was potently inhibited by tricyclic antidepressants (TA) and phenothiazine tranquilizers (PT). In leukocytes, preincubation of the cells with 5×10^{-4} M TA and 1×10^{-4} M PT resulted in up to 87% and 94% inhibition of the ATPase activity. Among TA, dibenzocycloheptadienes were somewhat more potent inhibitors than were derivatives of dibenzazepine. Substitution, at the position 2 of the phenothiazine nucleus, by a halogen and particularly by the CF_3 group, increased the inhibitory strength of PT. However, most effective in inhibiting ATPase was thioridazine, structurally differing from mesoridazine, the weakest inhibitor in this study, by a methylmercapto group instead of a methylsulfinyl substituent. The inhibition by the drugs was markedly reduced in the presence of millimolar ATP. The results indicate a possible adverse effect of these drugs on the cellular energy-yielding capacity.

Current views on the mechanism of action of TA primarily focus on the adrenergic effects of these compounds, involving their interactions with catecholamines (1,2). Recently, reviewed was the structure-activity relationship of TA as the basis for their pharmacologic activity, as well as clinical effectiveness (3). Various other effects of these drugs have been reported, e.g., their action on synaptosomal components such as the Na,K-ATPase and acetylcholinesterase (4), on microsomal Na,K-ATPase (5), as well as on platelet

aggregation (6). The tranquilizing effect of PT has been correlated with their inhibition of Na,K-ATPase in vitro (7,8) and evidence for the mechanism of this interaction presented (9,10). We previously investigated and reported the strong inhibition by TA of the Na,K-ATPase from cortexes of both brain and kidney (11). In pursuing observations made during that study, we now present evidence for the strong effect of TA and PT on the energy metabolism by their potent inhibition of the overall ATP-hydrolyzing activity in various cellular and subcellular preparations. Particularly evaluated was the drug inhibitable ecto-ATPase activity in leukocytes.

MATERIALS AND METHODS

Materials. The drugs used in this study were kindly provided by Drs. E. F. Domino, H. H. Swain and J. H. Woods, Department of Pharmacology, The University of Michigan. The biochemicals and enzymes were purchased from Sigma Chemical Co., St. Louis, Mo. and from Boehringer-Mannheim Co., New York, N. Y. Plasmagel, a modified gelatin solution, was obtained from HTI Corporation, Buffalo, N. Y. All other chemicals were of reagent grade.

Isolation of cellular and subcellular fractions. Leukocytes and platelets were separated from blood of 300 g male, Sprague-Dawley rats by a previously described procedure, yielding fractions of viable cells with cross-contamination less than 5% (12). The isolation involves the selective sedimentation of erythrocytes in the presence of Plasmagel, and the subsequent separation of leukocytes and platelets by differential centrifugation, after the addition of adenosine diphosphate to induce aggregation of the latter cells.

Neuronal perikarya from cerebral cortexes of 100 g, male rats were obtained by a bulk-isolation technique (13). The procedure is based on a gentle homogenization of the tissue by sieving through nylon gauze, followed by sucrose density gradient centrifugation, yielding a pellet consisting mainly of neuronal cell bodies.

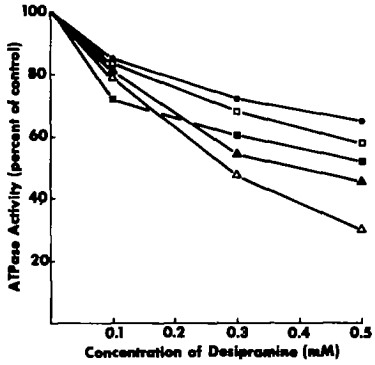
Microsomal fractions from cortexes of beef brain and kidney were prepared as described previously (11).

Assay of ATPase activity. The isolated fractions were suspended in a buffer medium containing the following concentrations (mM): Tris. HCl, 35; NaCl, 120; KCl, 5; $MgCl_2$, 2.5 and glucose, 10. The pH of the medium was adjusted to 7.4. Aliquots of the above suspension were subjected to the assay of enzymatic activity. ATPase activity was quantitated by determining the inorganic phosphate (Pi) released from ATP. The latter estimation was carried out by two methods: a colorimetric determination of Pi at 700 nm as described previously (14) or by an enzymatic method using phosphorylase a, phosphoglucomutase and glucose 6-phosphate dehydrogenase to fluorometrically measure the formation of reduced nicotinamide adenine dinucleotide phosphate (15). The latter procedure was necessary due to the interference of some of the drugs in the colorimetric determination of Pi. The ATPase assay was carried out as described (14) with the following modifications in case of the enzymatic determination of Pi: the ATPase reaction at 37° was stopped by placing the tubes in a bioling water bath for 8 min, followed by centrifugation at 1900 x g for 10 min. Aliquots of the supernatants were added to 1 ml of the prepared reagent

(15) containing all the necessary components except for phosphorylase. After mixing and taking the initial reading of fluorescense, the reaction was started by the addition of the latter enzyme. The rate of the enzymatic reaction was monitored on a recorder. After its completion, the second readings of the samples were taken. Appropriate standards and blanks were included in each set of analysis.

Protein was estimated according to Lowry et al. (16).

FIG 1

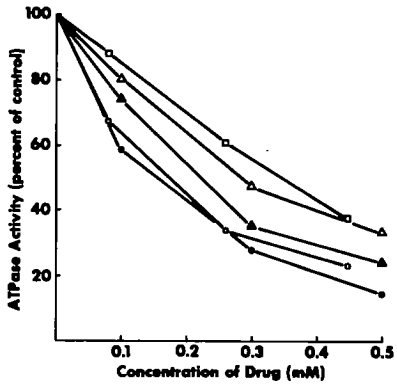


Inhibition of the ATP-hydrolyzing activity in various cellular and subcellular preparations by desipramine: platelets, ●—●; kidney cortex microsomes, □—□; brain cortex microsomes, ■—■; neuronal perikarya ▲—▲; leukocytes, Δ—Δ. The fractions were separated as outlined under Materials and Methods. Suspensions of the biologic material in the medium described in the text were incubated for 15 min at 37° with varying concentrations of desipramine. Subsequently, ATP was added to give a final concentration of 3 mM and the samples incubated for additional 30 min. The further handling of the samples and the colorimetric determination of Pi was as described under Materials and Methods. Each point represents the mean of at least 12 individual determination.

RESULTS

In a variety of tissue preparations the total ATP-hydrolyzing activity was inhibited by TA, as exemplified in Fig. 1 by the action of desipramine. The most potent effect was obtained in leukocytes and these cells were used to further

FIG 2

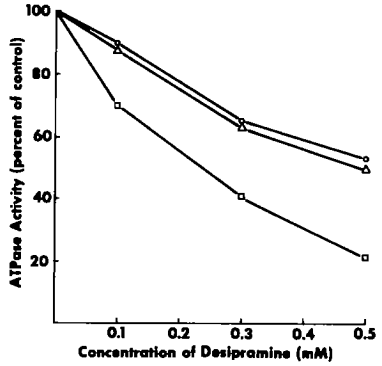


Effect of various homologues of tricyclic antidepressants on the ATP-hydrolyzing activity in leukocytes: imipramine, □—□; desipramine, Δ—Δ; amitriptyline, ▲—▲; protriptyline, o—o; nortriptyline, ●—●. Suspensions of freshly isolated rat leukocytes were incubated for 15 min at 37° with the indicated concentrations of the listed compounds. After the addition of ATP and renewed incubation for 30 min, the released Pi was determined enzymatically as described under Materials and Methods. Presented are mean values of results obtained in at least 4 experiments using different preparations of leukocytes.

characterize the nature of this inhibition. Whereas all the investigated homologues of the TA were strong inhibitors of the total ATP-hydrolyzing activity in leukocytes, dibenzocycloheptadienes were somewhat more potent than were derivatives of dibenzazepines (Fig. 2). Nortriptyline, at concentrations of 0.1 and 0.5 mM, inhibited the ATPase activity 40% and 85%, respectively. These potent effects were obtained if the cells were preincubated with the drug in the absence of ATP. In the presence of 3 mM ATP, a concentration corresponding to ten times the Km value for this compound (17), the inhibition by desipramine of the ATPase decreased by 30% (Fig. 3). Higher concentrations of ATP had no further protective effect. The ATP-drug interaction was observed with both TA and PT.

In general, all the investigated PT inhibited the ATPase activity in leukocytes, however with a wide range of

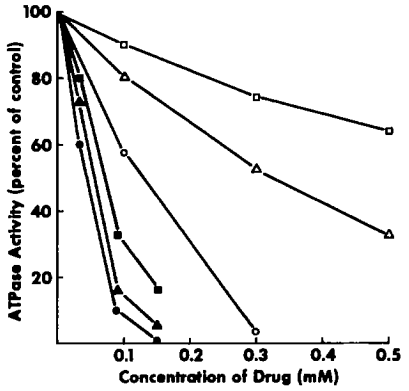
FIG 3



Effect of ATP on the inhibition by desipramine of the ATP-hydrolyzing activity in leukocytes. Suspensions of cells were incubated for 30 min at 37° with 3 mM (o—o) or 13.5 mM (Δ—Δ) ATP and desipramine, present at concentrations as indicated. In other experiments, the cells were first incubated for 15 min with the drug (□—□), then 3 mM ATP added and the tubes further incubated for 30 min at 37°. The released Pi in all samples was determined using the colorimetric assay described in the text. Presented are mean values of 3 experiments carried out with different preparations of leukocytes.

potency (Fig. 4). Whereas mesoridazine was a weaker inhibitor

FIG 4



Effect of phenothiazine tranquilizers on the ATP-hydrolyzing activity in leukocytes: mesoridazine, □—□; promazine, Δ—Δ; chlorpromazine, o—o; perphenazine, ■—■; trifluoperazine, ▲—▲; thioridazine, ●—●. Suspensions of freshly isolated rat leukocytes were incubated for 15 min at 37° with the indicated concentrations of the listed compounds. After the addition of ATP and renewed incubation for 30 min, the released Pi was determined enzymatically as described under Materials and Methods. Presented are mean values of results obtained in 3 experiments using different preparations of leukocytes.

than TA, promazine exhibited a comparable effect and the other PT were even considerably stronger inhibitors. In the presence of 3×10^{-5} M thioridazine, the ATPase activity was decreased by 40%. The inhibitory potency increased with substitution, at position 2 of the phenothiazine nucleus, by Cl and particularly by the CF_3 group. However, the strongest inhibition was obtained with thioridazine, containing instead CH_3S- substituent.

In addition to TA and PT, the effects of various other CNS drugs on the ATPase activity in leukocytes were investigated. The results showed relatively little effect of these compounds on the total ATP-hydrolyzing activity. Highest inhibition was obtained with methadone and amphetamine which, at concentrations of 0.4 mM, inhibited the enzyme activity 25% and 15%, respectively (Table 1).

DISCUSSION

Interaction of TA and PT with the Na,K-ATPase has been discussed as a possible mechanism involved in inducing the CNS effects of these compounds (4,5,7,8). The basis for these considerations was the well documented involvement of the Na,K-ATPase in the membrane transport of Na^+ and K^+ (18), the release and uptake of which is linked to nerve conduction. Results of our previous study on the inhibition by various CNS drugs of the Na,K-ATPase in microsomal preparations from brain and kidney showed a potent effect of TA on the enzyme from both organs (11). In that study other investigated drugs, e.g., benzomorphans, were more selective, inhibiting the Na,K-ATPase from brain whereas having little effect on that enzyme enriched from kidney. In the present study, the potent inhibitory effects of these drugs on the total ATP-hydrolyzing activity in

TABLE 1

Inhibition of ATPase Activity by CNS Drugs

Compound	Inhibition of ATPase activity (% of control) at drug concentration (mM)		
	0.1	0.3	0.5
Amphetamine	6	10(0.25)	15(0.4)
Cocaine	1	4	11
Desipramine	28	59	78(0.4)
Levorphanol	4	6	10
Methadone	14	20(0.25)	25(0.36)
Morphine	0	5	11
Nardil	0	0	0
Nialamide	1	5	10
Phenobarbital	8	10	--
Thebaine	8	8	9
Thioridazine	94	100	--
Trifluoperazine	83	100	--

Suspensions of leukocytes in the medium described in the text were incubated for 15 min at 37° with the indicated concentrations of the listed drugs (the numbers in parenthesis represent concentrations of the particular drugs if different from those used throughout the experiment). Subsequently, ATP was added and the suspensions reincubated for an additional 30 min. The quantitation of the released Pi was carried out enzymatically using phosphorylase a and auxiliary enzymes as described under Materials and Methods. Presented are mean values of results obtained in 3 experiments in which different preparations of leukocytes were used.

various cellular and subcellular preparations were revealed. The inhibitory action of both TA and PT was particularly strong in leukocytes in which the Na,K-activated enzyme was only a small component of the ATPase system (17). The marked inhibition of the ATPase activity in leukocytes was caused by a variety of homologues of TA, among which the derivatives of dibenzocycloheptadienes were somewhat more effective.

Whereas, in general, PT were stronger inhibitors of leukocyte ATPase as compared to TA, their potency varied considerably. Halogen substituents as well as the presence of the CF_3

group at position 2 of the phenothiazine nucleus, increased the inhibitory potency. Of particularly interest was the marked difference in inhibition exhibited by thioridazine and mesoridazine, structurally closely related compounds. The former drug, carrying at position 2 a methylmercapto group, was the most potent inhibitor in this study, significantly affecting the ATPase already at a concentration of 3×10^{-5} M. Mesoridazine, on the other hand, containing instead a methylsulfinyl substituent, was effective only at concentrations higher than 3×10^{-4} M. Interestingly, the order of inhibitory strength agreed quite closely with that reported by Davis and Brody for the interaction of PT with Na,K-ATPase from brain microsomes (7). In leukocytes, however, the Na,K-activated component of the ATPase amounted to only 7% of the total enzymatic activity (17).

The magnitude of the inhibitory effects raises the question of their metabolic consequences. The energy metabolism, in which the ATPase system has an essential role, could be severely impaired under conditions of such strong inhibition of this energy-generating capacity. In view of the reported adverse side effects of TA (19-25), frequently associated with cardiac function (21,22,24,25), the here described effect of these drugs on the cellular ATP-hydrolyzing capacity in various tissue preparations becomes of special interest. The numerous side effects of PT have been reviewed extensively (26,27). It should be emphasized, that ECG anomalities and ventricular tachycardia were most frequently associated with thioridazine (28), the most potent inhibitor of the ATP-hydrolyzing activity in this study. Interestingly, among various tranquilizers investigated, trifluoperazine and thioridazine

were reported as strongest inhibitors of O_2 uptake in yeast (29). Recently reported was the pronounced cytotoxicity of TA, as determined by enzyme leakage from isolated hepatocytes (30). Unfortunately however, no data was presented on the energy state of the cells. Since inhibition of the ATPase activity by TA and PT was partially prevented by ATP, the inhibitory action of these drugs becomes particularly pronounced at low levels of ATP, possibly corresponding to depleted energy stores in vivo, e.g., local ischemic conditions.

The specificity of the action of TA and PT on the ATPase activity in leukocytes is underlined by the lack of comparable inhibition by a variety of CNS drugs investigated in this study. A misinterpretation of the data on the basis of a possible inhibition by the drugs of phosphorylase (31), utilized in this study to determine inorganic phosphate, was ruled out by results of control experiments.

In view of the unknown concentration of most drugs at their cellular site of action, it is difficult to evaluate the significance of the difference between reported therapeutic levels of the drugs in plasma and their effectiveness in vitro. Inhibition of ATPase was observed with drug concentrations ranging from 10^{-5} M to 10^{-4} M. Maximum therapeutic plasma levels of TA and PT, e.g., amitriptyline and chlorpromazine, were reported to be 220 ng/ml (32) and 100 ng/ml (33), corresponding to about 0.8×10^{-6} M and 0.3×10^{-6} M, respectively. However, both TA and PT as basic amines are likely to accumulate markedly in tissues (34-36). Independently of such considerations, the here reported potent inhibition of the ATP-hydrolyzing activity by relatively low in vitro concentrations of TA and PT indicates a possible adverse effect of these drugs

on the cellular metabolism. In leukocytes, in which the ATP-ase activity appears to be localized on the outer side of the cell membrane (17), the effects of these drugs could result in an impairment of the, biologically most important, surface activity of these cells.

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