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EFFECTS OF SOME ANALGESICS AND ANTIDEPRESSANTS ON THE ($Na^+ + K^+$)-ADENOSINE TRIPHOSPHATASE FROM CORTICES OF BRAIN AND KIDNEY

FEDOR MEDZIHRADSKY and PRANEE S. NANDHASRI

Department of Biological Chemistry, and the Upjohn Center for Clinical Pharmacology, The University of Michigan, Ann Arbor, Mich. 48104, U.S.A.

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Abstract—The effects of benzomorphans, tricyclic antidepressants, monoamine oxidase (MAO) inhibitors and chlorpromazine on the microsomal ($Na^+ + K^+$)-adenosine triphosphatase (ATPase) from beef cerebral cortex were studied. As a comparison, the interaction of these drugs with the corresponding enzyme from kidney cortex was also investigated. In addition to chlorpromazine, the benzomorphans and the tricyclic antidepressants inhibited the brain enzyme considerably, whereas the MAO inhibitors had little effect. The ($Na^+ + K^+$)-ATPase from kidney was not affected by the benzomorphans or MAO inhibitors, but its activity diminished in the presence of the tricyclic antidepressants and chlorpromazine.

THE EXISTENCE of an ($Na^+ + K^+$)-activated adenosine triphosphatase (ATPase) in a variety of tissues is well documented.[1] The highest activities of this enzyme were found in nervous tissue.[2] The enzyme is intimately associated with membranes, but its solubilization has recently been achieved.[3] Considerable evidence indicates the involvement of this enzyme in the active transmembrane transport of Na^+ and K^+.[4,5] Lower aliphatic alcohols, including ethanol, as well as several general depressants have been reported to inhibit the brain ($Na^+ + K^+$)-ATPase.[6–9] It has also been shown that the lower alcohols produced an inhibition of cation transport in brain slices.[7] Inhibitory effects of imipramine on the brain microsomal ($Na^+ + K^+$)-ATPase under various experimental conditions were reported previously.[10,11]

In view of the apparent interaction of general depressants with the cell membrane, including the brain ($Na^+ + K^+$)-ATPase, it was of interest to investigate the effect of drugs having analgesic or antidepressant activity on this enzyme. As a comparison, the interaction of these compounds with the ($Na^+ + K^+$)-ATPase isolated from kidney cortex was also studied.

EXPERIMENTAL PROCEDURE

Materials. Beef brains and kidneys were generously supplied by Standard Beef Co., Detroit, and Russell B. Slaughter House, Ypsilanti, Mich. respectively. All common chemicals were of reagent grade. The sources of drugs were: Merck (amitriptyline), Geigy (desipramine, imipramine), Smith, Kline & French (chlorpromazine, phenazocine, tranylcypromine), Winthrop (pentazocine, cyclazocine), Ciba (hydralazine)

and Warner–Chilcott (phenelzine). Etazocine was kindly supplied by Dr. J. E. Villarreal, Department of Pharmacology, The University of Michigan.

Preparation of enzymes. The organs from beef were transported in ice to the laboratory within 1 hr after slaughter. The brain ($Na^+ + K^+$)-ATPase was prepared as described previously.[12] The specific activity of the enzyme in the "heavy" microsomal fraction was 16·6 μmoles Pi/mg of protein/hr. The same procedure was used to prepare the ($Na^+ + K^+$)-ATPase from kidney cortex, except that the pH of the suspending medium during the isolation of the microsomal fraction was 7·5. The specific activity of the kidney enzyme was 7·9 μmoles Pi/mg of protein/hr. Protein was determined according to Lowry *et al.*[13] The microsomal enzyme preparations from both organs contained approximately 10 mg/ml of protein and were stored at − 70°.

Enzyme assays. Aqueous solutions of the drugs (5 mg/ml) were prepared fresh prior to each experiment. If needed to achieve solubilization, the pH of the solution was adjusted to 4 with HCl. Chlorpromazine was protected from light.

The incubation medium (pH 7·0) was prepared "double strength" and contained respective millimolar concentrations of: NaCl, 180, KCl, 6, $MgCl_2$, 6, ATP, 6 and imidazol buffer, 50. The same basic incubation medium was used for both the brain and kidney enzyme, except that the latter was assayed at pH 7·5.[14] The activity of the ($Na^+ + K^+$)-ATPase was calculated by subtracting the ouabain-insensitive ATPase from the total ATPase activity. Therefore, in addition to the basic incubation medium described above (medium A), a second medium containing in addition 0·2 mM ouabain (medium B) was prepared.

Three different incubations were performed with each drug studied. The enzyme preparations from brain and kidney were diluted 1:80 with 50 mM imidazol-HCl, pH 7·0 and 7·5 respectively. To 200 μl of the diluted suspensions were added 200 μl of the incubation medium A, the set (four tubes) incubated for 10 min at 37°, then placed into an ice–water bath, and 5–55 μg of the investigated drug contained in 1–10 μl of aqueous solution, added. The final concentration of the drugs during the incubation ranged from $0{\cdot}4 \times 10^{-4}$ to 1×10^{-3} M. The tubes were then incubated for 60 min at 37° (incubation set A). Further experimental details of the enzyme assay were described previously.[12] Briefly, the enzymatic reaction was terminated with 0·6 M $HClO_4$ and the inorganic phosphate liberated was determined in the protein-free supernatant by measuring the absorbance at 700 nm after the additon of a medium containing $FeSO_4$, H_2SO_4 and NH_4-molybdate. The inorganic phosphate was quantitated from a standard curve obtained with known amounts of KH_2PO_4.

In another set of tubes, the diluted enzyme preparation was preincubated with medium B, then the drug under study was added and the tubes were reincubated for 60 min (incubation set B).

In a third group of tubes, the enzyme was preincubated for 10 min with the ouabain-free medium (medium A) in the presence of the individual drug in concentrations as indicated, followed by the addition of ouabain to give a final concentration of 1×10^{-4} M and an additional incubation of 60 min (incubation set C).

RESULTS

In view of the antagonistic effect of K^+ on the inhibition of the ($Na^+ + K^+$)-ATPase by cardiac glycosides,[12,15] its most potent inhibitors, all the experiments

reported in the present study were performed in the presence of 3 mM K$^+$. This concentration was selected as a compromise between optimal conditions for measuring (Na$^+$ + K$^+$)-ATPase activity and for measuring its inhibition.

The effect of tricyclic antidepressants on the (Na$^+$ + K$^+$)-ATPase isolated from the cortices of brain and kidney is shown in Fig. 1. Total inhibition of the enzyme by 0·1 mM ouabain was used as a reference for plotting the per cent inhibition caused by various concentrations of the drugs. The indicated amounts (0·4–5 × 10^{-4} M) are those actually present during the incubation. Although a similar pattern of inhibition was obtained for both enzymes, the (Na$^+$ + K$^+$)-ATPase from brain was more susceptible to these drugs. Amitriptyline (0·2 × 10^{-3} M), the most potent inhibitor of both enzymes, inhibited the brain enzyme twice as much as the enzyme from kidney. Interestingly, whereas the inhibition of both enzymes by amitriptyline and imipramine increased with rising concentrations of the drugs, the inhibition by desipramine showed saturation. The concentrations of imipramine which inhibited the microsomal (Na$^+$ + K$^+$)-ATPase from rat brain 9 and 25 per cent, respectively, were reported [10,11] as 1 × 10^{-4} and 0·5 × 10^{-3} M.

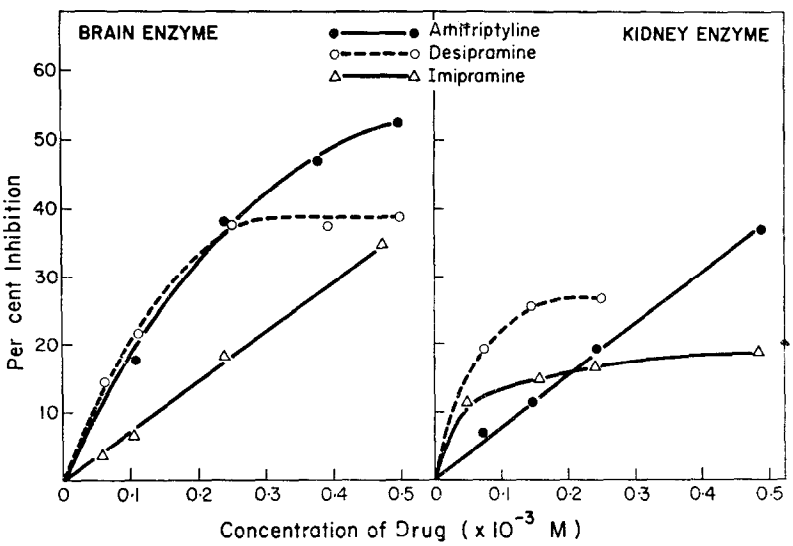

FIG. 1. Effect of tricyclic antidepressants on the microsomal (Na$^+$ + K$^+$)-ATPase from cortices of brain and kidney. The enzyme preparations were incubated with the indicated amounts of the drugs in the medium, as described in the text, containing 3 mM K$^+$. The incubations were carried out and the enzyme activity was calculated as described in the text. Each experiment, using the same enzyme preparation, was run in quadruplicate and the values presented are the averages of three experiments. The standard deviation about these averages did not exceed 5 per cent of the mean value.

In contrast to the tricyclic antidepressants, the drugs having antidepressant activity but belonging to the group of monoamine oxidase (MAO) inhibitors, present at concentrations of 0·1–1 × 10^{-3} M, showed little inhibition of the brain enzyme (Fig. 2). Only 10 per cent inhibition of the (Na$^+$ + K$^+$)-ATPase from brain was obtained with hydralazine at a concentration of 0·8 × 10^{-3} M. These drugs had no effect on the enzyme from kidney over the concentration range stated above.

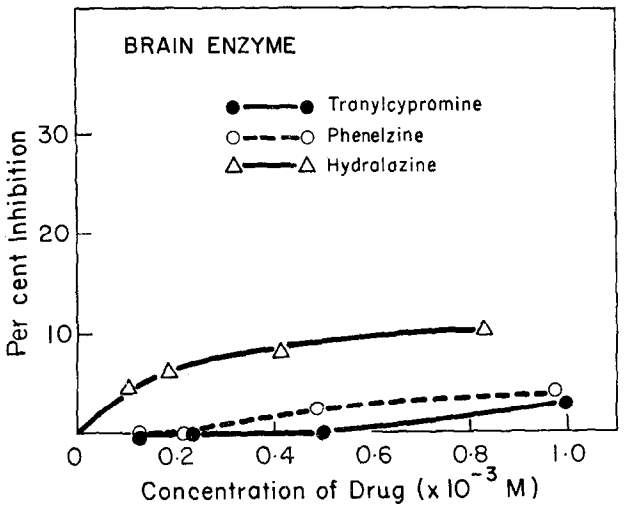

FIG. 2. Effect of MAO inhibitors on the microsomal ($Na^+ + K^+$)-ATPase from brain cortex. The enzyme preparation was incubated with the indicated amounts of the drugs in the medium, as described in the text, containing 3 mM K^+. The incubations were carried out and the enzyme activity was calculated as described in the text. Each experiment, using the same enzyme preparation, was run in quadruplicate and the values presented are the averages of three experiments. The standard deviation about these averages did not exceed 5 per cent of the mean value.

Striking differences in the inhibition of both enzymes were obtained with the benzomorphans,[16] (Fig. 3). Whereas all four analogues inhibited the brain enzyme, none of them affected the ($Na^+ + K^+$)-ATPase from kidney at concentrations shown

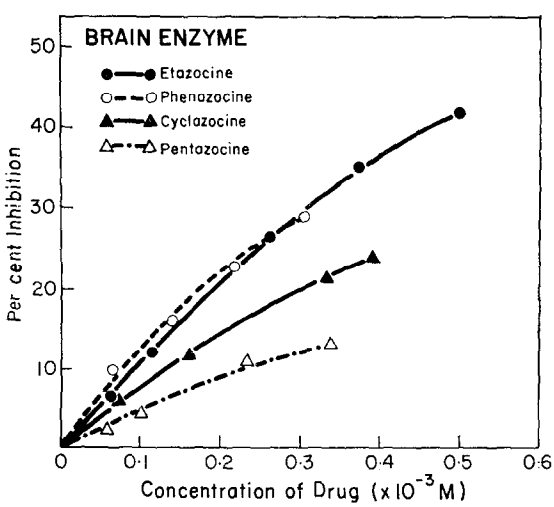

FIG. 3. Effect of benzomorphans on the microsomal ($Na^+ + K^+$)-ATPase from brain cortex. The enzyme preparation was incubated with the indicated amounts of the drugs in the medium, as described in the text, containing 3 mM K^+. The incubations were carried out and the enzyme activity was calculated as described in the text. Each experiment, using the same enzyme preparation, was run in quadruplicate and the values presented are the averages of three experiments. The standard deviation about these averages did not exceed 5 per cent of the mean value.

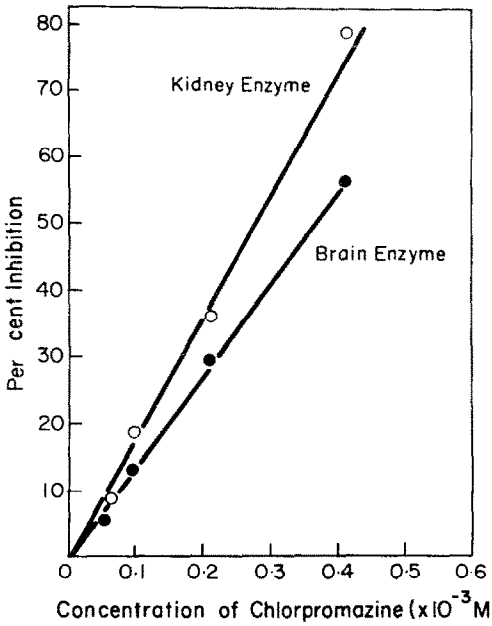

FIG. 4. Effect of chlorpromazine on the microsomal (Na$^+$ + K$^+$)-ATPase from cortices of brain and kidney. The enzyme preparations were incubated with the indicated amounts of the drug in the medium, as described in the text, containing 3 mM K$^+$. The incubations were carried out and the enzyme activity was calculated as described in the text. Each experiment, using the same enzyme preparation, was run in quadruplicate and the values presented are the averages of three experiments. The standard deviation about these averages did not exceed 5 per cent of the mean value.

in Fig. 3. The most potent inhibitor of the (Na$^+$ + K$^+$)-ATPase from brain was etazocine; 0·5 × 10^{-3} M of the drug caused more than 40 per cent inhibition.

In agreement with previous reports,[17,18] chlorpromazine was a potent inhibitor of the brain (Na$^+$ + K$^+$)-ATPase. In addition, it also strongly inhibited the enzyme from kidney (Fig. 4). The inhibition of both enzymes was linear with increasing concentrations of the drug in the investigated range of 0·1–4 × 10^{-4} M.

DISCUSSION

The reported plasma levels of the drugs producing pharmacologic activity in man[19,20] are much lower than those shown to inhibit the (Na$^+$ + K$^+$)-ATPase in the present study. However, tissue levels of these basic amines can be expected to be considerably higher than their concentrations in plasma.[21,22] The concentrations of the central nervous system drugs which significantly inhibited the brain (Na$^+$ + K$^+$)-ATPase *in vitro*[8,23] ranged from 10^{-1} to 10^{-4} M. The tension of diethylether and of halothane which produced inhibition of the microsomal (Na$^+$ + K$^+$)-ATPase from brain was ten times that of clinical concentrations.[9]

There is no direct evidence for the involvement of the brain (Na$^+$ + K$^+$)-ATPase in the mechanism of action of analgesics. When assayed in the absence of ions, the activity of the microsomal (Na$^+$ + K$^+$)-ATPase from brain decreased after chronic

morphine treatment of rats, suggesting an interaction between these ions and the drug at the active site of the enzyme.[24] A similar relation between the presence of Na^+ and K^+ and the inhibition of this enzyme was reported with imipramine.[11] The correlation between the effects of these drugs on the $(Na^+ + K^+)$-ATPase from the brain and their pharmacologic activity has been discussed on the basis of a drug-induced change in membrane permeability.[10,25] Since both the general depressants[8] and the antidepressant drugs reported here inhibit the brain $(Na^+ + K^+)$-ATPase, a common phase in the mechanism of action of these two groups of drugs may exist. In view of the difference in psychological effects of tricyclic antidepressants and of MAO inhibitors,[26,27] the distinctly different interaction of these two groups of compounds with the $(Na^+ + K^+)$-ATPase from brain becomes of particular interest.

The differential sensitivity of the kidney enzyme to the tricyclic antidepressants and benzomorphans is of particular interest and its elucidation deserves further work. Concerning the relatively strong inhibition of the kidney enzyme by the tricyclic antidepressants, there is an increasing number of reports describing various side effects of these drugs.[28–30] It is possible that the effects observed in the present study on the $(Na^+ + K^+)$-ATPase from kidney are a reflection of the multi-organ pharmacologic actions of these drugs.

Chlorpromazine, which is known to be a potent inhibitor of the $(Na^+ + K^+)$-ATPase from brain,[17,18] was used in this study as a reference compound in order to compare the viability of the two enzyme preparations. As can be seen in Fig. 4, the drug strongly inhibited the $(Na^+ + K^+)$-ATPase from kidney in addition to its described effects on the brain enzyme. Interestingly, a linear relation between inhibition and concentration of chlorpromazine was obtained with both enzymes. This is in contrast to the sigmoidal dose–response curve obtained by the interaction of chlorpromazine free radical with the microsomal $(Na^+ + K^+)$-ATPase previously treated with deoxycholate and NaI.[18]

The interaction of the drugs with the enzyme preparations was studied under three different experimental conditions. In incubation series A, both the $(Na^+ + K^+)$-ATPase and the ouabain-insensitive ATPase were exposed to drug action. In series B, prior to the addition of the drugs, the $(Na^+ + K^+)$-ATPase was totally inhibited by ouabain. This order of addition was reversed in series C, i.e. the enzyme preparation was first exposed to the drug, followed by the addition of ouabain. Inhibition of the $(Na^+ + K^+)$-ATPase by each drug was obtained by subtracting enzyme activity in series B or C from that obtained in series A. Except with chlorpromazine, the enzyme activities obtained in series C were higher than those in series B in all other instances of observed drug inhibition of the $(Na^+ + K^+)$-ATPase, suggesting partial blocking of the cardiac glycoside site of the enzyme by the drugs. With the brain enzyme, the observed differences averaged to 16 ± 3 per cent for both the tricyclic antidepressants and the benzomorphans. Interestingly, the activity of the $(Na^+ + K^+)$-ATPase from kidney revealed no difference when assayed in series B and C in the presence of the tricyclic antidepressants.

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