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KINETICS OF CATECHOLAMINE SENSITIVE Na^+-K^+ ATPase ACTIVITY IN MOUSE BRAIN SYNAPTOSOMES

D. DESAIAH and I. K. HO

Department of Pharmacology and Toxicology, The University of Mississippi Medical Center, Jackson, MS 39216, U.S.A.

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Abstract—The effects of several neurotransmitters on mouse brain synaptosomal ATPase activities were determined *in vitro*. Both dopamine and norepinephrine activated Na^+-K^+ and Mg^{2+} ATPase activities in a dose-dependent manner. Na^+-K^+ ATPase was more sensitive to the catecholamines than was Mg^{2+} ATPase activity. Acetylcholine, γ-aminobutyric acid, L-glutamic acid and serotonin were without effect up to 10^{-3} M concentration. Chlorpromazine, an antipsychotic agent, which has been shown to block the dopamine-receptor site, totally inhibited the dopamine-stimulated Na^+-K^+ and Mg^{2+} ATPase activities in mouse brain synaptosomes. Further, catecholamine-sensitive ATPase activities from mouse brain synaptosomal preparation were determined in relation to the substrate, pH and ionic concentrations in the reaction medium. The results indicate that Na^+-K^+ and Mg^{2+} ATPases were activated by dopamine (DA) and norepinephrine (NE) at different concentrations of ATP. Lineweaver–Burk plots reveal that Na^+-K^+ ATPase was activated non-competitively to ATP with a K_m value of 5.5×10^{-4} M, whereas Mg^{2+} ATPase exhibited a mixed type of activation in that K_m was decreased and V_{max} increased in the presence of DA or NE. A maximum stimulation occurred by catecholamines at the optimum pH of 7.5 for Na^+-K^+ and 8.0 for Mg^{2+} ATPase activities. Both catecholamines increased the Na^+-K^+ ATPase activity in the presence of Na^+ and K^+ in the reaction medium. However, in the absence of Na^+ ions the K^+-ATPase activity was stimulated by DA and NE but in the absence of K^+ ions the Na^+ ATPase, was not activated by DA or NE, indicating that the ATPase activity was more sensitive to catecholamines in the presence of K^+ than Na^+.

Na^+-K^+ ATPase (EC 3.6.1.3) is a component of the plasma membranes of animal cells [1, 2]. It is well established that this enzyme has been implicated in the active ion transport across the cell membrane [1, 3–5] and the Na^+-K^+ ATPase is considered synonymous with the sodium pump [4]. Na^+-K^+ Activated ATPase is known to be concentrated in synaptic membranes of central nervous system [6]. Grenell [7] has pointed out that changes in the status of synaptic membranes would markedly modify information transmission and coding. The transducer property of synapse is critical to central nervous system functioning. Certain neurotransmitters are shown to influence the membrane bound Na^+-K^+ ATPase activity in rat brain microsomes [8–11], and more recently in rabbit brain synaptic membrane preparations [12]. In a previous study [13], we showed that dopamine and norepinephrine enhanced the ATPase activity in mouse brain crude nerve ending preparation but not in kidney and liver tissues. Recently, a dopamine-sensitive adenylate cyclase has been found in homogenates of rat caudate nucleus and olfactory tubercle and has been suggested as the dopamine receptor in mammalian brain [14–16]. The activation of adenylate cyclase was inhibited by antipsychotic agents [17]. However, no attempts have been made so far to study the interaction between dopamine-stimulated ATPase and antipsychotic drugs. Since the process of neurotransmitter release or uptake occurs at synapse and Na^+-K^+ activated and Mg^{2+} ATPase may be involved in this process [6], the present studies were undertaken to examine the effects of various neurotransmitters on ATPase activity and the kinetic properties and ion requirements of the catecholamine-sensitive Na^+-K^+ ATPase activity in mouse brain synaptosomes, and to study the interaction between dopamine-sensitive ATPase and chlorpromazine, an antipsychotic drug.

MATERIALS AND METHODS

Materials. Male ICR mice weighing 25–30 g obtained from Charles River, Wilmington, MA, were used. Neurotransmitters and their suppliers were as follows: acetylcholine bromide (ACh) from Eastman Organic Chemical Co., Rochester, NY; dopamine (DA), γ-aminobutyric acid (GABA), and L-glutamic acid (GLU) from Calbiochem. LaJolla, CA; norepinephrine (NE) and serotonin (5-HT) from Sigma Chemical Co., St. Louis, MO; and chlorpromazine from Elkins-Sinn, Inc., Cherry Hill, NJ. The rest of the chemicals used in this study were obtained from Sigma Chemical Co., St. Louis, MO.

Preparation of synaptosomes. Synaptosomes were prepared from mouse brain using a slightly modified procedure of Cotman and Matthews [18]. Mice were decapitated, the whole brains were quickly removed and kept in ice cold sucrose solution (0.32 M sucrose, with 1 mM EDTA and 10 mM imidazole, pH = 7.5). The tissue was homogenized in 9 volumes of sucrose solution using a ground glass homogenizer. The homogenate was centrifuged at 750 *g* for 10 min and the pellet was discarded. The supernatant was centrifuged at 17,000 *g* for 10 min and the pellet was resus-

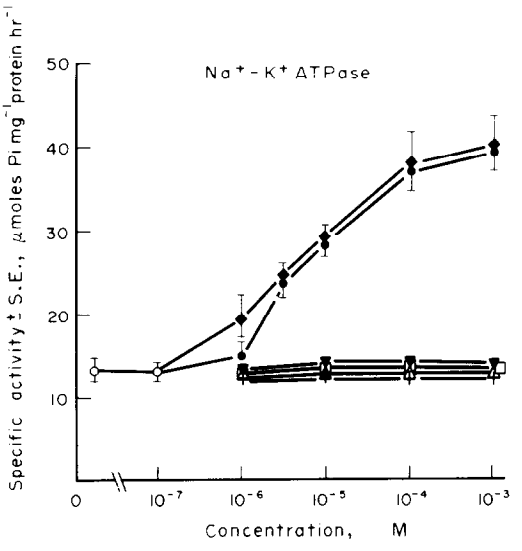

Fig. 1. Effect of various concentrations of different neurotransmitters on Na^+-K^+ ATPase activity in mouse brain synaptosomal fraction. Each point was the mean of three different preparations and each preparation was assayed 2–3 times and the averages were taken. (○) control; (●) dopamine; (◆) norepinephrine; (▲) γ-aminobutyric acid; (□) L-glutamic acid; (△) acetylcholine; (✣) serotonin.

pended in sucrose solution and re-centrifuged at 17,000 *g* for 10 min. The pellet was resuspended in 10 ml sucrose solution and layered on a two step discontinuous ficoll-sucrose gradient, consisting of 13% (w/v) ficoll in 0.32 M sucrose and 7.5% ficoll (w/v) in 0.32 M sucrose. After centrifugation in a Beckman L5-65 centrifuge with SW 27 rotor at 22,000 rpm for 45 min, the synaptosomal fraction was obtained at the interface of 7.5–13% ficoll-sucrose layer. The synaptosome band was removed, diluted with 9 volumes of sucrose solution and centrifuged at 17,000 *g* for 10 min in a Sorval RC5 centrifuge. The synaptosomal pellet was resuspended in sucrose solution, divided into small aliquots and quick frozen in liquid N_2. The frozen samples were stored at −85° until used for ATPase assay.

Determination of ATPase activity. ATPase activity was measured essentially according to the method of Fritz and Hamrick [19] and as reported previously [20]. A 3 ml reaction mixture (unless otherwise mentioned) contained: 4.5 mM ATP, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 135 mM imidazole-Cl buffer, pH 7.5, 0.2 mM NADH, 0.5 mM phosphoenol pyruvate, 0.02% bovine serum albumin, approximately 9 units of pyruvate kinase and 12 units of lactic acid dehydrogenase. A 25 μl synaptosomal preparation was used with a protein content of 20–30 μg. Absorbance changes in reaction mixture were measured at 340 nm using a Beckman Acta III recording spectrophotometer with temperature controlled at 37°. The change in optical density at 340 nm over a period of 10 min was used in calculating the specific activity. Enzyme activities were expressed as μmoles P_i mg protein^{-1} hr^{-1}. Protein was determined by the method of Lowry *et al.* [21], using bovine serum albumin as standard.

Total ATPase activity was measured with Mg^{2+}, Na^+ and K^+ present in the reaction mixture. Mg^{2+} ATPase activity was measured in the presence of 1 mM ouabain, which is a specific inhibitor of Na^+-K^+ ATPase [22]. Na^+-K^+ ATPase activity (ouabain-sensitive) was obtained by the difference between total ATPase activity and Mg^{2+} ATPase activity.

Effects of catecholamines and other neurotransmitters on ATPase activities. Fresh stock solutions of neurotransmitters used were made daily in glass distilled water. Desired amounts of these stock solutions were added to the reaction mixture to give the final concentrations as shown in the figures. The ATPase activities were determined in the presence and absence of different neurotransmitters and percent effect was calculated. Standard errors were calculated where three or more enzyme preparations were used. In other experiments triplicate assays were made using two different enzyme preparations and the difference between the replicates was less than 5 percent of the average. All compounds at 1 mM concentrations did not show any effect on the reaction medium in the absence of synaptosomal preparation.

RESULTS

Several neurotransmitters were tested for their influence on Na^+-K^+ ATPase activity in mouse brain synaptosomal preparation *in vitro* and the results are presented in Fig. 1. Both DA and NE significantly enhanced the Na^+-K^+ ATPase activity. The increase in ATPase activity by these two catecholamines was dose dependent reaching a maximum effect of 200 per cent at 10^{-3} M. A 100 per cent increase in Na^+-K^+ ATPase activity was obtained at 10^{-5} M concentration of DA and NE. GABA, GLU, ACh and 5-HT were without appreciable effect on Na^+-K^+ ATPase activity in mouse brain synaptosomal preparation (Fig. 1).

Mg^{2+}-ATPase activity (ouabain-insensitive) in mouse brain synaptosomes was also activated by DA and NE (Fig. 2). A 50 per cent increase in enzyme activity was observed at 10^{-5} M concentration of DA

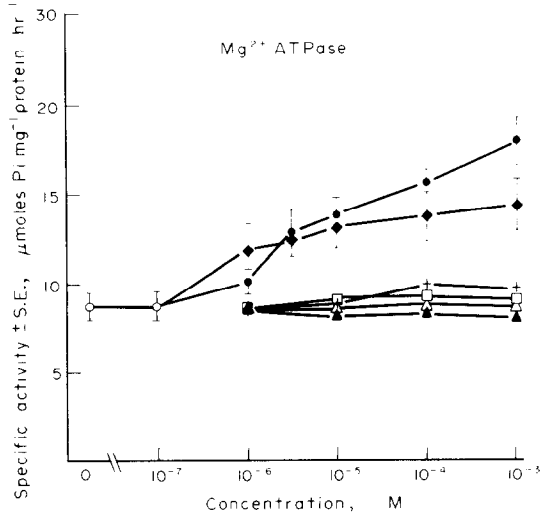

Fig. 2. Effect of various concentrations of different neurotransmitters on Mg^{2+} ATPase (ouabain-insensitive) activity in mouse brain synaptosomal fraction. The other details as in the legend of Fig. 1.

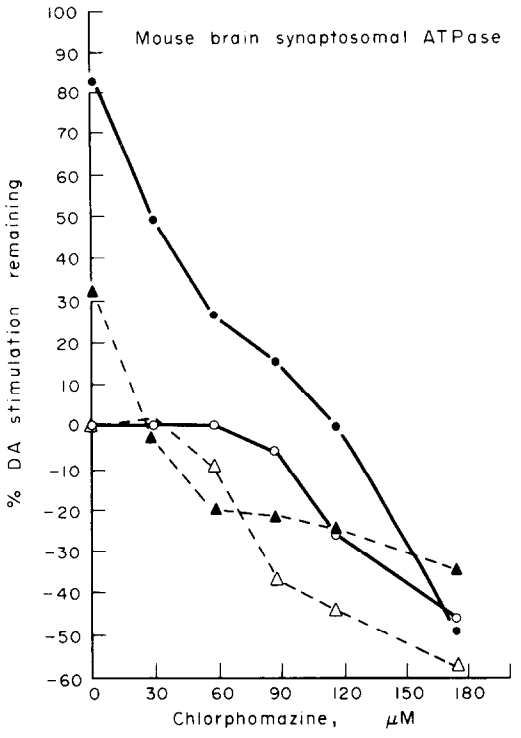

Fig. 3. Effect of different concentrations of chlorpromazine on dopamine-stimulated ATPase activities in mouse brain synaptosomal fraction. Each point was the average of three different enzyme assays and the difference was less than 2–5%. Solid line represents Na^+-K^+ ATPase activity in the presence (●) and absence (○) of dopamine. Broken line represents the Mg^{2+} ATPase activity in the presence (●) and absence (○) of dopamine.

and NE. Like Na^+-K^+ ATPase activity, the Mg^{2+} ATPase activity was not altered by the other neurotransmitters tested, i.e., GABA, GLU, ACh and 5-HT (Fig. 2).

Since DA and NE stimulated both Na^+-K^+ and Mg^{2+} ATPase activities at low concentrations, we determined the interaction of chlorpromazine, an antipsychotic agent, and DA-stimulated ATPase activities in mouse brain synaptosomes. The results in Fig. 3 show that chlorpromazine blocked both Na^+-K^+ and Mg^{2+} ATPase activities stimulated by DA. A 50 per cent inhibition of DA-stimulated Na^+-K^+ ATPase activity occurred with 40 μM chlorpromazine at which concentration the drug has no effect on basal Na^+-K^+ ATPase activity. At higher concentrations, chlorpormazine inhibited basal Na^+-K^+ ATPase activity. A similar effect was also observed with Mg^{2+} ATPase activity. However, it is interesting to note that basal Mg^{2+} ATPase activity was inhibited more than Na^+-K^+ ATPase activity by chlorpromazine.

Since the DA and NE activated Na^+-K^+ ATPase activity in mouse brain synaptosomal preparation, we have studied the properties of the catecholamine-sensitive ATPase activities in relation to the substrate, pH and ionic requirements.

The effect of ATP concentration on Na^+-K^+ *and* Mg^{2+} *ATPase activities in the absence and presence of DA and NE.* In the present investigation, kinetic studies were conducted to evaluate the nature of the activation of mouse brain synaptosomal Na^+-K^+ ATPase activity by DA and NE at 10^{-5} M concentration. The results in Fig. 4(a) show that the mouse brain synaptosomal Na-K ATPase activity was increased with the increase in concentration of the substrate, ATP. It is also evident that Na^+-K^+ ATPase activity was activated by the addition of 10^{-5} M dopamine or norepinephrine at different concentrations of ATP. The data in Fig. 4(a) were replotted as double reciprocal plots (Lineweaver–Burk) and presented in Fig. 4(b). The results in Fig. 4(b) show that both catecholamines activated Na^+-K^+ ATPase activity in a non-competitive manner; the K_m value for ATP was 5.5×10^{-4} M both in the absence and presence of catecholamines.

The data in Fig. 5(a) show that the mouse brain synaptosomal Mg^{2+} ATPase activity was increased with increase in ATP concentration and the data also

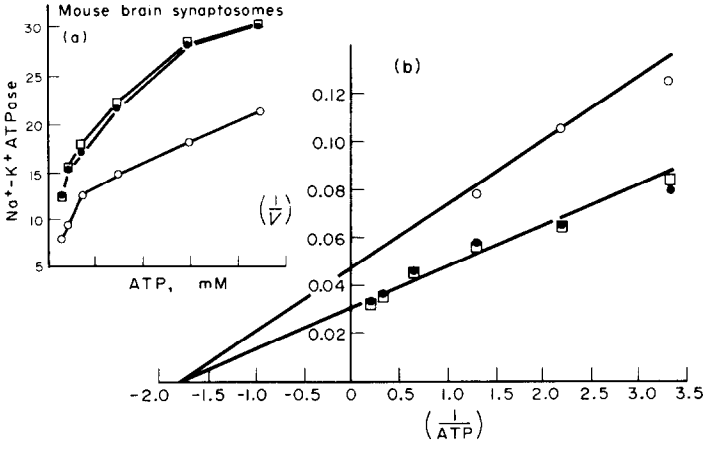

Fig. 4. (a) Mouse brain synaptosomal Na^+-K^+ ATPase activity in relation to the concentration of ATP in the absence (○—○) and presence of 10^{-5} M dopamine (●—●) or norepinephrine (□—□). (b) Lineweaver-Burk plots of mouse brain synaptosomal Na^+-K^+ ATPase activity in the absence (○—○) and presence of 10^{-5} M dopamine (●—●) and norepinephrine (□—□). ATP in mM concentration and V = specific activity expressed as μmoles P_i mg protein^{-1} hr^{-1}. V_{max} for control was 20.8 and in the presence of DA or NE was 33.3 and K_m was 5.5×10^{-4} M.

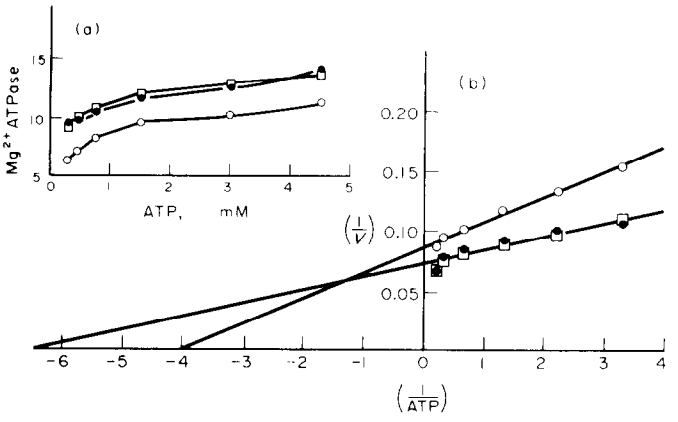

Fig. 5. (a) Mouse brain synaptosomal Mg^{2+} ATPase activity in relation to the concentration of ATP in the absence (○) and presence of 10^{-5} M dopamine (●) or norepinephrine (□). (b) Lineweaver–Burk plots of mouse brain synaptosomal Mg^{2+} ATPase activity in the absence (○—○) and presence of 10^{-5} M dopamine (●—●) or norepinephrine (□—□). ATP in mM concentration. V_{max} values for control = 11.6; dopamine = 13.6 and norepinephrine = 13.9 μmoles P_i mg protein^{-1} hr^{-1}. K_m values were: control = 2.5×10^{-4} M; dopamine = 1.5×10^{-4} M and norepinephrine = 1.6×10^{-4} M.

show that both catecholamines stimulated Mg^{2+} ATPase activity. The data in Fig. 5(a) were replotted as double reciprocal plots and presented in Fig. 5(b). The activation was of a mixed type because the K_m was decreased and V_{max} was increased in the presence of DA or NE.

The effect of pH on Na^+-K^+ *and* Mg^{2+} *ATPase activities in the absence and presence of DA and NE.*

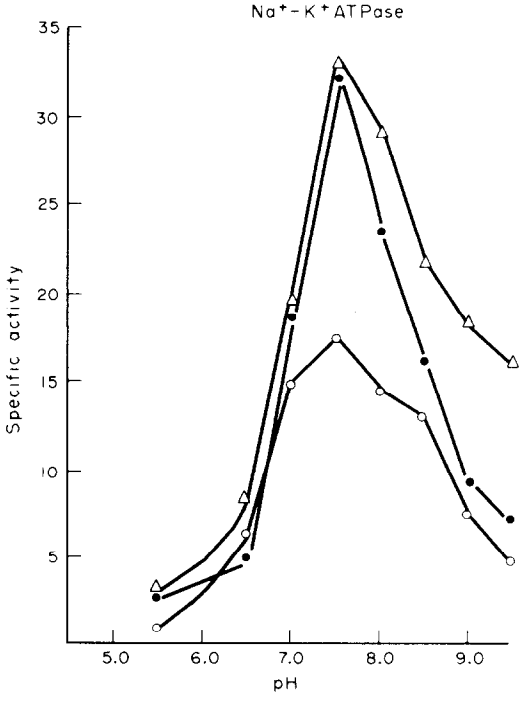

Fig. 6. Relation between Na^+-K^+ ATPase activity (ouabain-sensitive in mouse brain synaptosomes and pH. (○—○) control; (●—●) dopamine (10^{-5} M); (△—△) norepinephrine (10^{-5} M).

Na^+-K^+ ATPase activity was determined in the absence and presence of DA and NE (10^{-5} M) over a wide range of pH. As shown in Fig. 6, the pH optimum was 7.5 for control activity. Both DA and NE did not stimulate the ATPase activity in acid pH but showed their effect at pH 7.5. However, NE enhanced the Na^+-K^+ ATPase activity in alkaline pH whereas DA did not stimulate in this pH range (Fig. 6).

Mouse brain synaptosomal Mg^{2+} ATPase activity was also measured in the absence and presence of DA and NE (10^{-5}) over a wide range of pH. The results in Fig. 7 show that the optimum pH for this enzyme activity was 8.0. Both DA and NE were effective in pH 7.5–8.0 and were without appreciable effect in acid and alkaline pH.

The effect of K^+ *concentration on* Na^+-K^+ *ATPase activity in the absence and presence of DA and NE.* ATPase activity was measured in the presence of 100 mM Na^+ and with varying amounts of K^+ in the reaction medium. As shown in Fig. 8 without K^+ present, the ATPase activity was minimal but increased with the addition of K^+ ions. A maximum activity was observed with 5 mM K^+ and a decrease in activity was seen with further increase in concentration of K^+ ions. Both DA and NE (10^{-5} M) showed no effect on Na^+-K^+ ATPase activity without adding K^+ ions but the stimulatory effect of these catecholamines was evident with increase in concentration of K^+ ions. Although the control activity decreased with high K^+ concentration the stimulatory effect of DA or NE was not decreased up to 20 mM K^+.

The effect of Na^+ *concentration on* Na^+-K^+ *ATPase activity in the absence and presence of DA and NE.* Since maximum Na^+-K^+ ATPase activity was obtained at 5 mM K^+ and 100 mM Na^+, we have determined the ATPase activity with constant K^+ concentration at 5 mM and varying the amount of Na^+ in the reaction medium (Fig. 7). The ATPase activity at 0 Na^+ was minimal and gradually an in-

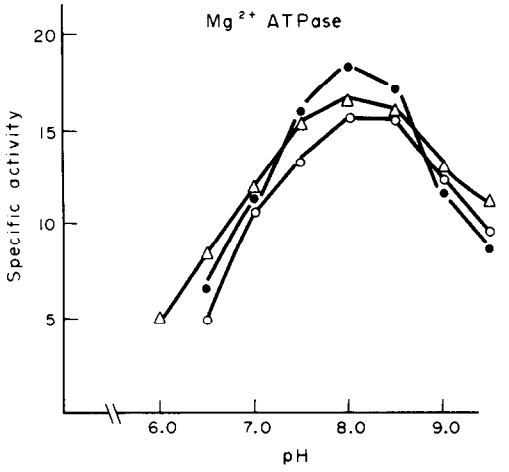

Fig. 7. Relation between Mg^{2+} ATPase activity (ouabain-insensitive) in mouse brain synaptosomes and pH. (O—O) control; (●—●) dopamine (10^{-5} M); (△—△) norepinephrine (10^{-5} M).

creased ATPase activity was obtained attaining a maximum activity at 100 mM Na^+ in the reaction. When DA or NE (10^{-5} M) was added into the reaction mixture the ATPase activity was increased considerably at 0 Na^+ and further increase in ATPase activity was seen with the addition of Na^+ ion up to 100 mM. However, with further increase in Na^+ ion beyond 100 mM the effect of DA and NE was decreased to zero Na^+ level as the Na^+ concentration reached 200 mM (Fig. 9).

The effect of DA and NE on K^+-ATPase activity. The ATPase activity of mouse brain synaptosomes was stimulated by either DA or NE in the presence of K^+ alone. As seen in Fig. 10 the control activity

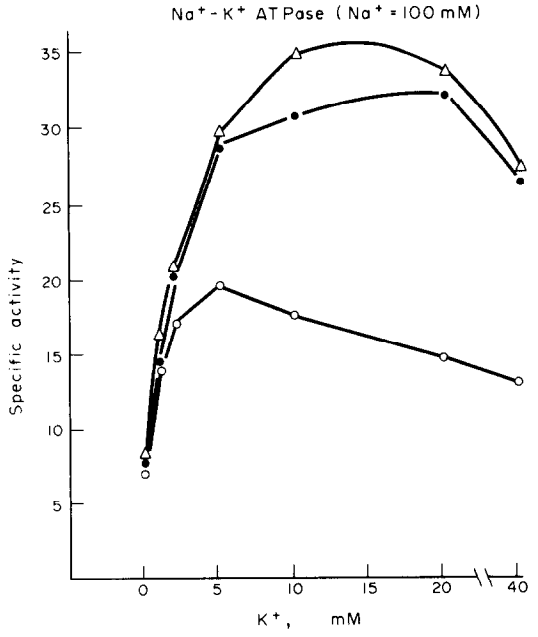

Fig. 8. Na^+-K^+ ATPase activity (μmoles P_i mg protein^{-1} hr^{-1}) in relation to the various concentrations of K^+ at constant 100 mM concentration of Na^+. (O—O) control; (●—●) dopamine (10^{-5} M); (△—△) norepinephrine (10^{-5} M).

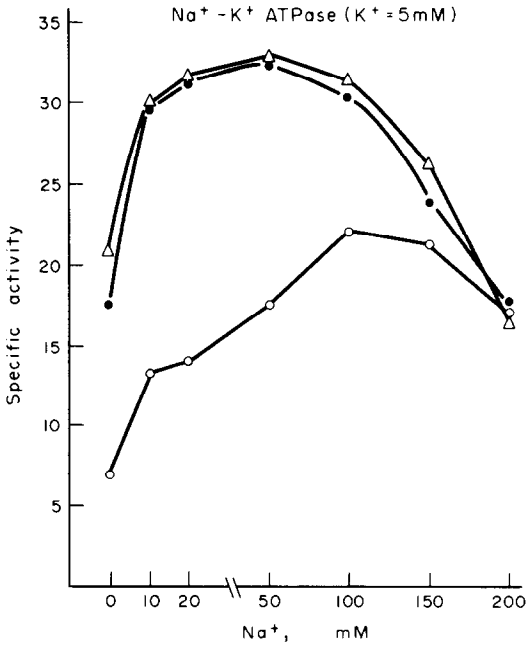

Fig. 9. Na^+-K^+ ATPase activity (μmoles P_i mg protein^{-1} hr^{-1}) in relation to the various concentrations of Na^+ at constant 5 mM concentration of K^+. (O—O) control; (●—●) dopamine (10^{-5} M); (△—△) norepinephrine (10^{-5} M).

was not changed although the ATPase activity was minimal. However, DA and NE considerably increased the ATPase activity up to 20 mM K^+ concentration in the reaction. The stimulatory effect was decreased as the K^+ ion concentration increased to 40 mM. GABA (10^{-4} M), on the other hand, apparently decreased the ATPase activity in the presence of K^+ alone and a greater decrease was evident at 40 mM K^+ (Fig. 10).

DISCUSSION

From the present results it is clear that catecholamines stimulate both Na^+-K^+ and Mg^{2+} ATPase activities in mouse brain synaptosomal fraction. The activation of ATPase activities was dose dependent. These results are in good agreement with those reported earlier using rat brain microsomes [8, 9], whole homogenate [10] and mouse brain crude nerve ending preparation [13]. In the present experiments we demonstrated that ATPase activity in brain synaptosomes is more sensitive to catecholamine stimulation. ACh, GLU, GABA and 5-HT affect neither Na^+-K^+ nor Mg^{2+} ATPase activities. This observation is consistent with our earlier findings that GABA and 5-HT were without effect on ATPase activities in brain nerve ending preparation [13]. Further, the present results show that chlorpromazine was an effective inhibitor of dopamine-stimulated ATPase activities. The present results also show that the activation of Na^+-K^+ ATPase activity by DA and NE was a non-competitive in nature and Mg^{2+} ATPase activity was a mixed type as evidenced by Lineweaver–Burk plots of the data. The optimum pH for Na^+-K^+ ATPase in synaptosomes was 7.5 and 8.0 for Mg^{2+} ATPase activity. The enzyme activities are

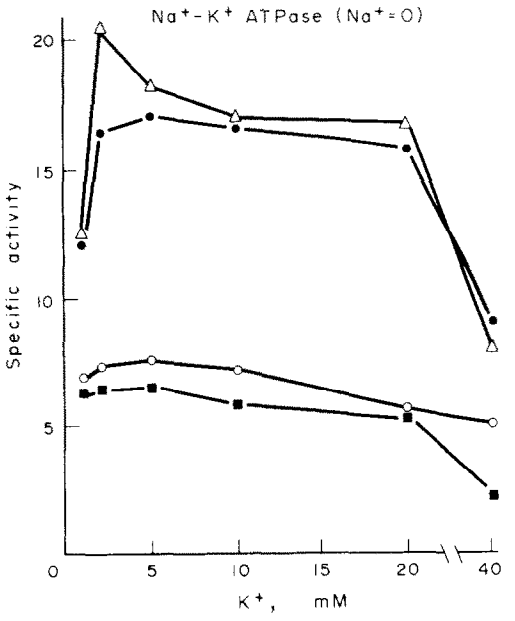

Fig. 10. ATPase activity (μmoles P_i mg protein^{-1} hr^{-1}) in relation to the various concentrations of K^+ in the absence of Na^+ ions in the medium. (O—O) control; (●—●) dopamine (10^{-5} M); (△—△) norepinephrine (10^{-5} M); (■—■) GABA (10^{-4} M).

more sensitive to DA and NE at the optimum pH and less sensitive in acidic and alkaline pH media.

ATPase activity is known to be activated by Na^+ and K^+ ions [1]. Similarly, in the present experiments, we observed the maximum activation of ATPase activity at 100 mM Na^+ and 5 mM K^+. In the absence of either Na^+ or K^+ the enzyme activity is very low. In the absence of K^+ and in the presence of 100 mM Na^+, DA or NE (10^{-5} M) did not stimulate the ATPase activity. By the addition of K^+ ions the two catecholamines increased the Na^+-K^+ ATPase activity. The increase in enzyme activity by DA and NE was dose dependent of K^+ ions up to 20 mM and a decrease was seen at 40 mM K^+. On the other hand, in the absence of Na^+ and in the presence of 5 mM K^+, DA and NE enhance the enzyme activity and with inclusion of Na^+ ion in the reaction the stimulation is further increased. However, at higher concentration of Na^+ beyond 100 mM the stimulatory effect of DA and NE was decreased to zero level. These results suggest that the Na^+-K^+ ATPase in the mouse brain synaptosomes was more sensitive to DA and NE in the presence of K^+ than in the presence of Na^+ ions in the medium.

It is generally believed that the partial reactions of the Na^+-K^+ ATPase involve a phosphorylation step yielding a phosphoenzyme (E_1-P) in the presence of Na^+ and Mg^{2+}, and a second intermediate (E_2-P) formed by conformational change of E_1-P; the E_2-P has a high affinity to K^+ and undergoes hydrolysis (2,4,23,24). Since DA and NE were more effective on Na^+-K^+ ATPase in the presence of K^+ than Na^+ it may be possible that these catecholamines are acting on E_2-P form of Na^+-K^+ ATPase. The demonstration that the Na^+-K^+ ATPase in mouse brain synaptosomal preparation was more sensitive to DA and NE in the presence of K^+ than Na^+, it may be possible that these catecholamines are acting directly at or near the K^+ site. Further work is warranted in support of this hypothesis. The Lineweaver-Burk plots show that Na^+-K^+ ATPase was activated by DA and NE non-competitively when assayed in the presence of 100 mM Na^+, 20 mM K^+, and 5 mM Mg^{2+} in the reaction mixture. However, our preliminary results show that by reducing the K^+ concentration to 5 mM in the reaction medium the activation by DA and NE was neither competitive nor non-competitive suggesting a major role of K^+ in the activation of Na^+-K^+ ATPase by DA and NE.

An alternative approach for explaining the observed effects of DA and NE on ATPase activity is by implicating adenyl cyclase system. It has been proposed that a possible relationship exists between adenyl cyclase and Na^+-K^+ ATPase in the membrane [5]. They suggested that the c-AMP dependent protein kinase may be involved in the phosphorylation of ATPase system. DA has been shown to stimulate adenyl cyclase resulting in the elevated production of c-AMP and stimulated c-AMP dependent protein kinase, which would increase the Na^+-K^+ ATPase activity. However, our preliminary results did not show any effect of c-AMP (up to 10^{-4} M) on ATPase activity in mouse brain synaptosomes suggesting a direct modulation of catecholamines on ATPase system rather than via c-AMP. However, our results demonstrate that chlorpromazine can block the dopamine stimulation of ATPase activity. These results would suggest a direct interaction of Na^+-K^+ ATPase and catecholamines. The demonstration that the activation of Na^+-K^+ and Mg^{2+} ATPases by DA and NE in synaptosomes may indicate that these enzymes are serving as receptors or located at the receptor site of the catecholamines on the synaptic membrane.

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