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A COMPARATIVE STUDY OF HISTAMINE AND K^+ EFFECTS ON (Ca^{2+}–Mg^{2+})-ATPase ACTIVITY IN SYNAPTOSOMES

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Abstract—Histamine (10^{-4} M) and 60 mM K^+, but not 60 mM Na^+ or 60 mM choline$^+$, increased the maximal synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity by 15 and 36% respectively and decreased the extrasynaptosomal Ca^{2+} concentration necessary to reach it. Histamine and K^+ enhanced the synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity in a concentration-dependent manner. In synaptic plasma membranes histamine (10^{-4} M) and 60 mM choline$^+$ were not able to alter the enzymatic activity, however 60 mM K^+ and 60 mM Na^+ elevated (Ca^{2+}–Mg^{2+})-ATPase activity by 20 and 15%, repectively, without altering the affinity for Ca^{2+}. Histamine effects in synaptosomes were mediated by H_2 receptor stimulation. 3-Isobutyl-1-methyl-xanthine (10^{-4} M) potentiated (15%) the maximal histamine effect. The slow Ca^{2+} channel antagonists verapamil and diltiazem, both at 10^{-6} M, completely inhibited K^+ effects in synaptosomes, however histamine effects were only blocked by verapamil. The data suggest that K^+ and histamine effects on synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity are mediated by increases of intrasynaptosomal Ca^{2+} levels. Moreover, histamine effects on synaptosomal enzyme activity were mediated by cAMP.

The regulation of cytosolic free Ca^{2+} concentration is particularly important for various aspects of synaptic transmission [1]. In synaptosomes Ca^{2+} can be stored in the endoplasmic reticulum and mitochondria [2–4]. Ca^{2+}-extrusion across the synaptic plasma membrane (SPM)† by a Na^+/Ca^{2+} exchange system [5, 6] and an ATP-driven Ca^{2+} pump, (Ca^{2+}–Mg^{2+})-ATPase [7, 8], have also been described.

The (Ca^{2+}–Mg^{2+})-ATPase activity in synaptosomes has been shown to be stimulated by calmodulin and with high affinity by Ca^{2+} [8–10]. On the other hand, synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity is modulated by cAMP [11].

It has been reported that stimulation of histamine (HA) H_2 receptors increases cAMP levels in mammalian brain [12, 13]. Moreover, stimulation of these receptors, as well as depolarizing K^+ concentrations, increase Ca^{2+} uptake in rat brain synaptosomes [14, 17]. Thus, it could be suggested that both HA and depolarizing K^+ act on Ca^{2+} extrusion mechanisms to maintain intrasynaptosomal Ca^{2+} homeostasis.

In this work we compared the effects of HA and K^+ on synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity.

MATERIALS AND METHODS

Chemicals. Histamine, mepyramine, 3-isobutyl-1-methyl-xanthine (IBMX), and verapamil were obtained from the Sigma Chemical Co. (Deisenhofen, F.R.G.). 2-Thiazolylethylamine and dimaprit were kindly supplied by Smith Kline and French (Philadelphia, PA, U.S.A.). Ranitidine was a gift from Lesvi Laboratorios (Spain). Diltiazem was purchased from Laboratorios Dr Esteve (Spain). ATP disodium salt was from Merck (Darmstadt, F.R.G.). All other reagents were of analytical grade.

Isolation of synaptosomes and SPM. Synaptosomes and SPM were prepared from rat brains as described by Dodd *et al.* [18] and Jones and Matus [19], respectively. Synaptosomal and SPM pellets were resuspended in 20 mM Tris–HCl, pH 7.4, with and without 0.32 M sucrose respectively, at concentrations of 2.0–2.5 mg protein/mL for synaptosomes and 0.5–1.0 mg protein/mL for SPM and immediately used for ATPase assays.

ATPase assays. ATPase activity was measured by the colorimetric determination of P_i hydrolysed from ATP by the method of Fiske and Subbarrow as modified by Lebel *et al.* [20].

ATPase assay was performed as described previously [11]. Briefly, synaptosomes (0.20–0.25 mg protein) or SPM (0.05–0.10 mg protein) were suspended in incubation media (final volume 1 mL) containing 20 mM Tris–HCl, pH 7.4; 0.75 mM $MgCl_2$; 0.4 mM ethyleneglycolbis(aminoethyl-ether)tetra-acetate (EGTA); 0.1 mM ouabain; 0.5 mM sodium azide; with (for total ATPase activity) or without (for basal Mg^{2+}-ATPase activity) addition of varying concentrations of $CaCl_2$ (0.1–0.4 mM) which correspond to (0.075–9.500 μM) free Ca^{2+} concentrations as calculated by the method described by Bartfai [21]. When enzymatic activity was investigated in intact synaptosomes, the isotonicity of the reaction media was maintained by the addition of sucrose. Reaction mixtures were preincubated at 37° for 10 min. The reaction was

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† Abbreviations: HA, histamine; SPM, synaptic plasma membranes; IBMX, 3-isobutyl-1-methyl-xanthine; Ch$^+$, choline$^+$.

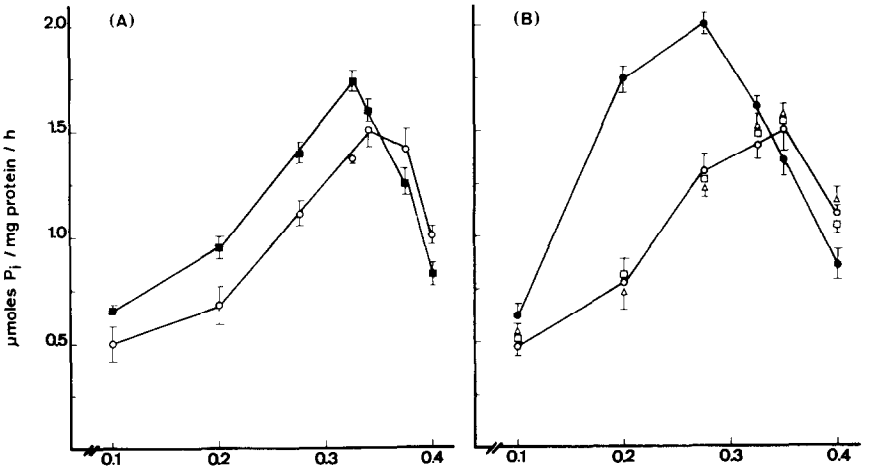

Fig. 1. Effects of HA and monovalent cations on (Ca^{2+}–Mg^{2+})-ATPase activity in synaptosomes as a function of Ca^{2+} concentrations. Synaptosomes were incubated in reaction mixtures containing varying $CaCl_2$ concentrations (0.1–0.4 mM) (0.075–9.5 µM free Ca^{2+} concentrations), in the presence or absence of 10^{-4} M HA, 60 mM K^+, 60 mM Na^+ or 60 mM Ch^+. (A) Control (○), HA (■). (B) Control (○), K^+ (●), Na^+ (□), Ch^+ (△). Each point represents a mean ± SEM, (N = 6) or (N = 3) for (A) or (B) respectively. Statistically significant differences were found between HA vs control ($P < 0.01$) and K^+ vs control ($P < 0.001$), as determined by two way ANOVA.

started by the addition of 1.5 mM ATP (final concentration). After 20 min incubation the reaction was stopped with 1 mL ice-cold 10% (w/v) trichloroacetic acid. Drugs were added just before incubation.

The assay was linear with the amount of protein over the range between 0.1 and 0.4 mg protein for synaptosomes and 0.025 and 0.100 mg protein for SPM. Phosphate hydrolysed from ATP was linear between 5 and 30 min. In the presence of HA (10^{-4} M) or K^+ (60 mM) the assay was also linear with respect to amount of protein and to time. (Ca^{2+}–Mg^{2+})-ATPase activity represents the difference between total ATPase and basal Mg^{2+}-ATPase activities and was expressed as µmoles P_i liberated/mg protein/hr.

Protein determination. The protein content was determined by the method of Lowry *et al.* [22] with bovine serum albumin as a standard.

Statistics. The data are presented as means ± SEM of separate determinations each performed in triplicate. The mean values were compared by variance analysis. In some cases, when F was significant, the difference between means was determined using the Scheffe's test.

RESULTS

Synaptosomal $CaCl_2$ concentration response curves (0.1–0.4 mM) in the presence or absence of 10^{-4} M HA are represented in Fig. 1A. Control (Ca^{2+}–Mg^{2+})-ATPase activity increased from 0.1 to 0.34 mM $CaCl_2$. Maximal activity (1.514 ± 0.088, N = 6) was achieved at 0.34 mM $CaCl_2$. Higher $CaCl_2$ concentrations caused decreases in ATPase activity suggesting an inhibitory action of excess

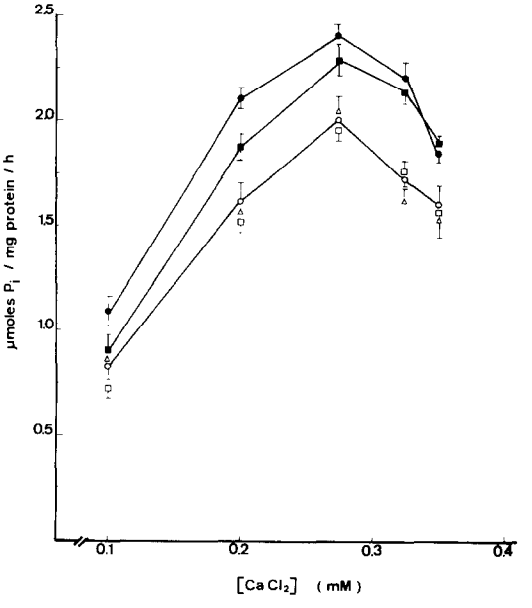

Fig. 2. Effects of HA and monovalent cations on SPM (Ca^{2+}–Mg^{2+})-ATPase activity, as a function of Ca^{2+} concentrations. SPM Ca^{2+} concentration–response curves were run in the presence of increasing $CaCl_2$ concentrations (0.1–0.35 mM) (0.075–1.54 µM free Ca^{2+} concentrations), with or without 10^{-4} M HA, 60 mM K^+, 60 mM Na^+ or 60 mM Ch^+, as described in Materials and Methods. Control (○), HA (△), K^+ (●), Na^+ (■), Ch^+ (□). Results are presented as means ± SEM (N = 3). Statistically significant differences were found in K^+ or Na^+ vs control ($P < 0.01$) and ($P < 0.05$), respectively. Two way ANOVA.

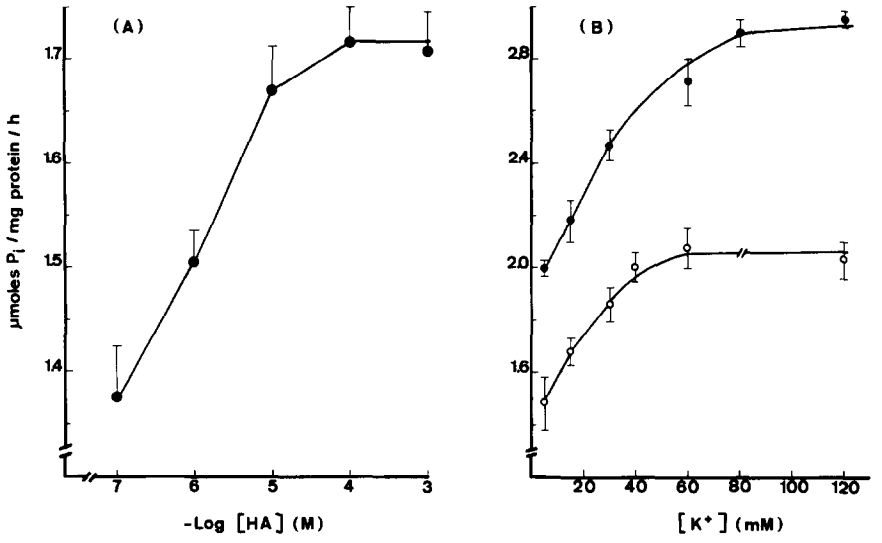

Fig. 3. (A) Concentration-dependent effect of HA on synaptosomal $(Ca^{2+}$–$Mg^{2+})$-ATPase activity. The assay medium contained 0.325 mM $CaCl_2$ (1 μM free Ca^{2+}), 0.75 mM $MgCl_2$ and 1.5 mM ATP. Synaptosomes were incubated in the presence or absence of various HA concentrations (10^{-7}–10^{-3} M). The control value was 1.393 ± 0.027 (N = 4). Results are means ± SEM (N = 4). Significant differences became apparent at 10^{-6} M HA ($P < 0.001$). Scheffe's test. (B) Concentration-dependent effect of K^+ on SPM and synaptosomal $(Ca^{2+}$–$Mg^{2+})$-ATPase activity. Synaptosomes (○) or SPM (●) were incubated in reaction media containing 0.275 mM $CaCl_2$ (0.5 μM free Ca^{2+}), in the presence of increasing K^+ concentrations (5–120 mM). The control values were 1.261 ± 0.071 (N = 3) for synaptosomes and 1.992 ± 0.052 (N = 3) for SPM. Each point represents a mean ± SEM (N = 3). Statistically significant differences became apparent at 5 mM and 15 mM ($P < 0.001$) for synaptosomes and SPM, respectively. Scheffe's test.

Ca^{2+} on the Ca^{2+} high-affinity pump in accordance with Duncan [10]. In the presence of 10^{-4} M HA, $CaCl_2$ concentration response curve had similar shape as the control but shifted to the left. HA increased the maximal synaptosomal $(Ca^{2+}$–$Mg^{2+})$-ATPase activity (1.738 ± 0.035, N = 6) and decreased the extrasynaptosomal $CaCl_2$ concentration necessary to reach it (0.325 mM $CaCl_2$).

On the other hand, the effects of monovalent cations, Na^+, K^+ and choline$^+$ (Ch^+) (all at 60 mM) on synaptosomal $(Ca^{2+}$–$Mg^{2+})$-ATPase activity were studied (Fig. 1B). Only K^+ was able to modify the control ATPase activity. K^+ effects were similar to those of HA, being the effects of K^+ more apparent. The maximal synaptosomal $(Ca^{2+}$–$Mg^{2+})$-ATPase activity was increased by 36% and the extrasynaptosomal $CaCl_2$ concentration to reach it was decreased to 0.275 mM $CaCl_2$.

In SPM, control $(Ca^{2+}$–$Mg^{2+})$-ATPase activity increased from 0.1 to 0.275 mM $CaCl_2$. Maximal activity was reached at 0.275 mM $CaCl_2$. At higher $CaCl_2$ concentrations $(Ca^{2+}$–$Mg^{2+})$-ATPase activity decreased. In this preparation HA (10^{-4} M) lacked of effects on ATPase activity. However the monovalent cations Na^+ and K^+, but not Ch^+ (all at 60 mM), increased the maximal $(Ca^{2+}$–$Mg^{2+})$-ATPase activity by 15 and 20%, repsectively, without modifying the affinity of enzyme for Ca^{2+} (Fig. 2).

HA and K^+ induced a concentration-dependent increase on synaptosomal $(Ca^{2+}$–$Mg^{2+})$-ATPase activity, as shown in Fig. 3A and Fig. 3B respectively. 5×10^{-5} M HA produced maximal increase of ATPase activity (around 23% over the control value) ($EC_{50} = 2.0 \times 10^{-6}$ M). Maximal K^+ enzyme stimulation was reached at 40 mM K^+, and represents around 66% over the control value. The EC_{50} was 15 mM K^+. Figure 3B also shows the concentration-dependent effect of K^+ on $(Ca^{2+}$–$Mg^{2+})$-ATPase activity in SPM. Maximal ATPase activity in membranes was reached at 80 mM K^+ (48% over the control value) (EC_{50} = 30 mM).

The effect of HA (10^{-4} M) on the synaptosomal $(Ca^{2+}$–$Mg^{2+})$-ATPase activity was reversed by the H_2 antagonist ranitidine (10^{-6} M) but not by the H_1 antagonist mepyramine (10^{-7} M). The H_2 agonist dimaprit (10^{-5} M) mimicked the effects of HA. In contrast the H_1 agonist 2-thiazolylethylamina (10^{-4} M) lacked effects. These results are represented in Fig. 4. The antagonists did not by themselves modify the $(Ca^{2+}$–$Mg^{2+})$-ATPase activity in synaptosomes. In no case did the chemicals used affect the basal Mg^{2+}-ATPase activity.

When synaptosomes were incubated in the presence of IBMX (10^{-4} M) and HA (10^{-4} M), the maximal effect of HA on the enzyme activity was enhanced by 15% (Fig. 5). IBMX alone did neither affect the basal Mg^{2+}-ATPase nor the $(Ca^{2+}$–$Mg^{2+})$-ATPase activities in synaptosomes.

On the other hand, we studied the effects of the slow Ca^{2+} channel antagonists verapamil (10^{-6} M) and diltiazem (10^{-6} M) on 10^{-4} M HA or 60 mM K^+ stimulated $(Ca^{2+}$–$Mg^{2+})$-ATPase activity in synaptosomes (Fig. 6 and Fig. 7, respectively). Verapamil completely antagonized the effects of HA

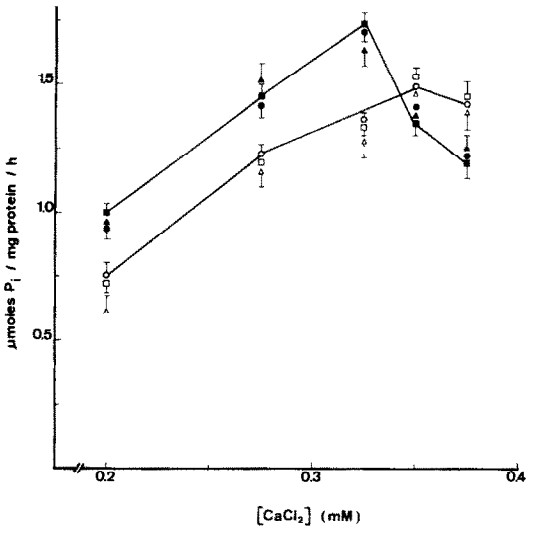

Fig. 4. Effect of HA, H_1 and H_2 agonists and antagonists. Synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity was assayed as described in methods, as a function of $CaCl_2$ concentrations (0.2–0.375 mM) (0.23–3.00 μM free Ca^{2+}). Control (○), HA (10^{-4} M) (■), ranitidine (10^{-6} M) + HA (10^{-4} M) (□), mepyramine (10^{-7} M) + HA (10^{-4} M) (▲), 2-thiazolylethylamine (10^{-4} M) (△), dimaprit (10^{-5} M) (●). Each point represents a mean ± SEM (N = 4). Statistically significant differences were found in: HA, mepyramine + HA or dimaprit vs contol ($P < 0.01$) as determined by two way ANOVA.

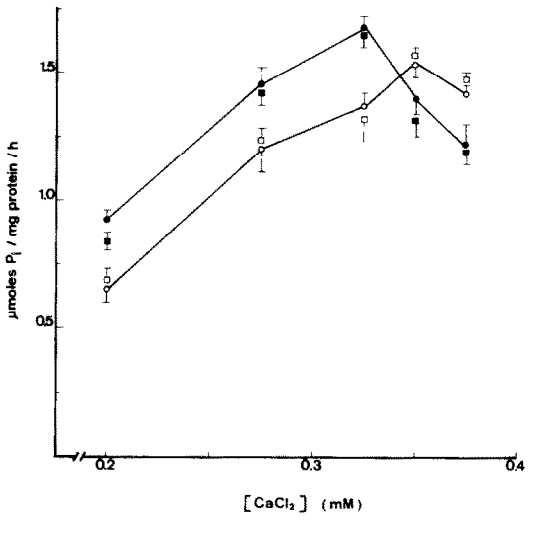

Fig. 6. Effect of slow Ca^{2+} channel antagonists on HA effects. The effect of various Ca^{2+} antagonists on $CaCl_2$ concentration–response curves (over the range of 0.2–0.375 mM) (0.23–3.00 μM free Ca^{2+}) in the presence or absence of HA (10^{-4} M) was assayed as described in methods. Control (○), HA (10^{-4} M) (●), verapamil (10^{-6} M) + HA (10^{-4} M) (□), diltaiazem (10^{-6} M) + HA (10^{-4} M) (■). Each point represents a mean ± SEM (N = 4). Statistical differences were significant in: HA or Diltiazem+HA vs control ($P < 0.01$). Two way ANOVA.

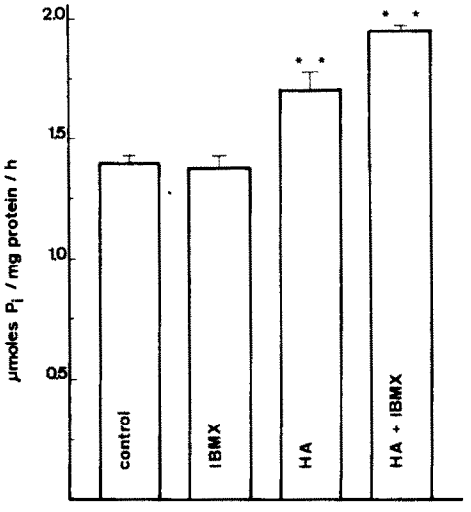

Fig. 5. Effect of IBMX on the increase of synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity induced by HA. The assay medium contained 0.325 mM $CaCl_2$ (1 μM free Ca^{2+}), 0.75 mM $MgCl_2$ and 1.5 mM ATP. Synaptosomes were incubated in the presence or absence of HA (10^{-4} M) and IBMX (10^{-4} M). The control value was 1.375 ± 0.047 (N = 4). Data are presented as means ± SEM (N = 4). (*) ($P < 0.001$), (**) ($P < 0.01$) vs control. (★) ($P < 0.05$) vs HA. Scheffe's test.

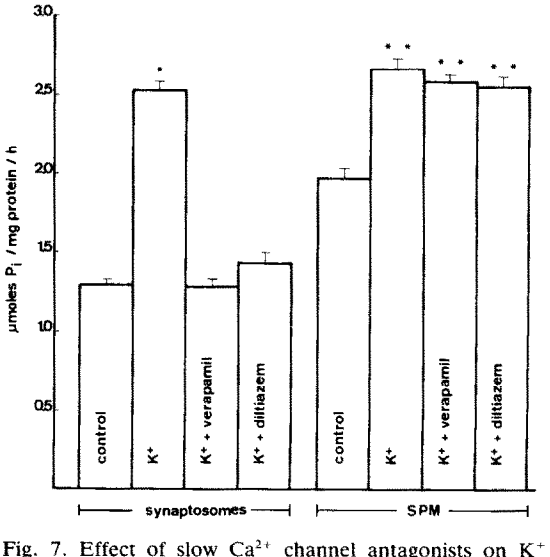

Fig. 7. Effect of slow Ca^{2+} channel antagonists on K^+ effects on (Ca^{2+}–Mg^{2+})-ATPase activity in synaptosomes and SPM. Synaptosomes or SPM were incubated in media containing 0.275 mM $CaCl_2$ (0.5 μM free Ca^{2+}), in the presence or absence of verapamil (10^{-6} M) or diltiazem (10^{-6} M) and 60 mM K^+. The control values were 1.242 ± 0.013 (N = 3) and 1.946 ± 0.083 (N = 3) for synaptosomes and SPM respectively. Results are means ± SEM (N = 3). (*) ($P < 0.001$) (**) ($P < 0.01$). Scheffe's test.

on ATPase activity, whereas diltiazem did not. However K^+ effects were inhibited by both antagonists. The slow Ca^{2+} channel antagonists alone did neither modify the Mg^{2+}-ATPase nor (Ca^{2+}–Mg^{2+})-ATPase activities. Figure 7 also shows the effect of these Ca^{2+} channel blockers on K^+ stimulated (Ca^{2+}–Mg^{2+})-ATPase activity in SPM. The increase on enzyme activity promoted by 60 mM K^+ was neither antagonized by verapamil nor by diltiazem.

DISCUSSION

HA and K^+ increased the maximal synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity and decreased the extrasynaptosomal Ca^{2+} concentration necessary to reach it. A direct interaction of HA with the enzyme is highly unlikely as HA did not alter SPM (Ca^{2+}–Mg^{2+})-ATPase activity and in addition HA effects were mediated by H_2 receptor stimulation.

Further evidence also suggests an indirect effect of K^+ on synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity. K^+ stimulates ATPase activity in synaptosomes and SPM with a different behaviour. In both cases the effect of K^+ is not due to ionic strength, as Ch^+ did not alter the enzyme activity in any preparation. On the other hand, Na^+ lacked effects on synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity, whereas in SPM Na^+ stimulates ATPase activity as observed by other authors [23, 24]. In synaptosomes K^+ decreased the extrasynaptosomal Ca^{2+} concentration necessary to reach the maximal activity, although in SPM did not.

We had previously described [11] that ATP added extrasynaptosomally could be used as substrate for the synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity. Furthermore the observation that in permeabilized synaptosomes with digitonin (Ca^{2+}–Mg^{2+})-ATPase activity was similar to ATPase activity measured in intact synaptosomes (data not shown), indicate that probably ATP can cross plasma membranes, as reported by other authors [27]. So the HA or K^+ effects on synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity could be due to a first action on ATP uptake. This possibility is, however, highly unlikely because on synaptosomal ATP concentration–response curves, HA or K^+ increased maximal activity without altering the extrasynaptosomal ATP concentration necessary to reach it (data not shown). This fact could explain the effect of HA or K^+ on maximal activity but not the decrease in the extrasynaptosomal calcium concentration necessary to reach it, as shown in Fig. 1 (A and B).

It has been reported that HA H_2 receptor stimulation [14] and depolarizing K^+ concentrations [15–17] increase Ca^{2+} uptake in synaptosomes through Ca^{2+} channels stimulation [17, 25]. Therefore, our present findings suggest that the observed decreases in the concentration of extrasynaptosomal Ca^{2+} necessary to reach the maximal (Ca^{2+}–Mg^{2+})-ATPase activity induced by HA or K^+ are due to the elevated intrasynaptosomal Ca^{2+} levels promoted by both agents. The fact that some Ca^{2+} channel antagonists inhibit the HA and K^+ effects further supports this interpretation. Although it has been widely described that synaptosomes are very resistant to Ca^{2+} channel antagonists on K^+ stimulation of Ca^{2+} uptake, it must be taken into account that in certain conditions (absence of external Na^+, as in our experiments), the Ca^{2+} channel blockers inhibit the effects of K^+ on Ca^{2+} entry to synaptosomes [28]. On the other hand, verapamil but not diltiazem antagonizes the effects of HA on synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity, could be attributed to the different potency of the Ca^{2+} channel antagonists to inhibit the increase on Ca^{2+} uptake in synaptosomes promoted by HA, as we previously reported [25]. The ability of diltiazem to inhibit K^+ effects but not HA effects could be interpreted as HA stimulates different Ca^{2+} channels that K^+ stimulates.

The increase in the maximal activity induced by 60 mM K^+ could be attributed to a modulatory action of this ion on (Ca^{2+}–Mg^{2+})-ATPase activity as reported by other authors [23, 24, 26]. Nevertheless, the fact that this effect is reversed by several calcium channel antagonists suggests that the increase in maximal activity in synaptosomes induced by K^+ is dependent on a previous rise in intrasynaptosomal Ca^{2+} levels promoted by K^+.

Several reasons point to the assumption that HA effects on maximal enzyme activity in synaptosomes are mediated by cAMP. In support of this view, IBMX, a potent phosphodiesterase inhibitor, potentiated (15%) the maximal HA effect. In addition, the results provide evidence that HA effect is mediated by H_2 receptor stimulation and it should be taken into account that the primary action of HA H_2 receptor stimulation in brain is an increase of intracellular cAMP concentration [12, 13]. On the other hand, it has been described that synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity is regulated by a cAMP-dependent protein kinase [11]. As it has been stated above for K^+ effects, the increase in maximal (Ca^{2+}–Mg^{2+})-ATPase activity induced by HA is presumably dependent on a previous rise in intrasynaptosomal Ca^{2+} levels promoted by the amine, since verapamil completely inhibited HA effect.

In summary, we suggest that the effects of HA and K^+ on synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity are mediated by increases in intrasynaptosomal Ca^{2+} levels promoted by both agents. Moreover, potassium ions directly as well as the HA increased intrasynaptosomal cAMP levels modulate the synaptosomal (Ca^{2+}–Mg^{2+})-ATPase activity.

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REFERENCES

1. Augustine GJ, Charlton MP and Smith SJ, Calcium action in synaptic transmitter release. *Annu Rev Neurosci* **10**: 633–693, 1987.
2. Alnaes E and Rahamimoff H, The role of mitochondria in transmitter release from motor nerve terminals. *J Physiol* **248**: 285–306, 1975.
3. Blaustein MP, Ratzlaff RW and Schweitzer ES, Calcium

buffering in presynaptic nerve terminals II: Kinetic properties of the nonmitochondrial Ca^{2+} sequestration mechanism. *J Gen Physiol* **72**: 43–66, 1978.
4. Nicholls DG and Scott ID, The role of mitochondria in the regulation of calcium transport in synaptosomes. *Biochem Soc Trans* **8**: 264–266, 1980.
5. Rahamimoff H and Spanier R, Sodium-dependent calcium uptake in membrane vesicles derived from rat brain synaptosomes. *FEBS Lett* **104**: 111–114, 1979.
6. Gill DL, Grollman EF and Kohn LD, Calcium transport mechanisms in membrane vesicles from guinea pig brain synaptosomes. *J Biol Chem* **256**: 184–192, 1981.
7. Javors MA, Bowden CL and Ross DH, Kinetic characterization of Ca^{2+} transport in synaptic membranes. *J Neurochem* **37**: 381–387, 1981.
8. Ross DH and Cardenas HL, Calmodulin stimulation of Ca^{2+}-dependent ATP hydrolysis and ATP-dependent Ca^{2+} transport in synaptic membranes. *J Neurochem* **41**: 161–171, 1983.
9. Sobue K, Ichida S, Yoshida H, Yamazaki R and Kakiuchi S, Occurrence of a Ca^{2+} and modulator protein activable ATPase in the synaptic plasma membranes of brain. *FEBS Lett* **99**: 199–202, 1979.
10. Duncan CJ, Properties of the Ca^{2+}-ATPase activity of mammalian synaptic membrane preparations. *J Neurochem* **27**: 1277–1279, 1976.
11. Rodriguez R, Toledo A, Sabrià J, Rodriguez J and Blanco I, Synpatosomal (Ca^{2+}–Mg^{2+})-ATPase activity modulation by cyclic AMP. *Biochem Pharmacol* **38**: 3219–3222, 1989.
12. Hegstrand LR, Kanof PD and Greengard P, Histamine-sensitive adenylate cyclase in mammalian brain. *Nature (London)* **260**: 163–164, 1976.
13. McNeill JH, Histamine receptors and cyclic AMP. *Can J Physiol Pharmacol* **58**: 1023–1030, 1980.
14. Rodriguez R, Brandner T, Sabrià J, Toledo A, Rodriguez J and Blanco I, Effect of histamine on $^{45}Ca^{2+}$ uptake in rat brain synaptosomes. *Biochem Pharmacol* **36**: 974–976, 1987.
15. Blaustein MP, Effect of potassium, veratridine and scorpion venom on calcium accumulation and transmitter release by nerve terminals *in vitro*. *J Physiol* **247**: 617–655, 1975.
16. Nachshen DA and Blaustein MP, Some properties of potassium-stimulated calcium influx in presynaptic nerve endings. *J Gen Physiol* **76**: 709–728, 1980.
17. Adam-Vizi V and Ligeti E, Calcium uptake of rat brain synaptosomes as a function of membrane potential under depolarizing conditions. *J Gen Physiol* **372**: 363–377, 1986.
18. Dodd PR, Hardy JA, Oakley AE, Edwardson JA, Perry EK and Delaunoy JP, A rapid method for preparing synaptosomes: Comparison with alternative procedures. *Brain Res* **226**: 107–118, 1981.
19. Jones DH and Matus AI, Isolation of synaptic plasma membrane from brain by combined flotation-sedimentation density gradient centrifugation. *Biochim Biophys Acta* **356**: 276–287, 1974.
20. Lebel D, Poirier GG and Beaudoin AR, A convenient method for the ATPase assay. *Anal Biochem* **85**: 86–89, 1978.
21. Bartfai T, Preparation of metal–chelate complexes and the design of steady-state kinetic experiments involving metal nucleotide complexes. In: *Advances in Cyclic Nucleotide Research* (Eds. Brooker G, Greengard P and Robinson GA), Vol. 10, pp. 219–242. Raven Press, New York, 1979.
22. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
23. Robinson JD, Effects of cations on (Ca^{2+}–Mg^{2+})-activated ATPase from rat brain. *J Neurochem* **37**: 140–146, 1981.
24. Roufogalis BD, Properties of a (Mg^{2+}–Ca^{2+})-dependent ATPase of bovine brain cortex. Effects of detergents, freezing, cations and local anaesthetics. *Biochim Biophys Acta* **318**: 360–370, 1973.
25. Rodriguez R, Toledo A, Brandner R, Sabrià J, Rodriguez J and Blanco I, Histamine stimulated synaptosomal Ca^{2+} uptake through activation of calcium channels. *Biochem Biophys Res Commun* **153**: 1136–1143, 1988.
26. Coutinho OP, Carvalho AP and Carvalho CAM, Effect of monovalent cations on Na^+/Ca^{2+} exchange and ATP-dependent Ca^{2+} transport in synaptic plasma membranes. *J Neurochem* **41**: 670–676, 1983.
27. Chaudry NIH, Clemens MG and Baue AE, Uptake of ATP by tissues. In: *Purines: Pharmacological and Physiological Roles* (Ed. Stone TW), pp. 115–124. Macmillan Press, London, 1985.
28. Turner TJ and Goldin SM, Calcium channels in rat brain synaptosomes: identification and pharmacological characterization. *J Neurosci* **5**: 841–849, 1985.