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INFLUENCE OF pH AND SODIUM ON THE INHIBITION OF GUINEA-PIG HEART ($Na^+ + K^+$)-ATPase BY CALCIUM

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Summary

The inhibition of guinea-pig heart ($Na^+ + K^+$)-ATPase (ATP phosphohydrolase EC 3.6.1.3) by calcium has been studied at pH 7.4, 6.8 and 6.4.

1. A decrease in pH reduced the threshold inhibitory concentration of calcium and the calcium concentration producing an inhibition of 50% of the enzyme activity.

2. Calcium reduced the apparent affinity of the enzyme for Na^+, this effect occurred only at pH 7.4.

3. Calcium increased the apparent affinity of the enzyme for K^+, this effect was enhanced at acidic pH.

4. Activation of the enzyme by Na^+ for a constant $Na^+ : K^+$ ratio has been studied at pH 7.4 and at pH 6.8 in the absence and in the presence of $3 \cdot 10^{-4}$ M Ca^{2+}; the results of this experiment indicate that Ca^{2+} effect at pH 7.4 was not influenced by $Na^+ - K^+$ competition and was probably due to a $Na^+ - Ca^{2+}$ interaction.

5. At pH 7.4, the calcium inhibitory threshold concentration and the concentration producing 50% inhibition were reduced when Na^+ was low; at pH 6.8, the calcium inhibition was not markedly modified by the change of Na^+ concentration.

6. The Ca^{2+}-activated ATPase of myosin B which is related to the contractile behaviour of muscle and the Ca^{2+}-ATPase of the sarcoplasmic reticulum which is related to the ability of this structure to accumulate calcium were activated in a range of calcium concentration producing an inhibition of ($Na^+ + K^+$)-ATPase.

The present results indicate that the increase by acidity of the ($Na^+ + K^+$)-ATPase sensitivity to calcium might be due to a suppression of a Na^+-Ca^{2+}

A preliminary report [13] of part of this work has been presented at the Belgian Society of Physiology and Pharmacology (meeting of May 1975).

interaction. On the basis of these observations, it is proposed that calcium might inhibit the Na^+-pump during the repolarization phase of the action potential and that, by this effect, it might control cell excitability.

Introduction

Calcium inhibits ($Na^+ + K^+$)-ATPase (ATP phosphohydrolase EC 3.6.1.3) in low concentrations, as shown since the first description of the properties of the enzyme [1]. Calcium does not inhibit the sodium pump from outside the cell [2], but it has been reported that the sodium pump in red blood cells was inhibited when calcium concentration was increased inside [3—5]. It has been proposed that calcium inhibition was due to a competition with magnesium [6—8]. The possibility that calcium might compete with sodium is a controversial matter as some reports favour this view [3,4,7,9] and other do not[6]. Recently it has been observed that calcium induces phosphorylation of ATPase in the same way as sodium [8,10].

In recent years, the interaction between calcium and the sodium pump has aroused interest since it was postulated that the positive inotropic effect of cardiac glycosides might be related in some way to this interaction [7,11]. Most of the previous experiments were done with calcium concentrations higher than those achieved in the cell during the contractile cycle. Furthermore, it has been shown that intracellular pH is lower than the extracellular one and is reduced during muscle contraction [12].

The present experiments were designed in order to study the action of calcium on ($Na^+ + K^+$)-ATPase and its sensitivity to changes in sodium concentration; the experiments were performed at pH 7.4, 6.8 and 6.4. The results show that calcium concentrations which are likely achieved in heart cell during the contractile cycle inhibit partly ($Na^+ + K^+$)-ATPase and that this inhibition is enhanced by the acidity of the incubation medium. This enhancement is likely due to the suppression of a $Na^+ - Ca^{2+}$ interaction.

Methods

Preparation and assay of the ($Na^+ + K^+$)-ATPase

A fraction enriched in ($Na^+ + K^+$)-ATPase has been prepared from homogenates of albino guinea-pig heart treated by NaI according to Nakao et al. [14]. The pellet obtained after 30 min of centrifugation at 100 000 × *g* was resuspended in 0.25 M sucrose containing 5 mM Tris/maleate (pH 7.4) and was washed twice. The resultant pellet was resuspended in 0.25 M sucrose containing 0.1% deoxycholate, 5 mM Tris/maleate (pH 7.4) and was stored overnight at —30° C. After thawing, the supernate of a 30-min spin at 100 000 × *g* was centrifuged for 5 h at 100 000 × *g*. The final pellet was resuspended in 0.25 M sucrose (pH 7.4) and stored at —30° C. The protein concentration was measured by the method of Lowry et al. [15] using bovine serum albumin as a standard.

The ATPase activity was determined by measuring the release of inorganic phosphate from ATP at 37° C in 1 ml assay medium containing, unless otherwise stated: 100 mM Na^+, 20 mM K^+, 3 mM Mg^{2+}, 2.5 mM Tris/ATP, 1 mM

EGTA (Ethylene glycol bis (β-aminoethylether)-*N*,*N'*-tetraacetic acid), 20 mM maleic acid (adjusted with Tris at indicated pH values). When the concentration of NaCl or KCl was lowered, the ionic force was maintained constant with choline chloride. The reaction was started by the addition of the enzyme (usually 5—10 μg protein) and was stopped after 60 min with 0.1 ml of 50% trichloroacetid acid. The tubes were placed at once in crushed ice before determination of inorganic phosphate. When the amount of protein was higher than 10 μg, the mixture was centrifuged and inorganic phosphate measured in the supernate. When incubating medium was supplemented with choline chloride, the reaction was stopped with 1 ml of 8% silicotungstic acid in 1.2 M $HClO_4$. The mixture was centrifuged and inorganic phosphate was measured in the supernate in order to avoid interference of choline chloride [16]. Inorganic phosphate was measured by the method of Fiske and SubbaRow [17] slightly modified. To 0.6 ml or 1 ml of sample we added 1.6 ml of 1.25% $(NH)_6\ MoO_{24} \cdot 4\ H_2O$ in 0.88 M H_2SO_4 and 0.4 ml of Fiske and SubbaRow reductor reagent. Absorbance was measured at 660 nm, exactly 15 min later, in a Kip-Scalar (Holland) digital photometer. Controls for acid ATP hydrolysis were run in the absence of enzyme. The amount of enzyme added to the reaction medium was adjusted in order to hydrolyse less than one tenth of ATP. Total activity of the preparation was equal to 30 $\mu mol\ P_i \cdot mg^{-1} \cdot h^{-1}$; 95% of the ATPase activity was ouabain-sensitive and corresponded to $(Na^+ + K^+)$-ATPase. The preparation did not contain Ca^{2+}-activated ATPase and it was not contaminated by cytochrome oxidase.

Preparation and assay of Ca^{2+}-ATPases from myosin B and sarcoplasmic reticulum

Myosin B of guinea-pig heart was prepared according to the method described for rabbit skeletal muscle [18].

Microsomes containing sarcoplasmic reticulum Ca^{2+}-ATPase were prepared from guinea-pig heart. The hearts were homogenised in 9 volumes of 0.25 M sucrose containing 5 mM Tris/maleate (pH 7.4). The homogenates were centrifuged successively at 1 000 × *g* for 10 min and at 10 000 × *g* for 15 min. The pellets were discarded and the supernate was submitted to a 60-min spin at 100 000 × *g*. The pellet was resuspended in buffered sucrose solution (2 mg protein per ml) and immediately assayed for ATPase activities.

The assay of Ca^{2+}-ATPase was carried out as following: 800 μg of either myosin B protein or 120 μg of microsomal protein were incubated respectively for 2 min of 30 min, at 37°C, in a medium containing 80 mM K^+, 20 mM Na^+, 3 mM Mg^{2+}, 2.5 mM ATP, 1 mM EGTA, 1 mM ouabain, 20 mM Tris/maleate (pH 6.8) and various concentrations of Ca^{2+}. Ca^{2+}-ATPase activity was estimated by subtracting the activity obtained in the absence of added Ca^{2+} from the one measured when the medium was supplemented with Ca^{2+}.

Ca-EGTA buffers

The inhibition of $(Na^+ + K^+)$-ATPase by calcium was studied in the presence of Ca^{2+}-EGTA buffers. Free Ca^{2+} concentrations have been calculated as reported previously [19], using association constants of $10^{5.72}\ M^{-1}$ at pH 7.4, $10^{5.24}\ M^{-1}$ at pH 6.8 and $10^{5.01}\ M^{-1}$ at pH 6.4. The Ca^{2+}-EGTA association con-

stants were determined according to the method of Murphy and Hasselbach [18] in solutions used for enzyme studies. The values were consistent with those reported by Ogawa [20,21]. Since total magnesium concentration in incubating solutions was in excess with respect to total ATP concentration, no significant pCa changes occurred during ATP hydrolysis, which was maintained within the previously stated limits.

The calculated free Ca^{2+} concentrations reported in this paper were nearly 10 times higher than those calculated taking into account the Schwarzenbach's association constants which are, in a non biological medium: $10^{7.10}\ M^{-1}$, $10^{6.09}\ M^{-1}$ and $10^{5.41}\ M^{-1}$ [22,23] respectively at pH 7.4, 6.8 and 6.4.

Reagents

All solutions were prepared using distilled, deionized water. EGTA and bovine serum albumin were puchased from Sigma Chemical Co, St. Louis. All other chemicals were analytical grade and purchased from E. Merck, Darmstadt.

Tris/ATP was prepared by passage of disodium ATP (Boehringer GmbH, Mannheim) through Dowex 50 in the H^+ form, followed by neutralisation with Tris.

K^+, Na^+ and Mg^{2+} were used as chloride salts. Ca^{2+} solutions were prepared using $CaCO_3$ titrated by HCl.

Statistical methods

The concentrations of cation producing 50% of maximal ($Na^+ + K^+$)-ATPase activity ($K_{0.5}$) were calculated from Hill plots by linear regression analysis. The curves showed a good linearity for values of $\log(v/V - v)$ between +0.5 and −0.5. At least 6 concentrations of cation stood within these limits. The error was given by the estimated residual standard deviation of the straight lines. Significance of the difference between two $K_{0.5}$ values was analysed using the limits of error of the relative potency, a method developped by Emmens for the relative potency of various drugs with unbalanced dosage groups [24].

Results

Influence of pH on the inhibition of ($Na^+ + K^+$)-ATPase by calcium

The inhibition of ($Na^+ + K^+$)-ATPase by calcium has been studied for calcium concentrations varying from 10^{-6} M up to $0.80 \cdot 10^{-2}$ M. The pH of the incubation medium was also varied from 7.4 down to 6.4. As Fig. 1 illustrates, the inhibition was dependent not only upon calcium concentration but also upon pH. At pH 7.4, the threshold inhibitory concentration of calcium was comprised between 10^{-5} and $3 \cdot 10^{-5}$ M; the concentration producing 50% inhibition was $5 \cdot 10^{-4}$ M. A decrease in pH reduced the concentration of calcium needed for inhibition. This reduction occurred both for threshold concentration and for the calcium concentration producing an inhibition of 50% of the enzyme activity. As shown in Table II and Fig. 2, pH change from 7.4 to 6.8 did not affect in the absence of calcium the parameters of enzyme activation by magnesium, sodium and potassium.

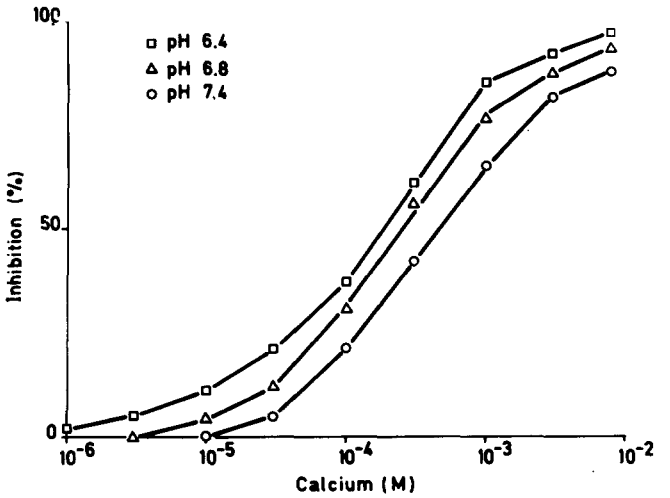

Fig. 1. Effect of pH on the inhibition of $(Na^+ + K^+)$-ATPase by Ca^{2+}. The enzyme (10 μg) was incubated for 60 min in the presence of 100 mM Na^+, 3 mM K^+, 3 mM Mg^{2+}, 2.5 mM ATP, 1 mM EGTA and 20 mM maleic acid. pH was adjusted with Tris at 37°C (pH 7.4: ○; 6.8: △; or 6.4: □). Calcium was added in order to obtain the indicated free Ca^{2+} concentrations. Each point is the mean value from 6 determinations. S.E. of means were contained within the circumference of the symbols.

Influence of pH on $Na^+ - Ca^{2+}$ and $K^+ - Ca^{2+}$ interactions

The influence of calcium on the activation of $(Na^+ + K^+)$-ATPase by monovalent cations has been studied at pH 7.4 and 6.8. The maximum activity of the enzyme was similar in both conditions. Activation curves were established without Ca^{2+} and in the presence of $3 \cdot 10^{-4}$ M Ca^{2+} which achieved an inhibition comprised between 20% and 80% depending on pH and the concentrations of monovalent cations. Fig. 3 illustrates such an experiment at pH 6.8 with Na^+

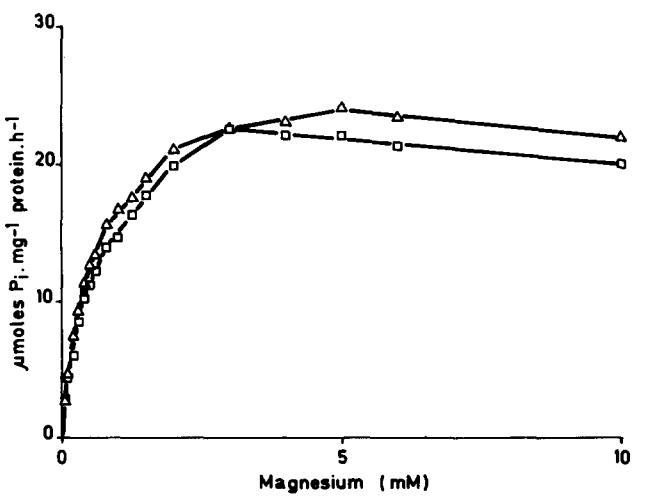

Fig. 2. Activation of $(Na^+ + K^+)$-ATPase by Mg^{2+}. The enzyme was incubated in the presence of 100 mM Na^+, 3 mM K^+, 2.5 mM ATP, 1 mM EGTA, 20 mM Tris/maleate (pH 7.4: □, or 6.8: △) and various concentrations of Mg^{2+}. Each point is the mean value from 4 experiments.

TABLE I

HALF-MAXIMAL ACTIVATION CONCENTRATIONS ($K_{0.5}$) OF Na^+, K^+, AND Mg^{2+} FOR (Na^+ + K^+)-ATPase

The enzyme was incubated in the presence of 100 mM Na^+, 20 mM K^+, 3 mM Mg^{2+}, 2.5 mM ATP, 1 mM EGTA, 20 mM Tris/maleate (pH 7.4 or 6.8). Na^+, K^+ and Mg^{2+} concentrations were varied keeping the other cations constant. Na^+ and K^+ concentrations were also varied simultanously with a constant ratio ($[Na^+]/[K^+] = 10$). In some experiments, the medium was supplemented with calcium in order to obtain $3 \cdot 10^{-4}$ M free Ca^{2+}. $K_{0.5}$ values were estimated from Hill plots. Standard deviations are indicated in brackets ($n = 6$).

Activation by	pH	$K_{0.5}$ (mM) without Ca^{2+}	$K_{0.5}$ (mM) with $3 \cdot 10^{-4}$ Ca^{2+}
K^+	7.4	1.0 (0.05)	0.8 (0.05)
	6.8	1.0 (0.06)	0.6 (0.06)
Na^+	7.4	15 (1)	22 (1)
	6.8	13 (0.7)	14 (1)
Na^+	7.4	12 (1)	15 (1)
($[Na^+]/[K^+] = 10$)	6.8	10 (1)	9 (0.8)
Mg^{2+}	7.4	0.50 (0.07)	
	6.8	0.44 (0.05)	

as an activator. Concentrations of monovalent cations producing a 50% activation of the enzyme ($K_{0.5}$) were estimated from Hill plots and are reported in Table I. Hill coefficients for Na^+ and K^+ were comprised between 1.5 and 1.8, an observation in agreement with data reported by others [25,26]. It was equal to 1.0 for Mg^{2+} as might be expected with a relatively purified (Na^+ + K^+)-ATPase [26]. For a K^+ concentration of 20 mM, $K_{0.5}$ for Na^+ in the absence of Ca^{2+} was equal to 15 mM at pH 7.4 and to 13 mM at 6.8. In the presence of $3 \cdot 10^{-4}$ M Ca^{2+}, $K_{0.5}$ for Na^+ was equal to 22 mM at pH 7.4 and to 14 mM at pH 6.8. This indicates that Ca^{2+} reduced ($P < 0.01$) the apparent affinity of the enzyme

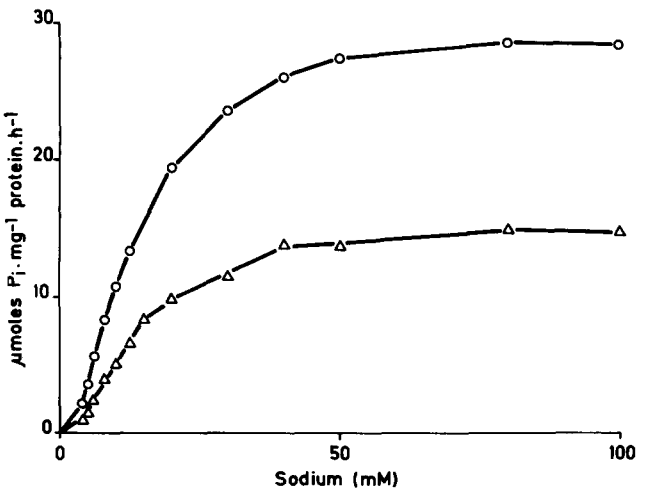

Fig. 3. Effect of Ca^{2+} on the activation of (Na^+ + K^+)-ATPase by Na^+. The enzyme was incubated in presence of 20 mM K^+, 3 mM Mg^{2+}, 2.5 mM ATP, 1 mM EGTA, 20 mM Tris/maleate (pH 6.8) and various concentrations of Na^+ with (△) or without (○) $3 \cdot 10^{-4}$ M Ca^{2+}. Each point is the mean value from 6 experiments.

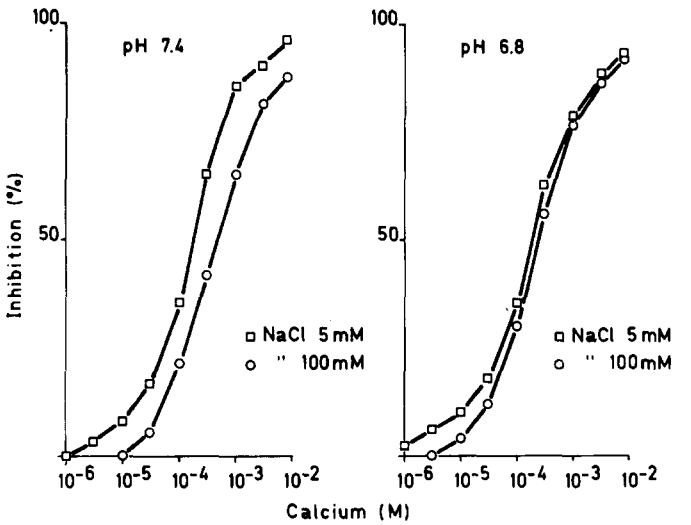

Fig. 4. Effect of Na^+ on the inhibition of $(Na^+ + K^+)$-ATPase by Ca^{2+} at pH 7.4. The enzyme was incubated in presence of 3 mM Mg^{2+}, 2.5 mM ATP, 3 mM K^+, 1 mM EGTA, 20 mM Tris/maleate, and various concentrations of Na^+ (□, 5 mM and ○, 100 mM) at pH 7.4 (left panel) or 6.8 (right panel). Calcium was added in order to obtain the indicated free Ca^{2+} concentrations. Each point is the mean value from 6 experiments.

for Na^+ and that this effect occurred only at pH 7.4. The Hill coefficient for Na^+ was not affected by pH change nor by the presence of Ca^{2+}. For a Na^+ concentration equal to 100 mM, $K_{0.5}$ for K^+ in the absence of Ca^{2+} was equal to 1.0 mM at pH 7.4 and was the same at pH 6.8. In the presence of $3 \cdot 10^{-4}$ M Ca^{2+}, $K_{0.5}$ for K^+ was equal to 0.8 mM at pH 7.4 and to 0.6 at pH 6.8. These results indicate that Ca^{2+} increased ($P < 0.01$) the apparent affinity of the enzyme for K^+ and that this effect was enhanced at acidic pH ($P < 0.01$).

It has been reported that Na^+ does compete with K^+ for K^+ activator sites and that reciprocally K^+ does alter the activation by Na^+ at Na^+ sites [27,28]. In order to examine whether the action of Ca^{2+} might be considered as a monovalent-divalent interaction and was not related to $Na^+ - K^+$ interaction, the activation by Na^+ has been studied for a $Na^+ : K^+$ ratio equal to 10. As shown in Table II, $K_{0.5}$ of Na^+ when $[Na^+]/[K^+] = 10$ and in the presence of $3 \cdot 10^{-4}$ M Ca^{2+} was lower at pH 6.8 than at pH 7.4 ($P < 0.01$). At pH 6.8, it was close to the value found in the absence of Ca^{2+}. In this experiment, Ca^{2+} effect at pH 7.4 was not influenced by $Na^+ - K^+$ competition and it was likely due to a $Na^+ - Ca^{2+}$ interaction.

The inhibition of $(Na^+ + K^+)$-ATPase by Ca^{2+} concentrations ranging from 10^{-6} to $8 \cdot 10^{-3}$ M was studied in the presence of high or low concentration of sodium at pH 7.4 and 6.8. Fig. 4 illustrates experiments at pH 7.4. The inhibitory threshold concentration and the concentration producing 50% inhibition were reduced when Na^+ was low. When the pH was equal to 6.8, the Ca^{2+} inhibition of $(Na^+ + K^+)$-ATPase was not markedly modified by the change of Na^+ concentration (Fig. 4b).

Activation of Ca^{2+}-ATPases from myosin B and sarcoplasmic reticulum

The activation of Ca^{2+}-ATPases from myosin B and from sarcoplasmic reti-

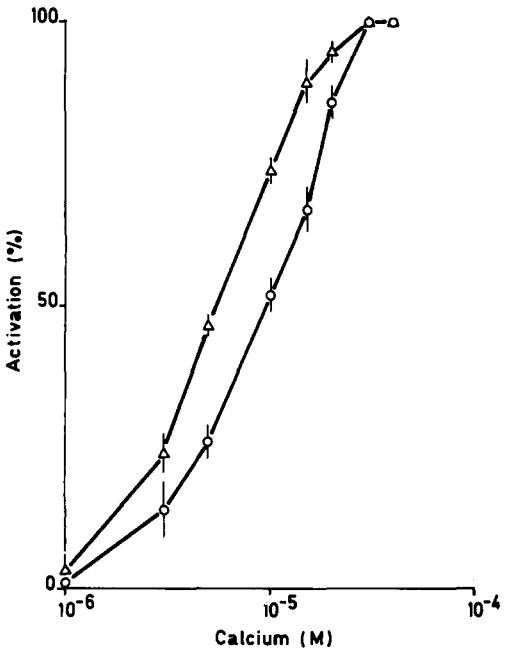

Fig. 5. Activation of Ca^{2+}-dependent ATPases from myosin B and sarcoplasmic reticulum. Myosin B (△) and sarcoplasmic reticulum (○) preparations were incubated in the presence of 80 mM K^+, 20 mM Na^+, 3 mM Mg^{2+}; 2.5 mM ATP, 1 mM EGTA, 1 mM ouabain, 20 mM Tris/maleate (pH 6.8). Calcium was added in order to obtain the indicated Ca^{2+}-free concentrations. Activities were expressed as per cent of maximal calcium activation. Each point is the mean value (±S.E.) from 8 determinations with 2 different preparations.

culum has been examined for calcium concentrations varying from 10^{-6} up to 10^{-4} M. The incubations were carried out at pH 6.8 for 2 min with myosin B and for 30 min with sarcoplasmic reticulum. Ca^{2+}-ATPase was estimated by subtracting the activity obtained in the absence of added Ca^{2+} from the one measured when the medium was supplemented with Ca^{2+}. The basal ATPase activities in the absence of added Ca^{2+} were equal to 20 ± 1 mol $P_i \cdot mg^{-1} \cdot h^{-1}$ (n = 8) for myosin B and to 32 ± 1 mol $P_i \cdot mg^{-1} \cdot h^{-1}$ (n = 8) for sarcoplasmic reticulum. In the presence of $5 \cdot 10^{-5}$ M Ca^{2+} which achieved the maximal activation, the activities were equal to 30 ± 2 for myosin B and to 37 ± 2 for sarcoplasmic reticulum. Fig. 5 illustrates activities expressed as per cent of maximal calcium activation. For both ATPases, the Ca^{2+} threshold concentrations were near 10^{-6} M. The Ca^{2+} concentration producing 50% of maximal activation of Ca^{2+}-ATPases was equal to $5 \cdot 10^{-6}$ M for myosin B and to 10^{-5} M for sarcoplasmic reticulum.

Discussion

The present results show, conforming other reports [1,29], that calcium does inhibit ($Na^+ + K^+$)-ATPase and that at pH 7.4, sodium reduces this inhibition [3,4,7,9]. In the present experiments, the action of calcium has been studied at acidic pH. It has been observed that the acidity of the incubation medium increases the sensitivity of ($Na^+ + K^+$)-ATPase to the inhibitory effect of

calcium. At pH 6.8, this increase in sensitivity is not associated with a change in the specific activity of the enzyme and in the parameters of activation by magnesium, sodium and potassium.

The calcium inhibitory effect is much more sensitive to sodium at pH 7.4 than at pH 6.8. Furthermore, the activation of $(Na^+ + K^+)$-ATPase by sodium is altered by calcium at pH 7.4 but not at 6.8, this effect being related to a $Na^+ - Ca^{2+}$ and not to a $Na^+ - K^+$ interaction. These observations indicate that the increase by acidity of the $(Na^+ + K^+)$-ATPase sensitivity to calcium might be due to the suppression of a $Na^+ - Ca^{2+}$ interaction.

The data reported here lead to reconsider the interaction between calcium and $(Na^+ + K^+)$-ATPase and consequently the Na Pump in the cell in vivo. Dunham and Glynn [29] have discarded such a possibility. Their experiments were carried out at pH 7.4. Intracellular pH is lower than extracellular and is still lower during excitation, being not far from pH 6.8 [12]. $(Na^+ + K^+)$-ATPase seems to be oriented in the membrane, the K^+ site facing extracellular fluid and the Na^+ site facing intracellular fluid [28]. As most of the experimental data [3—5] suggest that calcium inhibits from inside the cell, it is likely that, in vivo, the Ca^{2+} inhibitory sites are facing intracellular fluid.

In order to estimate if the free calcium concentrations here studied were within the range of the concentrations which might be achieved in the cell during the contractile cycle, we have examined their effect on other ATPases. These enzymes were the Ca^{2+}-activated ATPase of myosin B which is related to the contractile behaviour of muscle [30] and the Ca^{2+}-ATPase of the sarcoplasmic reticulum which is related to the ability of this structure to accumulate calcium [31,32]. The enzymes are activated in a range of calcium concentrations comprised between 10^{-6} and $3 \cdot 10^{-5}$ M. The latter evoked a 20% inhibition of $(Na^+ + K^+)$-ATPase at pH 6.4 in the presence of 100 mM Na^+ and 3 mM K^+. The Ca^{2+} concentrations activating Ca^{2+}-ATPases were higher than those reported by previous authors [18,21,33]. These discrepancies might be accounted for by a difference in the value of the apparent Ca^{2+}-EGTA association constant used for the calculation of the free Ca^{2+} concentrations [18,33]. Tissue and species variations as well as differences in medium composition and in experimental methodology might also be considered [21].

The present observations indicate that, in vivo, when Na^+ and Ca^{2+} concentrations increase in the cell during excitation process, some inhibition of $(Na^+ + K^+)$-ATPase might be achieved. This allows the proposition of a role for calcium in the regulation of membrane potential during excitation. It is generally admitted that sodium entry during the phase I of the action potential does trigger a release of intracellular calcium and that the plateau is supported by a slow inward calcium current [34]. The increase in free cytoplasmic calcium might be responsible for an inhibition of $(Na^+ + K^+)$-ATPase associated with the action potential. This inhibition could be enhanced by an acidification of the intracellular medium which occurs during the excitation-contraction cycle [35]. As a result, the reduction of the active transport of monovalent cations, namely potassium, might favour the increase of potassium conductance which has been attributed to intracellular calcium [36,37]. This might increase the rate of repolarisation of the cell membrane. Such a mechanism might be intensified by the factors such as catecholamines, anoxia or digitalis which increase intracel-

lular calcium [11,38—40]. It could be responsible for the reduction of the refractory period and for the induction of cardiac arrythmia. The activation of the sodium pump which follows the repolarization might be, at least in part, accounted for by the fading of calcium inhibition as a result of the cation translocation to calcium sequestering sites or to extracellular fluid.

In conclusion, the present results indicate that, beside its function as a second messenger in excitation-contraction coupling, calcium might play a role in the control of action potential and cell excitability.

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References

1 Skou, J.C. (1957) Biochim. Biophys. Acta 23, 394—401
2 Godfraind, T. (1973) in Proceedings of the 5th International Congress on Pharmacology, San Francisco, 1972, Vol. 4, pp. 344—358, Karger, Basel
3 Rummel, W., Seifen, E. and Baldauf, J. (1963) Biochem. Pharmacol. 12, 557—563
4 Hoffman, J.F. (1962) Circulation 26, 1201—1207
5 Wheeler, K.P. and Whittam, R. (1964) Biochem. J. 93, 349—354
6 Epstein, H.F. and Whittam, R. (1966) Biochem. J. 99, 232—238
7 Lindenmayer, G.E. and Schwartz, A. (1975) J. Mol. Cell. Cardiol. 7, 591—612
8 Hansen, O. (1974) Ann. N.Y. Acad. Sci. 242, 635—645
9 Repke, K. (1965) in Proceedings of the 2nd International Pharmacological Meeting, Prague, 1963, pp. 65—87, Pergamon Press, Oxford
10 Tobin, T., Akera, T., Baskin, S.I. and Brody, T.M. (1973) Mol. Pharmacol. 9, 336—349
11 Godfraind, T. (1975) Biochem. Pharmacol. 24, 823—827
12 Stephens, W.G.S. (1969) Nature 224, 547—548
13 Godfraind, T. and De Pover, A. (1975) Arch. Int. Physiol. Biochem. 83, 599—601
14 Nakao, T., Tashima, Y., Nagano, K. and Nakao, M. (1965) Biochem. Biophys. Res. Commun. 19, 755—758
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
16 Roufogalis, B.D. (1971) Anal. Biochem. 44, 325—328
17 Fiske, C.H. and SubbaRow, J. (1925) J. Biol. Chem. 66, 375—400
18 Murphy, R.A. and Hasselbach, W. (1968) J. Biol. Chem. 243, 5656—5662
19 Godfraind, T., Sturbois, X. and Verbeke, N. (1976) Biochim. Biophys. Acta 455, 254—268
20 Ogawa, Y. (1968) J. Biochem. 64, 255—257
21 Gattass, C.R. and De Meis, L. (1975) Biochim. Biophys. Acta 389, 506—515
22 Schwartzenbach, G., Senn, H. and Anderegg, G. (1957) Helv. Chim. Acta 40, 1886—1900
23 Schatzmann, H.J. (1973) J. Physiol. 235, 551—569
24 Emmens, C.W. (1948) Principles of Biological Assay, Chapman and Hall, London
25 Squires, R.F. (1965) Biochem. Biophys. Res. Commun. 19, 27—32
26 Robinson, J.D. (1967) Biochemistry 6, 3250—3258
27 Lindenmayer, G.E., Schwartz, A. and Thompson, H.K. (1974) J. Physiol. 236, 1—28
28 Skou, J.C. (1972) Bioenergetics 4, 203—232
29 Dunham, E.T. and Glynn, I.M. (1961) J. Physiol. 156, 274—293
30 Weber, A.M. and Herz, R. (1963) J. Biol. Chem. 238, 599—605
31 Weber, A.M., Herz, R. and Reiss, I. (1963) J. Gen. Physiol. 46, 679—702
32 Katz, A.M., Repke, D.I., Upshaw, J.E. and Polascik, M.A. (1970) Biochim. Biophys. Acta 205, 473—490
33 The, R. and Hasselbach, W. (1972) Eur. J. Biochem. 30, 319—324
34 Reuter, H. (1974) Circ. Res. 34, 599—605
35 Nakamaru, Y. and Schwartz, A. (1970) Biochem. Biophys. Res. Commun. 41, 830—836
36 Romero, P.J. and Whittam, R. (1971) J. Physiol. 214, 481—507
37 Meech, R.W. (1974) J. Physiol. 237, 259—277
38 Carmeliet, E. and Vereecke, J. (1969) Pflügers Arch. Eur. J. Physiol. 313, 300—315
39 Kushmerick, M.J. and Podolsky, R.J. (1969) Science 166, 1297—1298
40 Thyrum, P.T. (1974) J. Pharmacol. Exp. Ther. 188, 166—179