EFFECT OF SODIUM AND POTASSIUM ON ATP-DEPENDENT Ca^{2+} UPTAKE IN RAT PAROTID MICROSOMES

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1. Introduction

Previous studies suggested that calcium plays a key role in controlling secretory processes in parotid gland [1–3]. Some events induced by cholinergic or α-adrenergic agonists (e.g., K^+ efflux, Na^+ influx and amylase release) seem to be partially dependent on extracellular calcium [2–4]; other events like protein discharge induced through β-adrenergic receptors via the cAMP system or the 'phospholipidic effect' caused by cholinergic agonists, appear to be independent of extracellular calcium [2,5,6]. Some of the events induced by cholinergic and adrenergic agonists (e.g., amylase release [3], Ca^{2+} efflux [3,7], or modification of subcellular $^{45}Ca^{2+}$ distribution [8,9]) might involve or reflect the mobilisation of calcium from endogenous stores. Plasma membranes, mitochondria and endoplasmic reticulum are possible sequestration sites [8–10]. However, when muscarinic receptors are activated, sodium and potassium intracellular levels are modified and amylase secretion seems to be dependent on the extracellular sodium concentration [11,12]; sodium increases calcium efflux from the mitochondria of various tissues, including parotid glands [13]. Taken together, these data suggest that the variations of sodium (and/or potassium) intracellular levels might be involved in some regulation of secretory processes, presumably by affecting calcium intracellular stores.

Here, we investigated the effect of sodium and potassium on calcium transport in a microsomal fraction isolated from parotid gland; we showed that calcium uptake was stimulated by sodium and potassium but not significantly affected by lithium.

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2. Materials and methods

Microsomal fractions were obtained from homogenate of parotid gland by differential centrifugation, according to the following modified procedure [14]: the tissue was homogenized with a Dounce in a medium containing 0.3 M sucrose, 0.1% fatty acid-free serum albumin and 2 mM Tris–HCl (pH 7.5) at 4°C; after 6000 × *g* centrifugation, the supernatant was centrifuged at 16 000 × *g* for 20 min and the pellet discarded; after further centrifugation at 48 000 × *g* for 1 h, the sediment was suspended in homogenization medium and stored in liquid nitrogen from 1–7 days and was used as the microsomal fraction. Ca^{2+} binding to microsomal fraction (30–60 μg protein in 1 ml of incubation medium comprising 60 or 110 mM Tris–HCl buffer (pH 7.3)) was measured at 20°C in the absence of oxalate, and Ca^{2+}-uptake was measured in the presence of 4.5 mM oxalate under the same conditions; 1 or 10 μM $^{45}CaCl_2$ was added to the incubation medium, and, as indicated, lithium, sodium or potassium as their respective chloride salts. The reaction was initiated by adding ATP disodium or magnesium salt, 2 or 10 min after the microsomal proteins, and stopped by filtration through millipore filter (pore size: 0.45 μm) [15]; filters were washed with 5 ml of a solution containing 100 mM $CaCl_2$ and 2 mM Tris–HCl (pH 7.5) at 4°C [16]. We verified that the same results were obtained when filters were washed with Ca^{2+}-free incubation buffer. Filters were counted in vials which contained 5 ml instagel and were placed in a scintillation counter (Packard model 3375). The protein concentrations were measured by the Lowry method [17] using bovine serum albumin as standard.

The following compounds were purchased from Sigma Chemical Co: ATP (disodium and magnesium

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salts, vanadium-free), fatty acid-free serum albumin, phosphocreatine and creatine phosphokinase, oxalic acid, oligomycin, ruthenium red and sodium azide. $CaCl_2$, $MgCl_2$, lithium, sodium and potassium chloride salts and Tris were obtained from Merck, and $^{45}CaCl_2$ from IRE (Belgium).

3. Results

3.1. *Properties of the calcium transport system*

Fig.1 shows that the transport of Ca^{2+} was ATP-dependent and was increased by oxalate. During short incubation times (up to 1 min), addition of oxalate did not affect calcium binding. Several experiments were carried out to determine the app. K_m for ATP, which, for equimolar ATP and Mg^{2+} concentrations, was found to be 15 μM. Calcium transport was shown to be temperature-sensitive. These data are in agreement with those reported in [10] for a microsomal fraction from parotid gland. A similar system was characterized in microsomes from pancreatic exocrine cells [18]. The microsomal fraction was examined for possible contamination by mitochondria, but since both ruthenium red (10 μg/ml) and sodium azide (20 mM) failed to inhibit microsomal Ca^{2+} uptake, it was concluded that the microsomal fraction contained no intact mitochondria. The amount of cytochrome *c* oxidase detected, which was ~7% of that in the mitochondrial fraction, only revealed very slight contamination, possibly due to mitochondrial fragments.

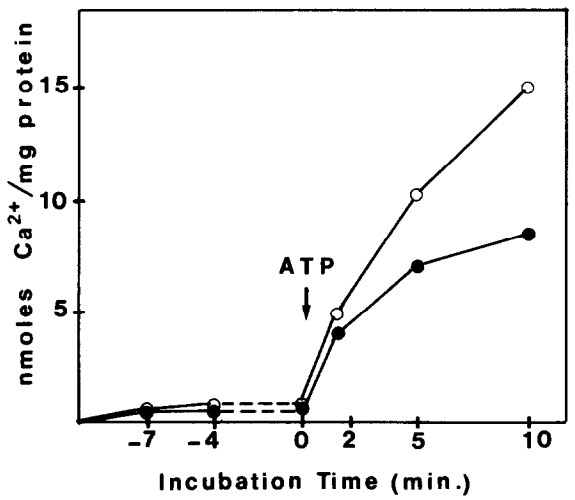

Fig.1. Ca^{2+}-binding and Ca^{2+} uptake by a parotid microsomal fraction. 2 mM MgATP was added after 10 min preincubation of microsomal fraction in the assay medium (110 mM Tris–HCl and 10 μM $^{45}CaCl_2$) with (○) or without (●) 4.5 mM Tris–oxalate. Results are expressed as nmol $^{45}Ca^{2+}$ incorp./mg microsomal protein.

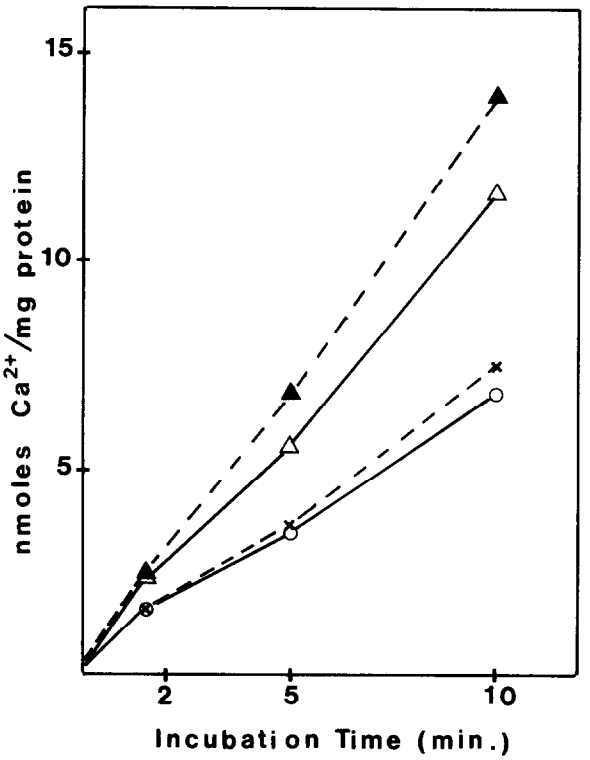

Fig.2. Effect of Li^+, Na^+ or K^+ on parotid microsomal Ca^{2+} uptake. Ca^{2+} uptake was measured in Tris–HCl buffer (pH 7.3) containing 4.5 mM Tris–oxalate, 1 μM $^{45}CaCl_2$, 3 mM $MgCl_2$, 0.2 mM ATP and an ATP regenerating system (1.5 mM creatine phosphate and 20 IU/ml creatine phosphokinase). ATP was added after 10 min preincubation of microsomes. Incubations were carried out in 130 mM Tris–HCl (○) or 80 mM Tris–HCl, with 50 mM LiCl (×), 50 mM NaCl (△) or 50 mM KCl (▲). Each point represents the mean of the results for 2 membrane preparations.

3.2. *Effects of sodium and potassium*

Fig.2 shows the time course for Ca^{2+} uptake by a microsomal fraction in the presence of 4.5 mM Tris–oxalate, 1 μM $^{45}CaCl_2$, 3 mM $MgCl_2$, 0.2 mM ATP and an ATP regenerating system. The ATP-driven Ca^{2+} uptake was linear and was hardly affected by the addition of 50 mM LiCl or Tris–HCl, whereas the addition of 50 mM NaCl or KCl stimulated such uptake by 70% and 100%, respectively. These results

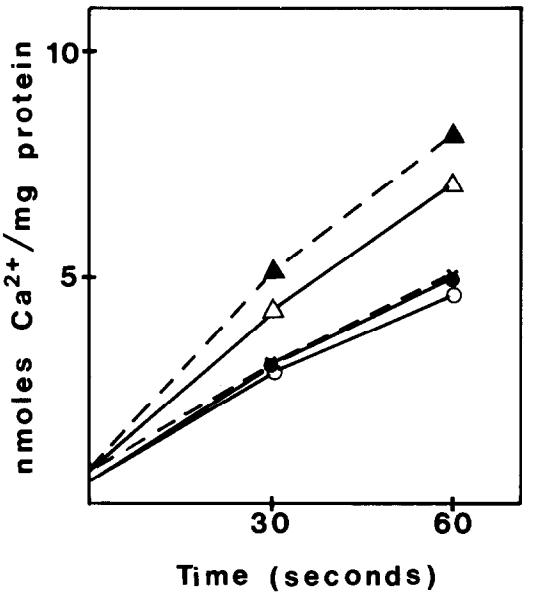

Fig.3. Effect of Li^+, Na^+ or K^+ on parotid microsomal Ca^{2+} uptake during short incubation times. Ca^{2+} uptake was measured in a Tris–HCl buffer (pH 7.3) containing 4.5 mM Tris–oxalate, 10 μM $^{45}CaCl_2$ and 2 mM MgATP (added 2 min after microsomes) in one of the 5 following mediums: 110 mM Tris–HCl (○), 60 mM Tris–HCl (●) or 60 mM Tris–HCl plus 50 mM LiCl (×), 50 mM NaCl (△) or 50 mM KCl (▲). Each point represents the average of 3 or 4 preparations.

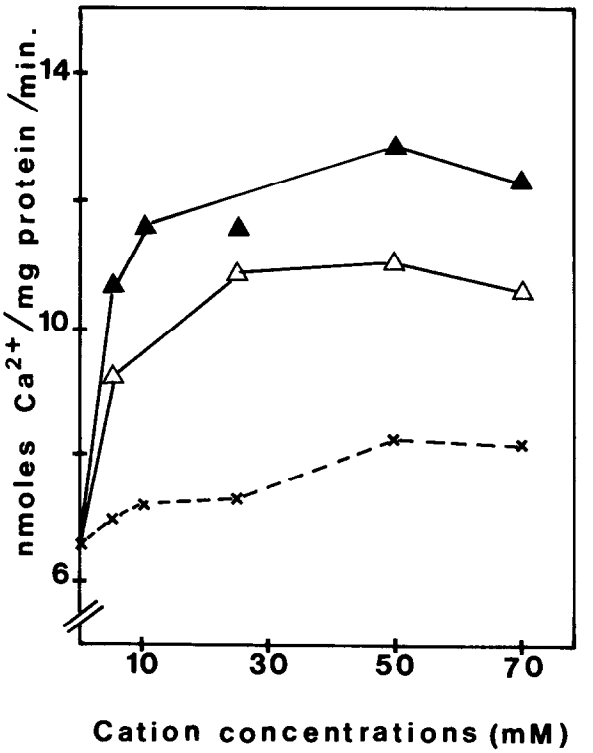

Fig.4. Dependence of microsomal Ca^{2+} uptake on concentration of Li^+, Na^+ or K^+. Ca^{2+} uptake proceeded for 1 min at 20°C in Tris–HCl buffer containing 10 μM $^{45}CaCl_2$, 4.5 mM Tris–oxalate and 2 mM MgATP (added 2 min after microsomes) plus LiCl (×), NaCl (△) or KCl (▲). Points from a representative experiment.

indicate that this stimulation occurred independently of possible effects at the nucleotide level, kept constant by the ATP regenerating system.

Fig.3 gives the results obtained for 30 s or 60 s periods of incubation in the presence of 4.5 mM Tris–oxalate, 2 mM Mg ATP and 10 μM $^{45}CaCl_2$. Under these experimental conditions, it has been shown that the Ca^{2+} uptake was linearly related to the protein content of 15–90 μg/ml, and that no Ca^{2+} efflux seemed to take place since oxalate had no effect. The stimulatory effect of Na^+ and K^+ obtained under these conditions (50% and 80%, respectively, after 1 min) indicates that these cations do act on ATP-dependent Ca^{2+} binding and/or Ca^{2+} uptake. In the absence of ATP, no effect by Na^+ or K^+ was detected (not shown).

Fig.4 indicates the effects of different concentrations of LiCl, NaCl and KCl on microsomal Ca^{2+} uptake. In these experiments, the osmolarity of the medium was kept constant by addition of Tris–HCl buffer. The specific effects of Na^+ and K^+ were detectable at as low as 5 mM.

4. Conclusion

These data indicate that Na^+ and K^+ specifically stimulate calcium transport in isolated microsomal fractions. It is unlikely that contaminant mitochondria were responsible for these effects, since they were also obtained in the presence of 20 μg oligomycin/ml and 10 μg ruthenium red/ml (not shown). However, the involvement of plasma membranes cannot be ruled out.

It is suggested that these effects reflect stimulation of the Ca^{2+} transport system; similar stimulation has been shown on the Ca^{2+}-transport system of sarcoplasmic reticulum [19–21] and the Ca^{2+}-ATPase of sarcolemma [22].

The effects observed in this study were obtained for concentrations of Na^+ and K^+ compatible with the intracellular concentrations of these cations mea-

sured in parotid cells [11], although their cytosolic concentrations are not known.

The stimulation of Ca^{2+} uptake by Na^+ and K^+ reported in this work suggests that these ions might help to regulate the intracellular calcium concentration, mainly during cholinergic stimulation. On the one hand, Na^+ can induce a calcium efflux from mitochondria [13], and on the other, Na^+ and/or K^+ (following rise or fall in their intracellular concentrations), can also modulate the calcium uptake in other compartments such as the endoplasmic reticulum.

The observations reported in this work are also of interest for the investigation of the part the endoplasmic reticulum might play in any intracellular calcium movements involved in the regulation of secretory processes.

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References

[1] Putney, J. W. jr (1976) J. Pharmacol. Exp. Ther. 198, 375–384.
[2] Herman, G., Busson, S., Ovtracht, L., Maurs, C. and Rossignol, B. (1978) Biol. Cell. 31, 255–264.
[3] Butcher, F. R. and Putney, J. W. jr (1980) Adv. Cyclic Nucl. Res. 13, 215–249.
[4] Keryer, G. and Rossignol, B. (1980) Am. J. Physiol. 239, G183–G189.
[5] Michell, R. H. (1975) Biochim. Biophys. Acta 415, 81–147.
[6] Oron, Y., Lowe, M. and Selinger, Z. (1975) Mol. Pharmacol. 11, 79–86.
[7] Butcher, F. R. (1980) Biochim. Biophys. Acta 630, 254–260.
[8] Dormer, R. L. and A. Schroft, S. J. H. (1974) Biochem. J. 144, 543–550.
[9] Kanagasuntheram, P. and Randle, P. J. (1976) Biochem. J. 160, 547–564.
[10] Selinger, Z., Naim, E. and Lasser, M. (1970) Biochim. Biophys. Acta 203, 326–334.
[11] Landis, C. A. and Putney, J. W. jr (1979) J. Physiol. 297, 369–377.
[12] Keryer, G., Herman, G. and Rossignol, B. (1979) FEBS Lett. 102, 4–8.
[13] Crompton, M., Moser, R., Lüdi, H. and Carafoli, E. (1978) Eur. J. Biochem. 82, 25–31.
[14] Amsterdam, A., Schramm, M., Ohad, I., Salomon, Y. and Selinger, Z. (1971) J. Cell. Biol. 50, 187–200.
[15] Martonosi, A. and Feretos, R. (1964) J. Biol. Chem. 239, 648–658.
[16] Alonso, G. L., Bazerque, P. M., Arrigo, D. M. and Tumilasci, O. R. (1971) J. Gen. Physiol. 58, 340–350.
[17] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[18] Ponnapa, B. C., Dormer, R. L. and Williams, J. A. (1981) Am. J. Physiol. 240, G122–G129.
[19] Shigekawa, M. and Pearl, L. J. (1976) J. Biol. Chem. 251, 6947–6952.
[20] Duggan, P. F. (1977) J. Biol. Chem. 252, 1620–1627.
[21] Jones, L. R., Besch, H. R. and Watanabe, A. M. (1977) J. Biol. Chem. 252, 3315–3323.
[22] Morcos, N. C. and Drummond, G. I. (1980) Biochim. Biophys. Acta 598, 27–39.