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CALCIUM BINDING BY A PLASMA MEMBRANE FRACTION ISOLATED FROM RAT SUBMANDIBULAR GLANDS

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SUMMARY

Ca^{2+} binds to isolated plasma membranes and the binding was augmented by ATP. Epinephrine further enhanced the Ca^{2+} binding in the presence of ATP without a parallel increase in the release of inorganic phosphate. Acetylcholine and NaF did not alter Ca^{2+} binding. Cyclic AMP increased Ca^{2+} binding more than either of ATP or epinephrine. These results are discussed in relation to the role of Ca^{2+} in the process of exocrine secretion.

INTRODUCTION

The requirement of Ca^{2+} for both exocrine[1–4] and endocrine[5–12] secretion is well-known, although its exact mode of action is unknown. Ca^{2+} could alter the permeability of cell membranes by binding to them and altering their structure or by stimulating (or inhibiting) an enzyme(s) concerned with secretion[13]. In this communication, some of the Ca^{2+}-binding properties of a plasma membrane fraction isolated from rat submandibular salivary glands are described.

METHODS

(1) *Isolation of plasma membranes*

Three distinct plasma membrane fractions were obtained from the $4000 \times g$ supernatant of a hypotonic homogenate of adult male rat submandibular salivary glands by discontinuous sucrose density gradient centrifugation as described previously[14]. One of these fractions, which appeared relatively little contaminated with other subcellular fractions, sedimenting between the 15 and 20% (w/v) sucrose layers (approximate density 1.12), was used throughout these studies.

(2) *Estimation of Ca^{2+} binding to plasma membranes*

Ca^{2+} binding to plasma membranes was estimated essentially as described by Sulakhe and Dhalla[15]. Approx. 50 μg of fresh plasma membrane protein was incubated in 0.2 ml medium containing 50 mM Tris–HCl buffer (pH 7.4), 0.1 mM $CaCl_2$ containing sufficient $^{45}CaCl_2$ to give a spec. act. of 1000–2000 dpm/nmole

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$CaCl_2$. Other additions are described in the legends to figures. Incubations were carried out for 10 min at 25 °C and were terminated by filtering through 0.45 μm Millipore filters (HAWP 01300, Millipore Filter Corporation, Boston, Mass., U.S.A.). Controls contained all the reactants except membranes. A 50-μl aliquot of the clear filtrate was mixed with 10 ml of Bray's solution[16] and the radioactivity of $^{45}Ca^{2+}$ measured with a Nuclear Chicago Unilux 11 scintillation spectrometer. The amount of Ca^{2+} bound to plasma membranes was calculated as the difference in the radioactivity between the filtrates from the control and test samples:

dpm bound Ca^{2+} = (total dpm in control) − (total dpm in test)

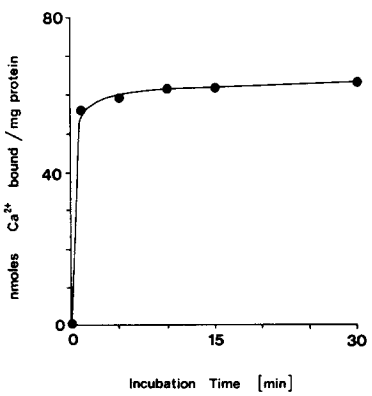
Protein was estimated by the procedure of Lowry *et al.*[17].

RESULTS

Ca^{2+} binding to plasma membranes was quite rapid and complete in 3–5 min of incubation (Fig. 1). Maximum Ca^{2+} binding occurred at pH between 7.5 and 8.5 and the binding was influenced by the ionic environment (unpublished observations). These results were similar to those reported for the liver plasma membranes[18,19] and for red blood cell membranes[20].

Ca^{2+} binding to plasma membranes increased linearly with increasing concentrations of Ca^{2+} up to 150 μM. When ATP was present, Ca^{2+} binding was enhanced (Fig. 2). These observations were confirmed when Ca^{2+} binding was measured as a function of ATP (Fig. 3). Apparently, Ca^{2+} binding was markedly increased by low concentrations of ATP reaching the maximum at 1 mM ATP. At higher concentrations, ATP reduced Ca^{2+} binding to plasma membranes (Fig. 3). Similar stimulatory effects of ATP on Ca^{2+} binding to plasma membranes from liver[19] and rabbit skeletal muscle[29] have been noted by other investigators.

Epinephrine enhanced Ca^{2+} binding (Table I) just as has been reported recently



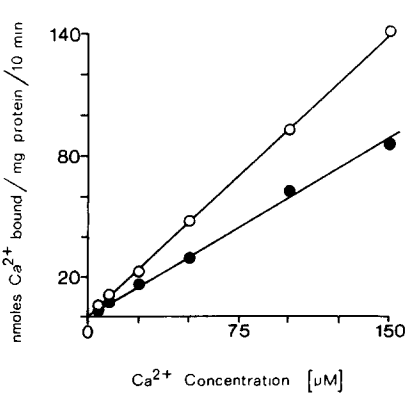


Fig. 1. Ca^{2+} binding to plasma membranes as a function of incubation time. The incubation medium contained 50 mM Tris–HCl buffer, pH 7.4; 0.1 mM $^{45}CaCl_2$ (2000 dpm/nmole $CaCl_2$); 100 mM KCl; 10 mM $MgCl_2$ and 50 μg of plasma membrane protein in a final volume of 0.2 ml. Incubation was carried out at 25 °C for the indicated time period.

Fig. 2. Ca^{2+} binding as a function of Ca^{2+} concentration in the absence (●—●) and presence (○—○) of 1 mM ATP. The incubation time was 10 min and other conditions were exactly as described in Methods and in the legend to Fig. 1.

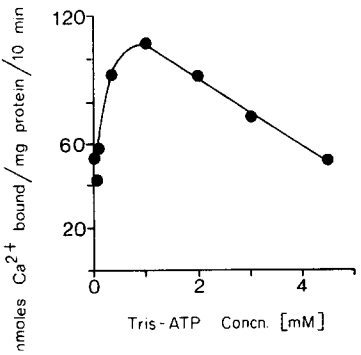


Fig. 3. Influence of Tris–ATP on Ca^{2+} binding. Incubation conditions were similar to those described in the legend to Fig. 1 except Tris–ATP concentration was varied.

TABLE I

EFFECT OF SOME METABOLIC EFFECTORS ON Ca^{2+} BINDING TO SUBMANDIBULAR GLAND PLASMA MEMBRANES

Incubation medium as in Fig. 1 except 1 mM Tris–ATP was present and the incubation time was 10 min. Each value represents the mean of two observations ± S. D.

$CaCl_2$ concn (μM)	*nmoles Ca^{2+} bound/mg protein per 10 min*				
	No additions (Control)	*Epinephrine bitartrate (0.25 mM)*	*Cyclic* AMP (1.00 mM)*	*NaF (1.25 mM)*	*Acetylcholine (0.5 mM)*
5	5.9 ± 3.7	8.0 ± 3.1	16.2 ± 1.6	6.0 ± 1.1	7.7 ± 4.5
10	11.7 ± 1.4	22.7 ± 5.0	37.1 ± 8.2	14.9 ± 2.0	4.1 ± 1.7
25	22.5 ± 2.0	40.9 ± 3.0	116.7 ± 10.9	31.5 ± 9.2	10.6 ± 3.8
50	48.1 ± 3.4	57.2 ± 3.8	159.2 ± 7.5	58.5 ± 10.2	49.4 ± 5.5

* Cyclic AMP replaced Tris–ATP

for isolated liver plasma membranes[18]. Since the plasma membrane fraction used in this study contains adenyl cyclase activity[14] and it is known that epinephrine apparently acts through the adenyl cyclase system in many tissues[21] including salivary gland[22], the effects of cyclic adenosine-3′,5′-monophosphate (cyclic AMP) on Ca^{2+} binding to submandibular gland plasma membranes were examined. It is seen that cyclic AMP did increase Ca^{2+} binding (Table I). Acetylcholine (0.5 mM) did not alter Ca^{2+} binding.

DISCUSSION

The results of this study show that low concentrations of ATP augment Ca^{2+} binding to submandibular gland plasma membrane fragments. ATP could provide substrate for a Ca^{2+}-ATPase system such as has been considered responsible for Ca^{2+} transport in red blood cells[20], and/or bind to plasma membranes inducing conformational changes in their structure such that more external Ca^{2+} binding sites are exposed[23].

The first possibility seems highly unlikely in view of the findings that the liberation of inorganic phosphate was uninfluenced by conditions which enhanced Ca^{2+} binding (present study, unpublished data). This inference is further substantiated by the finding that NaF, an inhibitor of ATPases, did not inhibit Ca^{2+} binding (Table I). An inhibition would have been expected if a Ca^{2+}-ATPase system was involved. The Ca^{2+}-binding process has been shown to be distinct from the ATPase system in dog brain[25] and rabbit muscle[26].

It has been demonstrated that ATP binds to isolated red cell membranes[27] and induces conformational changes in the membrane structure[23]. Such conformational changes might increase the number of Ca^{2+}-binding sites at the membrane surface. A recent report[28] states that ATP-treated red cell membranes have increased polyphosphoinositide levels that parallel the increase in Ca^{2+} binding. Inositides are well-known Ca^{2+} binders.

Thorpe and Seeman[29] reported that ATP slightly enhanced Ca^{2+} binding to isolated sarcolemma, but these authors suggested that this effect might be due to the microsomal contamination of their preparation. It is possible that the stimulatory effect of ATP on Ca^{2+} binding was due to microsomal contamination of the plasma membrane fraction used in the present study[30,31]. However, the effects of NaF and cyclic AMP. both of which are known to inhibit Ca^{2+} binding to microsomes[13], on the present plasma membranes preparation argue against this possibilty. Moreover, the enzymic, chemical and morphological characteristics of the present plasma membrane fraction[14] are comparable to many other plasma membrane preparations which have been used in various types of binding studies.

Rasmussen[13] has proposed a model for secretion wherein an extracellular secretory stimulant interacts with the outer cell membrane leading to an increase in Ca^{2+} permeability and an activation of adenyl cyclase. The fact that (i) adenyl cyclase activity is found in these plasma membranes[14]; (ii) epinephrine activates adenyl cyclase in a variety of tissues[21,22]; (iii) epinephrine and cyclic AMP enhanced Ca^{2+} binding to plasma membranes (present finding) and (iv) that Ca^{2+} is required for the epinephrine-induced, cyclic AMP-mediated secretion from submandibular gland[1,22,31] are reasonably consistent with the model of secretion proposed by Rasmussen[13].

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