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Invited paper

Regulation of calcium handling by rat parotid acinar cells

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Summary

Salivary gland fluid secretion following neurotransmitter stimulation is Ca^{2+}-dependent. We have studied the control of cellular Ca^{2+} following secretory stimuli in rat parotid gland acinar cells. After muscarinic-cholinergic receptor activation, cytosolic Ca^{2+} is elevated 4–5 fold, due to both intracellular Ca^{2+} pool mobilization and extracellular Ca^{2+} entry. Fluid movement ensues due to the Ca^{2+}-activated enhancement of membrane permeability to K^+ and Cl^-. Basal cytosolic Ca^{2+} levels are tightly controlled at ~150–200 nM through the action of high affinity and high capacity ATP-dependent Ca^{2+} transporters in the basolateral and endoplasmic reticulum membranes. Activity of these Ca^{2+} transporters can be modulated to facilitate rapid responsiveness and a sustained fluid secretory response necessary for alimentary function.

Introduction

It has been recognized for many years that Ca^{2+} is required for stimulus-secretion coupling in exocrine salivary glands [1]. Neurotransmitter-activated cell surface receptors (i.e. muscarinic-cholinergic, α_1-adrenergic) which elicit significant rates of fluid secretion, are coupled to the mobilization of intracellular Ca^{2+}, and to increased Ca^{2+} entry pathways in these tissues [2, 3]. It appears that intracellular Ca^{2+} is derived from a non-mitochondrial pool which is mobilized by inositol trisphosphate (IP_3) [4, 5]. IP_3 is generated following receptor activation through the phospholipase C-catalyzed hydrolysis of phosphatidylinositol-4,5-bisphosphate [6]. The manner of extracellular Ca^{2+} entry following receptor activation is as yet unclear [7].

Many studies have shown that basal levels of cytosolic Ca^{2+}, in non-excitable cells such as salivary glands, are in great part a result of the combined action of ATP-dependent Ca^{2+} transporters (presumably Ca^{2+}-ATPases) localized in the endoplasmic reticulum and plasma membrane [e.g., 8–10]. Thus the ability of salivary glands to realize their potential secretory function must, to a considerable degree, be set by the kinetic and regulatory characteristics of these transporters [11]. Resting salivary secretion is negligible but increases markedly (~100 fold) following receptor-induced Ca^{2+} activation. It is presently viewed that salivary transepithelial cell fluid movement requires the coordinated opening of two Ca^{2+}-activated plasma membrane channels, one for K^+ and the other for Cl^- [12–14].

We have studied the control of intracellular Ca^{2+}, as related to exocrine fluid secretion, using the rat parotid gland. This tissue is widely employed in studies of stimulus-secretion coupling [e.g., see 2, 3, 6, 12]. The fluid secretory capacity of the parotid is localized to its acinar cells [15] and following dis-

section and cell preparation, ~90% of the resulting cells are acinar [16]. Neurotransmitter-receptor controlled secretory events are well delineated in rat parotid acinar cells [e.g., 2, 3, 6, 12]. This cell is polarized, with most major plasma membrane ion channels and transporters apparently localized to the basolateral domain, along with neurotransmitter receptors and their associated signal transduction systems (Fig. 1). ATP-dependent Ca^{2+} transport in parotid endoplasmic reticulum-enriched vesicles (ERV) has been studied by several laboratories [e.g., 17–19]. Recently, we have been able to isolate basolateral membrane-enriched vesicles (BLV) from the parotid and to examine the ATP-dependent Ca^{2+} transporter [11, 20] as well as other key ion transporters in this system [e.g., 21]. In the present report, we provide a review of current understanding of the regulation of Ca^{2+} handling by rat parotid acinar cells as related to the fluid secretory process. We also suggest that the ATP-dependent Ca^{2+} transporters of the endoplasmic reticulum and basolateral plasma membranes play key roles in modulating the responsiveness of these cells.

Experimental procedures

The sources of animals, special chemicals and radiochemicals used in our studies were the same as previously reported [e.g., 11, 14, 20, 21]. All reagents used were the highest grade commercially available.

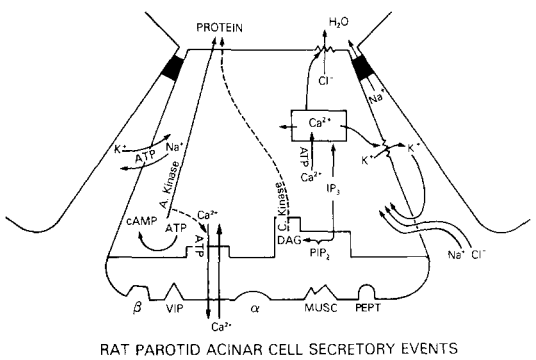

Fig. 1. Model of rat parotid acinar cell secretory events regulated by cell surface neurotransmitter receptors. β-Adrenergic (β) and vasoactive intestinal peptide (VIP) receptors are primarily coupled to cAMP, while α_1-adrenergic (α), muscarinic-cholinergic (MUSC) and substance P (PEPT) receptors are primarily coupled to hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) and subsequent mobilization of Ca^{2+}. See text for additional details.

Cell and membrane preparation

Enzymatically dispersed rat parotid acinar cells were prepared following digestion with collagenase and hyaluronidase in Hepes buffered (pH 7.4) Hanks' balanced salt solution (HBSS-H) essentially as described [14]. Throughout all preparation and incubation procedures, cells were gassed with 95% O_2/5% CO_2 at 20 min intervals. BLV were isolated from parotid homogenates (0.25 M sucrose, 0.1 M phenylmethane sulfonyl fluoride, 1 mM dithiothreitol, 10 mM Tris-Hepes, pH 7.5) using a self-generating Percoll (12% v/v) gradient during centrifugation at 41700 × g for 30 min, as reported previously [20]. ERV were prepared from parotid homogenates (same buffer as for BLV) after differential centrifugation. The ERV were obtained from a 41700 × g (45 min) pellet. Typically BLV were enriched in K^+-dependent phosphatase ~10 fold [20] while ERV were enriched in RNA ~2 fold [22].

Measurement of cytosolic Ca^{2+}

Cytosolic free Ca^{2+} levels ($[Ca^{2+}]_i$) were measured in parotid acinar cells loaded with Quin 2 as described [23]. Cells were incubated with Quin 2-AM at 25 °C for 45 min and then washed to remove extracellular probe. Ca^{2+} Mobilization was studied at 37 °C following addition of the muscarinic-cholinergic agonist carbachol. Fluorescence was monitored in an SLM 8000 spectrofluorimeter and $[Ca^{2+}]_i$ calculated according to Tsien *et al.* [24].

ATP-dependent Ca^{2+} transport

Uptake of $^{45}Ca^{2+}$ into BLV and ERV was measured using rapid filtration through a Millipore filter (HA, 0.45 μm). Typically reaction mixtures contained 12–18 μg membrane protein, 1 mM ATP, 150 mM KCl, 10 mM Tris/Hepes, pH 7.5, 1 mM Mg

gluconate and $^{45}Ca^{2+}$ (various concentrations) in a total volume of 100 μl. For kinetic experiments free Ca^{2+} was buffered with ethyleneglycol bis (β-aminoethyl)-N,N'tetracetic acid (EGTA) as described [11]. All results described here were obtained under initial rate conditions. Reactions were initiated by addition of vesicles. Legends to figures describe exact assay procedures. $^{45}Ca^{2+}$ Uptake was stopped by adding ice-cold reaction buffer (without Ca^{2+}, ATP). Reaction mixtures were then filtered and the filters washed with additional ice-cold buffer.

Results and discussion

A graphic summary of the status of $[Ca^{2+}]_i$, following exposure of rat parotid acinar cells *in vitro* to a continuous muscarinic stimulus (carbachol), is shown in Fig. 2. Under basal (unstimulated) conditions these cells exhibit a $[Ca^{2+}]_i$ of ~150–200 nM. When carbachol is added at a concentration which elicits a maximal secretory response (10 μM; [25, 26]), $[Ca^{2+}]_i$ rapidly increases, reaching ~800–900 nM in <10 sec (Fig. 2; [27]). In the presence of the agonist, peak $[Ca^{2+}]_i$ is maintained for 20–30 sec and thereafter gradually decreases, reaching basal levels 8–10 min after initial stimulation. If cells are stimulated with 10 μM carbachol in the absence of extracellular Ca^{2+} (i.e. HBSS-H + 5 mM EGTA), the initial rate and extent of elevation in $[Ca^{2+}]_i$ changes little (Fig. 2). However, $[Ca^{2+}]_i$ returns to basal level within 2 min. These results clearly demonstrate the involvement of both intracellular and extracellular Ca^{2+} in the changes in $[Ca^{2+}]_i$ which occur subsequent to muscarinic receptor activation.

It is important to remember that this aggregate picture is the end result of many separate events which may directly or indirectly influence $[Ca^{2+}]_i$ at any time. For example, to understand $[Ca^{2+}]_i$ homeostasis one must not only consider the functional characteristics of parotid ATP-dependent Ca^{2+} transporters (basolateral, endoplasmic reticulum) and Ca^{2+} channels (receptor-operated in basolateral membrane; IP_3-activated in the endoplasmic reticulum), but also the influence diverse post-receptor activation events may impose on these Ca^{2+} flux systems (e.g., cyclic AMP [9, 11]; calmodulin [10]; membrane potential [5, 22, 29]). In

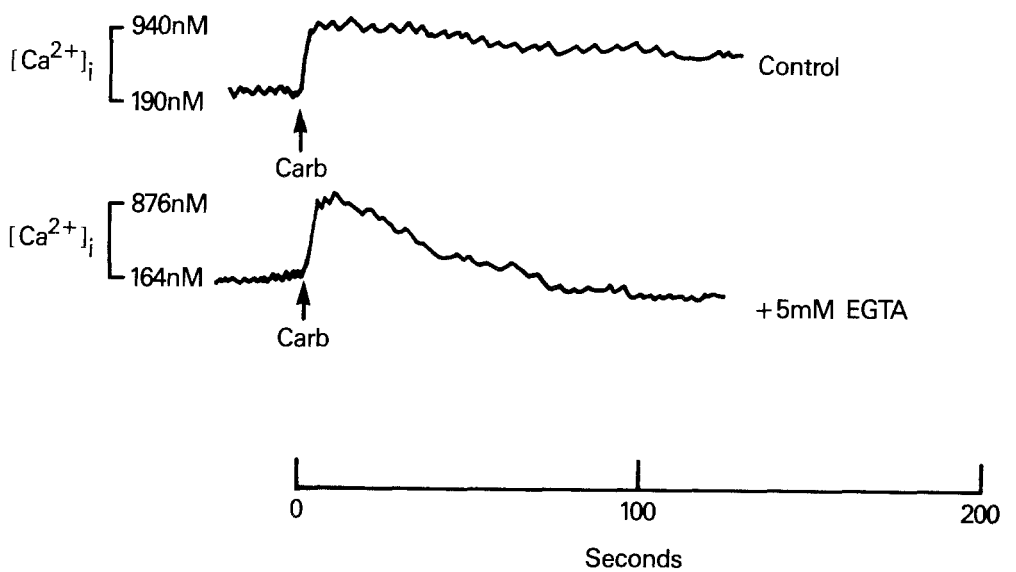

Fig. 2. Cytosolic Ca^{2+} levels in rat parotid acinar cells following muscarinic-cholinergic receptor stimulation. Parotid cells were loaded with Quin 2, and cytosolic Ca^{2+} ($[Ca^{2+}]_i$) measured, as described under Experimental procedures. Carbachol (Carb, 10 μM) was added at the arrow. Cells were incubated in HBSS-H (control, top trace) or HBSS-H + 5 mM EGTA (lower trace).

the present report, we focus attention on a major component of this Ca^{2+} homeostatic system; the possible influence of stimulus-induced changes in intracellular potential on parotid ATP-dependent Ca^{2+} transporters. This is a subject which generally has received limited attention in non-excitable tissues [e.g., 22, 29] but may well be of particular importance to understanding the physiology of exocrine glands.

Stimulus-secretion coupling in salivary glands is accompanied by dramatic alterations in membrane potential and membrane ion permeabilities [2, 12, 14, 28]. Under sustained stimulatory conditions this can result in significant fluid movement for more than 1 hr [e.g., 30, 31]. The mean resting potential across the basolateral membrane of rat parotid acinar cells is -73 mV, a value close to the K^+ diffusion potential [32]. After acute muscarinic-cholinergic stimulation, a characteristic biphasic change in membrane potential occurs [33]. Initially there is a rapid depolarization, thought to be due to enhanced Cl^- permeability. This is followed by a delayed hyperpolarization. This latter change is quite difficult to interpret. It cannot be readily attributed to plasma membrane permeability changes for a single ion, but probably involves multiple events such as changes in activities of the Na^+/K^+ pump, and of other ion transport pathways. Sustained receptor stimulation results in a prolonged hyperpolarization [33]. Given previous (albeit limited) studies which have shown that ATP-dependent Ca^{2+} transporters in the endoplasmic reticulum and plasma membrane of some epithelial tissues are electrogenic [22, 29], it is reasonable to hypothesize that the ionic events which underlie these electrophysiological changes will influence parotid Ca^{2+} handling and secretory responsiveness. Muscarinic-cholinergic stimulation of rat parotid acinar cells is accompanied by immediate, dramatic changes in K^+, Na^+ and Cl^- fluxes [13, 14, 28, 34]. In view of our hypothesis, it is of particular importance that both the temporal pattern and pharmacological characteristics of agonist-induced $[Ca^{2+}]_i$ changes (Fig. 2; [23, 35]) are closely related to agonist-induced changes in plasma membrane permeability to K^+ [28] and Cl^- [35]; the two ion movements most associated with parotid fluid secretion [12, 14].

Recently we have begun a series of studies which attempt to carefully characterize ATP-dependent Ca^{2+} transport processes in parotid BLV and ERV [e.g., 36, 37, 38]. In both membranes ATP-dependent Ca^{2+} transport is quite rapid, being linear with time for only ~ 20 sec under the conditions of assay utilized (see Methods). Both transporters are of high affinity and high capacity (Fig. 3). Typically, we have observed a Km of $\sim 80-100$ nM for ATP-dependent Ca^{2+} transport in BLV and $\sim 20-40$ nM in ERV. The observed Vmax values were $\sim 50-55$ and $\sim 25-30$ nmol Ca^{2+}/mg protein-min, respectively for BLV and ERV.

In order to initially examine the possible regulatory role of membrane potential on parotid ATP-dependent Ca^{2+} transport, we have examined the effect of altering vesicle K^+ diffusion potential by different experimental manipulations. Under con-

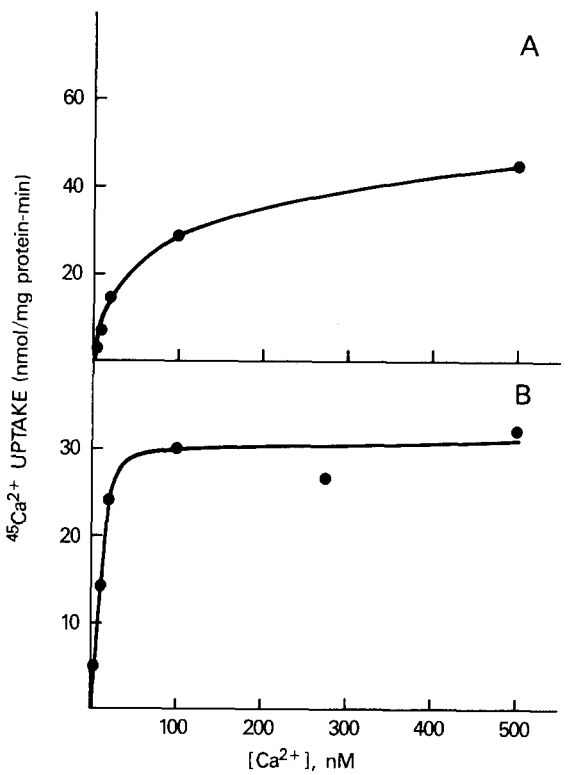

Fig. 3. Effect of $[Ca^{2+}]$ on ATP-dependent Ca^{2+} uptake in rat parotid acinar cell BLV (A) and ERV (B). Membrane vesicles were incubated as described under Experimental procedures in the presence of the indicated $[Ca^{2+}]$. Ca^{2+} was buffered with EGTA as described in reference 11. Results shown are based on initial rate determinations from representative experiments (presented in greater detail in references 37 and 38).

trol incubation conditions (viz. 300 mM sucrose-mannitol$_{in}$/150 mM KCl$_{out}$), the extent of ATP-dependent Ca^{2+} uptake after 20 sec in the presence of 12.5 μM total Ca^{2+} and 1 mM Mg-ATP, was ~10 nmol/mg protein for BLV, and 5 nmol/mg protein for ERV. When a positive inside membrane potential was induced by the presence of the K^+-ionophore, valinomycin (5 μM), the initial rate of Ca^{2+} uptake was reduced ~25% in both membrane preparations [37, 38]. Separate studies by us have shown that these results were not due to direct effects of the ionophore on the transporter. In another series of experiments we have altered vesicle K^+ diffusion potential by manipulating the loading and incubation conditions. Representative experiments are presented in Fig. 4. When BLV were subjected to a negative K^+ diffusion potential (KCl$_{in}$/trimethylammonium, TMA, Cl$_{out}$), ATP-dependent Ca^{2+} uptake was ~25–50% higher than observed in vesicles with zero membrane potential (KCl$_{in}$/KCl$_{out}$) (Fig. 4A). Conversely, when BLV were subjected to a positive K^+ diffusion potential (TMA Cl$_{in}$/KCl$_{out}$) ATP-dependent Ca^{2+} uptake was ~25% lower than at zero membrane potential (Fig. 4A). Qualitatively similar results were observed with ERV (Fig. 4B).

Do the observations reviewed here tell us anything about Ca^{2+} handling in the exocrine parotid cell following a fluid secretory stimulus? In the face of limited experimentation, both in our own and other laboratories, any interpretations should be narrow and cautious. However, it is possible to speculate about the nature of Ca^{2+} homeostasis and its relationship to membrane potential during this ionically complex process. As noted above, during secretory stimuli membrane potential is altered in a biphasic manner. Since ATP-dependent Ca^{2+} transporters in both the endoplasmic reticulum and basolateral plasma membrane of rat parotid acinar cells are electrogenic, during the initial transient depolarization, Ca^{2+} transport out of the cytosol would be stimulated at both membranes (assuming the membrane potential across the endoplasmic reticulum also depolarizes). The activation of these transporters would help the cell cope with the rapid burden of excess Ca^{2+} flooding the cytosol after stimulation (Fig. 2). Thereafter, while the cell is in the sustained

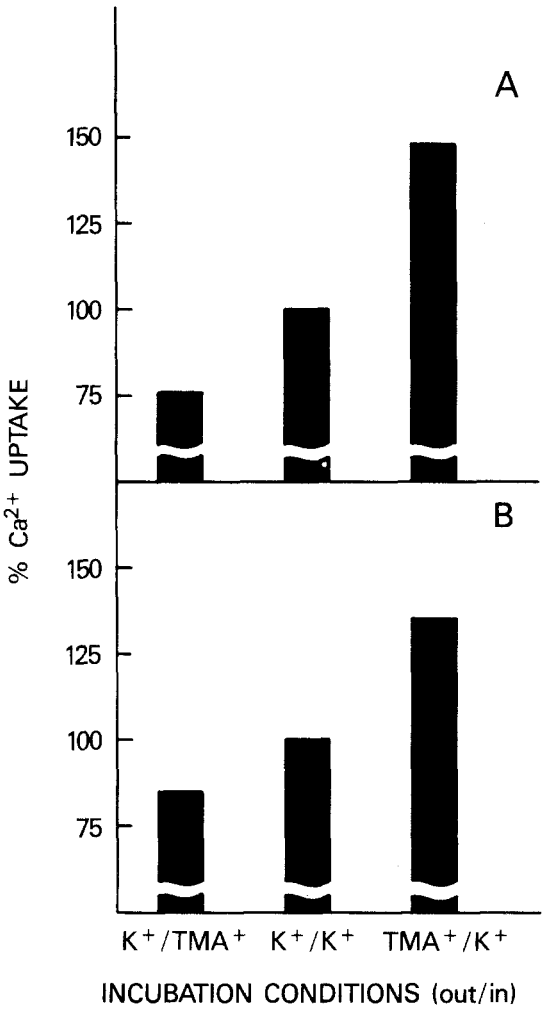

Fig. 4. Effect of K^+ diffusion potential on ATP-dependent Ca^{2+} uptake in rat parotid acinar cell BLV (A) and ERV (B). Membrane vesicles were incubated as described under Experimental procedures, with the loading (in) and incubation (out) conditions manipulated as described under Results and discussion. In the figure K^+ = 150 mM KCl and TMA^+ = 150 mM TMA Cl. Incubations with BLV also contained 5 μM valinomycin. Results are based on initial rate determinations from two experiments (A) or a representative experiment (B). Data are presented in greater detail in references 37 and 38.

hyperpolarized, secretory phase, and the rate of Ca^{2+} entry into the cytosol is reduced but still above basal levels, ATP-dependent Ca^{2+} transport would be relatively inhibited. This would help the cell maintain a level of $[Ca^{2+}]_i$, under continuous stimulation, which will sustain the prolonged fluid transport process but still be tolerable to the cell.

Thus changes in membrane potential which accompany secretory stimuli appear to influence the fine control of cellular Ca^{2+} homeostasis (i.e., ATP-dependent Ca^{2+} transporters) in a way which makes physiological sense. But this view, while reasonable, is simple (in great part because of the severely limited experimental data available). The control of $[Ca^{2+}]_i$, which is central to exocrine fluid secretion [1, 39], is a complicated process potentially influenced by many dynamic factors [9–11, 22]. A great deal more study is required to clarify exocrine intracellular Ca^{2+} homeostasis.

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