Hypothesis

Possible role of H^+–alkali cation countertransport in secretory granule swelling during exocytosis

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Studies in model systems, as well as observations in intact cells, suggest that osmotic swelling of secretory granules is an essential step in exocytosis. A model is proposed whereby the low pH recorded in most secretory organelles could provide the driving force for granule swelling. The model assumes that during stimulation an exchange of H^+ for alkali cations is triggered across the granule membrane. The outgoing H^+ are rapidly replaced by the internal buffering capacity with a concomitant osmotic gain. The exchange is independent of anions and could be triggered by cytoplasmic Ca^{2+}. The H^+-pump is responsible for the ΔpH but independent of the exchange mechanism.

Exocytosis *H^+ transport* *Countertransport* *Proton gradient* *Secretory granule* *Secretion* *Osmotic swelling*

1. INTRODUCTION

Evidence from a number of model systems indicates that Ca^{2+}-induced fusion of 'large' (>0.1 μm diam.) membrane vesicles with themselves [1] or with planar bilayers [2,3] will not occur spontaneously unless an osmotic gradient (inside > outside) is imposed across the vesicle bilayer. It is believed that the hydrostatic pressure generated by the osmotic disequilibrium provides the energy required for membrane destabilization and fusion.

Membrane fusion is an essential step in exocytotic secretion: the secretory granules must fuse with the plasma membrane to accomplish the delivery of their contents to the extracellular milieu. There is circumstantial evidence that osmotic swelling of secretory organelles precedes and is necessary for exocytosis to occur; granule enlargement has been observed just prior to fusion in *Paramecium* [4] and *Limulus* amebocytes [5]. Moreover, in a variety of systems, secretion can be gradually suppressed by increasing the tonicity of the bathing medium (and hence that of the cytoplasm), and therefore presumably eliminating the osmotic differential across the membrane of the secretory organelles [6–8]. The source of the solutes responsible for the purported increase in intragranular tonicity during secretion is unknown.

A remarkably constant feature of all the secretory granules reported to date is their extremely acidic interior (pH ≤ 5.5). Although some disagreement exists, it is generally believed that the low internal pH is the result of an inwardly directed H^+ pump in combination with a reduced H^+ permeability across the membrane [9–11]. In chromaffin granules (which have been intensively studied and will therefore be used as a prototype for the remainder of this paper), Pollard et al. [12,13] have tried to establish a connection between this H^+ accumulation and the osmotic swelling of the granules. In their view, extracellular Cl^-, which can permeate the granule membrane, is driven by its electrochemical gradient into the granule when the latter becomes juxtaposed with the plasma membrane. This hypothesis is supported by experiments of ion substitution in isolated granule suspensions and by inhibitor studies in both isolated granule and in-

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tact cell preparations [10,12,13]. The hypothesis is unfortunately incompatible with results of ion substitution experiments in intact [14] and electrically permeabilized chromaffin cells [8]. In these systems, exocytosis occurred normally in the absence of Cl^- or any other permeating anions outside, or both inside and outside the cell. Pollard's group attempted to explain similar observations in platelets by assuming that OH^- rather Cl^- is the transported species. However, it is not clear how uptake of OH^- and formation of intragranular water could swell the granule, considering that the membrane is very water-permeable.

2. PROPOSAL

Here, we present an alternative model to account for the osmotic swelling of secretory granules that is thought to precede secretion. The model assumes that during stimulation by secretagogues, a H^+-cation antiport becomes operative across the granule membrane, so that intragranular H^+ will be exchanged for extragranular (cytoplasmic or extracellular, see below) monovalent cations. In its simplest form, the exchanger would be tightly coupled and electrically neutral, transporting identical numbers of charges in both directions. Thus the reaction:

$$nH_i^+ + nC_o^+ \rightleftharpoons nH_o^+ + nC_i^+$$

where

n = an integral number
C^+ = a monovalent cation and
i and o = intra- and extragranular, respectively

will come to equilibrium when:

$$\left[\frac{H_i}{H_o}\right]^n = K_e \left[\frac{C_i}{C_o}\right]^n$$

where

K_e = the exchange equilibrium constant

An intrinsic feature of this type of coupled exchange system is that an ion can be driven away from its electrochemical equilibrium by the countertransported ion. This type of secondary active transport will occur for the monovalent cation if its electrochemical equilibrium (defined by the Nernst equation) is different from the exchange equilibrium defined above [15]. Thus net uptake or efflux of the cation can be propelled by a pH gradient. In the case of the internally acidic secretory granules, monovalent cations that were presumably at equilibrium prior to stimulation of secretion, could be concentrated in the intragranular compartment as the pH gradient tends to dissipate.

For a system with a finite ion content, it is the ion with the largest mole fraction that drives the other, minor ion. According to this view, only minor changes of monovalent cation concentration would occur since H^+ (in the 10^{-5} M range) would exchange for only a minute fraction of the total monovalent cations (in the 10^{-1}–10^{-2} M range). Moreover, the exchange of identical numbers of H^+ for cations would, in principle, be osmotically ineffectual. However, the presence in the intragranular space of organic molecules with a large buffering capacity becomes a key consideration. Upon exchange of an internal H^+ for an external cation, the buffering substances replace the lost H^+, resulting in a net osmotic gain inside the granule*. Under these circumstances the effective mole fraction of H^+ is determined by the buffering capacity of the granular contents, rather than by the intragranular pH.

Can the reported buffering capacity of secretory granules account for a substantial uptake of monovalent cations? In the case of the chromaffin granules, the buffering capacity in the pH 5.5 range has been estimated by two different methods at ~ 300 μmol H^+ . pH unit^{-1} . g dry wt^{-1} [16]. Assuming an intravesicular volume of ~ 4 μl/mg protein [10,16] and a protein/dry wt ratio of ~ 0.4 (calc. from [10]) the amount of cations required to raise the internal pH by one unit would increase the internal tonicity by, ideally, 187 mosM. If the granules conform to the ideal Boyle–Van't Hoff behavior, their volume could change by $>50\%$, assuming a starting osmolarity of 310 mosM.

The present model differs from that of Pollard et al. [13] in two important aspects. It is the pH rather than the electrical gradient that drives the osmotically active ion. As a consequence, it is the internal buffering capacity (which replenishes the H^+ gradient after an exchange cycle) rather than

* A major contribution of the H^+-pump in the replenishment of exchanged H^+ is unlikely, given the time course of secretion and the reported maximal rates of H^+-ATPase activity.

the H^+-pump (which restores the internally positive potential after anion influx) that ultimately propels swelling. This has important implications concerning the time course of exocytosis; dissociation of the intragranular buffer is practically instantaneous, unlike the H^+-pump, which is unlikely to transport sufficient charge to account for a significant swelling during the short times (fractions of a second) required for exocytosis.

Based on this secondary active cation uptake model, a number of predictions can be made and tested:

(i) Granule swelling is expected to be strictly dependent on extragranular pH, with more rapid and extensive swelling observed in the alkaline range where the pH gradient across the granule membrane is maximized. Experiments with intact platelets [6], parathyroid cells [17] and perfused adrenal medullas [8] show a dramatic decrease in secretory activity as the pH of the bathing medium approaches 5.5, the intragranular pH.

(ii) Secretion should be independent of the nature and concentration of extracellular anions. This has indeed been observed in chromaffin [8,14] and other cell types [6,7].

(iii) Addition of exogenous H^+–alkali cation exchangers in the presence of external cations could swell and potentially lyse secretory granules in isoosmotic media. Geisow and Burgoyne [19] recently reported that addition of Na^+ and monensin (a Na^+/H^+ exchanger) to chromaffin granules resulted in extensive lysis.

The observation by Baker and Knight [8] that exocytosis in permeabilized chromaffin cells is unimpaired by 30 mM NH_4Cl appears, in principle, to be incompatible with the present hypothesis; NH_4Cl was expected to collapse the pH gradient across the vesicle membrane. However, Johnson and Scarpa [11] had shown that this concentration of NH_4^+ dissipates only ~½ of the ΔpH in isolated chromaffin granules. Moreover, in the presence of ATP, an operative H^+-pump could maintain a ΔpH even in the presence of permeating bases. This has been reported for β-cell granules, which swell in the presence of benzylamine but maintain an orange fluorescent emission (indicative of an acidic interior) when incubated with acridine orange [20]. Finally, NH_4Cl-induced granule swelling could itself drive exocytosis, bypassing the cation/H^+ exchange mechanism; the fundamental swelling mechanism (replacement of a bound proton by an osmotically active cation) is the same in both cases.

How is the H^+-cation exchanger activated during secretion? At least two mechanisms can be envisaged:

(1) The system could already exist in an active form in the plasma membrane, and would be inserted into the granule membrane as the two become apposed just prior to fission. In this case, intragranular H^+ might be exchanged for extracellular Na^+, the main cation in the bathing solution. In this regard, H^+–Na^+ exchange systems have been described in the plasma membrane of a variety of cell types (review [21]).

(2) A normally latent exchanger on the granule membrane could be activated during stimulation. Cytoplasmic messengers could convey the activating information. It has been suggested that Ca^{2+}, an acknowledged intracellular mediator of secretion, can activate otherwise latent H^+–Na^+ countertransport systems [21].

In summary, a hypothesis is presented whereby an alkali cation influx would account for the secretory granule swelling required for fusion. The following temporal sequence can be envisaged: the granular H^+-pump reduces the internal pH; if acting electrogenically, the pump will render the granule inside positive, thereby excluding monovalent cations; the buffering molecules in the granule core become protonated as the H^+–pump maintains a low pH; during stimulation by secretagogues, a H^+–alkali cation exchanger is activated; H^+ that leave the granule in exchange for external cations are rapidly replenished by the internal buffer, whereby a new exchange cycle can be initiated; alkali cations are accumulated in the granule, and as osmotically obliged water is dragged in, the granule swells, swelling raises the surface free energy of the granule membrane thereby favoring granule–plasma membrane fusion.

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