The effect of permeant and impermeant osmoticants on exocytosis in guinea pig sperm

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Summary

Guinea pig sperm were suspended in calcium-containing medium supplemented with various concentrations of the tetrasaccharide, stachyose. At concentrations up to and including 0.6 M, stachyose was without effect on the A23187-induced acrosome reaction. At 1.0 M stachyose, >97% of sperm retained their acrosome after exposure to A23187, as judged by light microscopy. Electron microscopy demonstrated, however, that exocytotic membrane fusion had occurred, although with substantial retention of the acrosomal matrix. Sperm incubated in 1.0 M stachyose solutions also underwent exocytotic membrane fusion in the absence of A23187 and external calcium.

Sperm suspended in 0.175 M ammonium chloride solution progressively lost motility over 30 min, but without acrosomal swelling. By contrast, sperm in 0.19 M ammonium acetate underwent substantial swelling of the acrosome within 2–5 min. 70–80% of these sperm were able to exclude the vital dye propidium iodide with their acrosomes swollen. These sperm underwent acrosomal shrinkage if resuspended in normal medium within 5–10 min, and the majority (60–70%) recovered some motility. These sperm could undergo an A23187-induced acrosome reaction. Electron microscopy indicated that swelling in ammonium acetate solution solubilizes much of the acrosomal matrix and causes internal fusion between adjacent regions of the outer acrosomal membrane. There was no exocytotic membrane fusion in ammonium acetate solution, however.

The evidence suggests that there is no stachyose osmolality for guinea pig sperm which will suppress the membrane fusion associated with exocytosis, and that sufficiently high osmolalities cause exocytotic membrane fusion in the absence of calcium. The evidence also suggests that release of the normal osmotic compression of the acrosomal matrix does not cause local blebbing of the acrosomal membrane, nor does it render the membrane fusogenic. The data are consistent with the model for exocytosis in which no granule swelling occurs prior to membrane fusion.

Key words: guinea pig sperm, exocytosis, osmoticants.

Introduction

There has been much discussion in the past decade of the role that osmotic forces play in exocytosis (Finkelstein et al. 1986; Holz, 1986; Green, 1987; Lucy, 1989). On one view, secretory granules swell prior to fusion of their membrane with the overlying plasma membrane of the cell, and this swelling is the cause of membrane fusion (Zimmerberg et al. 1980; Hampton and Holz, 1983; Zimmerberg et al. 1985; Zimmerberg and Whitaker, 1985; Ahkong and Lucy, 1988; Lucy, 1989). On the other, granules have cores with latent osmotic activity which is expressed as swelling, after membrane fusion between the secretory granule membrane and the overlying plasma membrane has taken place (Green, 1978, 1982, 1987; Breckenridge and Almers, 1987; Whitaker and Zimmerberg, 1987; Zimmerberg et al. 1987). On this view, exocytotic membrane fusion is an event which is prior to, and independent of, any granule swelling.

The evidence for the first view is two-fold. Firstly, phospholipid vesicles fuse with planar phospholipid membranes much more readily if they are subject to osmotic swelling (Zimmerberg et al. 1980); secondly, secretion in many secretory cells is osmotically sensitive (Hampton and Holz, 1983; Finkelstein et al. 1986; Holz, 1986; Lucy, 1989), with increasing external osmolalities suppressing secretion. The osmotically sensitive event has been widely attributed to swelling of secretory granules (Zimmerberg and Whitaker, 1985; Finkelstein et al. 1986; Holz, 1989).

The evidence for the second view is also primarily two-fold. Firstly, iso-osmotic solutions of high Mr polymers do not arrest exocytotic membrane fusion but do arrest granule matrix swelling (Green, 1982; Whitaker and Zimmerberg, 1987; Chandler et al. 1989), suggesting that exocytotic pore formation is prior to, and independent of, swelling. Secondly, in mast cells with giant secretory granules, capacitance changes associated with integration of granule membrane into the plasma membrane of the cell take place before detectable swelling occurs, again suggesting that membrane fusion is an event prior to swelling (Breckenridge and Almers, 1987; Zimmerberg et al. 1987).

Recent evidence indicates that hyperosmolar solutions cause retraction of secretory granules from the cell cortex in sea urchin eggs (Merkle and Chandler, 1989). If this phenomenon is widespread, it would explain the osmotic...
sensitivity of much secretion without recourse to osmotically-sensitive granule swelling. The evidence, however, does not entirely eliminate a role for osmotic forces in membrane fusion since small, fusigenic blebs could develop under the granule membrane prior to fusion (Green, 1987). Blebs induced in erythrocytes, for example, cause cell fusion (Akhong and Lucy, 1988).

Guinea pig sperm possess a single large secretory granule, the acrosome, the size and position of which makes it possible to correlate light microscopy with ultrastructural examination by electron microscopy. Currently, evidence suggests that the acrosomal matrix swells after exocytotic membrane fusion (Green, 1978, 1982), although the possibility of local blebbing of the acrosome prior to exocytotic membrane fusion (Green, 1987) has not been ruled out. The experiments described in this paper fall into two parts: those in which stachyose solutions were used in an attempt to suppress an ionophore-induced secretory discharge, and those in which the acrosome was swollen by ammonium acetate and hypotonic solutions in an attempt to expose evidence for osmotic coupling between the granule matrix and the secretory granule membrane.

Materials and methods

Stachyose solutions

Reagents were analytical grade unless otherwise indicated. Washed epididymal sperm were obtained from guinea pigs as previously described (Green, 1978). Calcium or magnesium medium (Green, 1978) was supplemented with stachyose (Sigma, St Louis, MO) to give appropriate concentrations. Osmolalities were measured on a Wescor vapor pressure osmometer, Model 5500 (Wescor, Logan, UT). This machine is limited to displaying osmolalities of no greater than 1999 mmol kg⁻¹, although its inherent capability is somewhat above that. Osmolalities were therefore measured for solutions below 700 g l⁻¹ (0.972 mM stachyose, assuming an M_r for stachyose of 720) and correlated by the function y=271+1.34x+0.0005x², r²=1.00 (where y=stachyose mosmolality, x=stachyose molarity). On this basis, the calculated osmolality of the 1.0 M stachyose solution was 2144 mmol kg⁻¹. The osmolality of the 0.6 M stachyose/saline solution could be measured directly and was 1265 mmol kg⁻¹. Pellets of washed sperm were resuspended in stachyose solutions to give final counts of 10⁹ sperm ml⁻¹. Sperm pellets contain >95% water, and pellet sizes were used which resulted in no more than a 5% decrease in osmolality. Unfixed sperm were observed directly at 34–36°C in a heated chamber mounted on a Zeiss IM35 inverted microscope equipped with ×40 Nomarski optics. Sperm suspensions were fixed for electron microscopy by direct addition of 20 µl of 80% glutaraldehyde per ml of sperm suspension to give a final concentration of 1%, and the suspension was pelleted after 30 min. Pellets were washed 3 times with 0.15 M sodium cacodylate buffer, pH 7.2, before staining with 1% osmium tetroxide in 0.15 M sodium cacodylate buffer for 2 h. After poststaining overnight in 1% uranyl acetate, 0.15 M sodium acetate solution, pH 5.1, pellets were processed through graded ethanols and embedded in Spurr resin. Silver-to-gold sections were examined on a Philips 400 LS electron microscope. Acrosome reactions were induced in calcium-containing stachyose solutions with A23187 (Green, 1978).

Ammonium chloride and ammonium acetate solutions

Sperm were incubated with either 0.175 M ammonium chloride solution (310 mosmolal) or 0.19 M ammonium acetate solution (311 mosmolal) under continuous observation on the heated stage. Sperm in normal medium were placed in a central well, which was then mixed with solution placed in the surrounding part of the chamber. Under normal conditions, it is possible to maintain viability of sperm and oocytes for several hours in this chamber.

Solution changes could be made rapidly by emptying the chamber outside the well and then re-flooding it with fresh solution. In this way, direct observations could be made of the effect on sperm of ammonium acetate solutions, and their subsequent response to normal medium. In some experiments, the bathing solution was supplemented with the vital dye propidium iodide (PI, 10 µM), and sperm screened for fluorescence of their nuclei by fluorescence microscopy. For electron microscopy, washed sperm pellets were resuspended in 0.175 M ammonium chloride or 0.19 M ammonium acetate solutions, both pH 7.2, and fixed at various time intervals by direct addition of ~50% glutaraldehyde to give a final concentration of 1%. In some experiments with 0.19 M ammonium acetate solution, sperm suspensions were pelleted without fixation, and resuspended in either calcium- or magnesium-containing medium. Acrosome reactions were induced by addition of A23187 to sperm suspensions in calcium-containing medium (Green, 1978).

Results

The guinea pig sperm acrosome contains a matrix which is regionally differentiated in a characteristic way (Fig. 1): a broadly elliptical region a lies adjacent to the convex face of the acrosome; a broad band of matrix b runs from the anterior tip of the acrosome to the leading edge of the acrosomal cusp, e, the membrane fusion is unaccompanied by any obvious expansion of the acrosomal matrix (Fig. 5). Ultrastructural examination of these sperm reveals a pattern of membrane fusion which closely follows that seen in early stages of the normal acrosome reaction. It is marked by membrane fusion in the regions marked d and e of Fig. 1. In the region at the tip of the acrosomal cusp, e, the membrane fusion is unaccompanied by any obvious expansion of the acrosomal matrix (Fig. 5). The fate of the region d in Fig. 1 is more difficult to determine because of the disruptive effects which the spontaneous expansion and loss of region a has on this area of the acrosome.

When sperm are suspended in increasing concentrations of stachyose up to and including 0.6 M, the stachyose is without effect in suppressing the A23187-induced acrosome reaction, and sperm lose their acrosomes. However, in 0.8 M stachyose, approximately half the sperm retain their acrosomes, as judged by light microscopy, and in 1.0 M stachyose and above, >97% of acrosomes are retained. Ultrastructural examination of these sperm shows, however, that they have invariably undergone membrane fusion between the outer acrosomal and overlying plasma membrane. Sperm suspended in 1.0 M stachyose show no change by light microscopy either on suspension or during the post-fixation wash (Fig. 3A,B,C). Membrane fusion also occurs when sperm are suspended either in Ca²⁺-containing solutions without A23187, or in Ca²⁺-free solutions, as controls, both containing 1.0 M stachyose (Figs 4, 5). Ultrastructural examination of these sperm reveals a pattern of membrane fusion which closely follows that seen in early stages of the normal acrosome reaction. It is marked by membrane fusion in the regions marked d and e of Fig. 1. In the region at the tip of the acrosomal cusp, e, the membrane fusion is unaccompanied by any obvious expansion of the acrosomal matrix (Fig. 5).
examined by electron microscopy. By contrast, sperm suspended in 0.19 M ammonium acetate swell progressively over a period of 2–5 min (Fig. 2C,D,E). Initially, stacked sperm begin to separate as the acrosome and cytoplasm swell (Fig. 2C). Swelling in the acrosome is restricted, however, to the acrosome anterior to the leading edge of the sperm nucleus, with the acrosomal matrix pinning the outer acrosomal membrane at the base of the concave face of the acrosome (Fig. 2D). As the acrosome continues to swell further, the acrosome adopts a spherical shape (Fig. 2E). Ultrastructural examination of sperm swollen by ammonium acetate solution shows that the outer acrosomal membrane from the concave face is folded back against the posterior part of the outer acrosomal membrane (Fig. 6). The effect of overlaying these two adjacent parts of the outer acrosomal membrane on top of each other is to produce extensive membrane fusion between the two. This fusion occurs in the absence of external calcium. Much of the acrosomal matrix is solubilized in ammonium acetate, with only relatively small amounts of acrosomal matrix remaining (Fig. 6). The matrix which is left is the residuum of area b in Fig. 1. It is also possible to identify the former areas d and e of Fig. 1 in Fig. 6 and note that no matrix expansion or membrane blebbing has taken place. Sperm such as those in Fig. 2E continue to exclude the vital dye propidium iodide in 70–80% of cases, indicating that rupture of the plasma membrane has not occurred in these sperm. When sperm suspended in ammonium acetate solution are returned to ordinary saline solutions, the acrosome and cytoplasm shrink over a period of 1–2 min. Fig. 2F shows a partially shrunken acrosome in sagittal section, and Fig. 2G a shrunken acrosome viewed en face. In neither case has the acrosome returned to its former shape. Ultrastructurally, these two states have the appearances shown in Figs 6 and 7. Fig. 6 represents an early stage in shrinkage whereas Fig. 7 shows an acrosome in transverse section in which shrinkage is probably close to completion. An acrosomal cusp is still identifiable. Also notable is the trapping of the acrosome in a new, highly distorted state. This has apparently occurred as a consequence of the condensation of the acrosomal contents originally solubilized by ammonium acetate. Once acrosomes have shrunk, it is possible to induce an acrosome reaction in the conventional way with A23187 (Figs 2H, 8). The only difference between the morphology of a sperm which has undergone a normal acrosome reaction and one such as that shown in Fig. 8 is the folding of the outer acrosomal membrane back on itself. In this sperm, the folding has resulted in little membrane fusion between these adjacent regions of acrosomal membrane (cf. Fig. 6).

It is not possible to cause an acrosome reaction with A23187 in sperm swollen in 0.19 M ammonium acetate solution containing 2 mM CaCl₂: that is, in sperm having the appearance of Fig. 2E. Moreover, sperm held with their acrosomes fully swollen for more than a few minutes increasingly remain swollen when returned to normal calcium medium, and will not undergo an acrosome reaction on subsequent exposure to A23187. The cycle of decondensation and recondensation of acrosomal contents undergone with ammonium acetate solutions appears to make it easier for the contents to disperse once membrane fusion has occurred (Fig. 8).
Fig. 2. (A) Stack of normal guinea-pig sperm in sagittal section; the acrosome for one sperm is marked between arrows. (B) A single normal sperm en face; the nucleus is an approximately-flat plate with the acrosome forming a cusp around the anterior edge of the nucleus (arrow). (C,D,E) Sperm at stages after suspension in 0.19 M ammonium acetate solution, pH 7.4; (C) shortly after swelling has begun, (D) at the end of the first phase of swelling, with the acrosome pinned at the leading edge of the nucleus (arrow), and (E) after swelling is complete. A comparison of E with A shows the extent of swelling produced by the ammonium acetate. (F) Sagittal section of a sperm after suspension in 0.19 M ammonium acetate and return to normal medium: the acrosome (arrow) has shrunk, but not to its original shape. (G) En face view of sperm treated as in F; again, the acrosome has shrunk but not to its original shape. (H) Sperm which have undergone an A23187-induced acrosome reaction after a cycle of swelling in 0.19 M ammonium acetate solution and shrinkage in calcium medium showing disintegrating acrosomes (arrows). (I) Sperm after suspension in 25 mM saline, 10 mM Hepes; the extent of acrosomal swelling is more limited than that seen in ammonium acetate. Bar, 10 μm for all micrographs.

Fig. 3. Guinea-pig sperm (A) in 1.0 M stachyose, (B) after fixation as described in text and (C) after suspension in 0.15 M sodium cacodylate buffer. Bar, 10 μm for all micrographs.

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Fig. 4. The acrosomes of a stack of guinea pig sperm suspended in calcium-free 1.0 M stachyose solution. The sperm have undergone exocytotic membrane fusion: the area a has disappeared and the matrix b has undergone limited cavitation at its base. Bar, 1 µm.

Fig. 5. The tips of two acrosomal cusps after membrane fusion in calcium-free 1.0 M stachyose. The right hand acrosome shows membrane fusion at the tip, which is the predominant site in 1.0 M stachyose. The other sperm shows a less common form of membrane fusion, in which the fusion runs to one side of the tip. In neither case has the underlying matrix expanded. Bar, 0.5 µm.

Comparison of sperm swollen in 0.19 M ammonium acetate with those swollen in 25 mM NaCl solution (Figs 21 and 9) reveals some interesting differences. In 25 mM NaCl, the acrosomal matrix which forms the concave face of the acrosome neither dissolves nor undergoes deformation. Nor is the outer acrosomal membrane pinned at the anterior edge of the sperm nucleus, and the folding back of this membrane on itself is therefore absent. Although the convex face of the outer acrosomal membrane takes on a more rippled appearance, possibly following fixation, it fails to produce blebs, and the tip of the acrosome remains intact.

Discussion

Sperm possess a single secretory granule, the acrosome, which is large enough in guinea-pig sperm to be directly observable. It undergoes A23187-induced exocytosis in the presence of external calcium, but not its absence (Green, 1982). Evidence to date indicates that acrosomal matrix swelling follows exocytosis rather than precedes it (Green, 1978, 1982), and occurs by at least two mechanisms. One is cavitation, driven by a latent colloid osmotic pressure within the matrix (Green, 1978, 1982); this occurs in the matrix adjacent to the concave surface of the acrosome (Green, 1978). The other is a decondensation of the matrix close to the convex surface of the acrosome. The evidence suggests that the acrosome is compressed because the osmotic pressure within it is lower than it is outside (Green, 1982). Elimination of this deficit through exocytosis or other means, together with the activation of proteolysis, allows the granule matrix to expand and disperse.

If correct, this view of the acrosome would suggest that exposure of sperm to solutions of increased osmolality should leave acrosomal shape and volume relatively unaffected, because the acrosome is already in a state of compression. Exocytotic membrane fusion would also be unaffected because, on this model for the acrosome reaction, it is not osmotically driven. The effect of stachyose osmolarities of up to 0.6 M supports this view: no change occurs to the shape of the acrosome and the A23187-induced acrosome reaction proceeds normally. It could however, be argued that an insufficiently high external osmotic pressure was employed to suppress any

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localized regions of membrane blebbing which are the prelude to membrane fusion. The behaviour of guinea-pig sperm in stachyose osmolarities greater than 0.6 M does not resolve this question, since higher osmotic pressures apparently cause membrane fusion in their own right. It is possible, however, that the fusion occurs during the post-fixation wash, when the stachyose osmolality is falling. Although there is no way in which the latter can be ruled out using the experimental techniques described in this paper, it would entail that glutaraldehyde fixation has no effect on membrane fusion. In addition, the evidence of Fig. 3 suggests that the washing step produces no changes. Taken together, this might suggest that the fusion occurs following the initial exposure to 1.0 M stachyose. Either way, membrane fusion is occurring in the absence of external calcium, and since there is no internal calcium whose release can cause exocytosis (Green, 1978), the evidence suggests that the fusion is wholly independent of calcium. If it is directly due to the high osmolality of the 1.0 M stachyose solution, then it presumably occurs either because the activity of the water separating plasma and acrosomal membrane falls, or because the osmotic forces which push the plasma membrane against the acrosomal membrane overcome the repulsive forces which normally

Fig. 6. Guinea pig sperm after swelling in 0.19 M ammonium acetate, and partial shrinkage during fixation. The plasma membrane has lifted away from the acrosome during swelling, and the outer acrosomal membrane has folded back on itself and undergone extensive membrane fusion with itself (small arrows). The matrix (b in Fig. 1) has been substantially solubilized and the residue has undergone deformation. During the shrinkage, plasma membrane has been trapped in the folds of the outer acrosomal membrane as the acrosome itself shrinks. The tip of the acrosome and the region marked d in Fig. 1 are still present (large arrows). Bar, 1 μm.

Fig. 7. Transverse section through the head of a guinea-pig sperm after a cycle of swelling in 0.19 M ammonium acetate and shrinkage in saline. The acrosome is now marked by a highly irregular shape in which the acrosomal contents have initially undergone solubilization and then condensation. The tip of the acrosomal cusp is still visible (arrow). Bar, 1 μm.
Fig. 8. An A23187-induced acrosome reaction in a sperm after a cycle of swelling in 0.19 M ammonium acetate solution and shrinkage in calcium-containing medium. Membrane fusion has apparently proceeded normally with formation of cisternae, seen in section. The outer acrosomal membrane has folded over itself (arrows), a consequence of swelling in ammonium acetate solution (cf. Fig. 6). Bar, 1 μm.

Fig. 9. Guinea-pig sperm after suspension in 25 mM NaCl solution. The pattern of swelling is both more limited than that seen in ammonium acetate solution (Fig. 6) and different in detail. The acrosome is not pinned at the anterior tip of the nucleus, with the consequence that the outer acrosomal membrane peels away from the inner, rather than folding back on it, as happens in ammonium acetate. The tip of the acrosomal cusp remains intact, and the region d can be identified in some cases. Bar, 0.5 μm.

prevent the two membranes from fusing. The geometry of the sperm head makes this possible, since, prima facie, any stachyose-induced retraction of secretory granule from the plasma membrane (seen, for example, in sea urchin eggs (Chandler et al. 1989; Merkle and Chandler, 1989)) cannot occur. Areas of early membrane fusion in stachyose solutions are the same as those seen in the ionophore-induced acrosome reaction (Green, 1978; Flaherty and Olson, 1988), suggesting that there may be fusion-specific areas of acrosomal membrane.

If membrane blebs do arise before membrane fusion, the acrosomal matrix must be able to localize an osmotic response, either by acting as a semi-permeable membrane trapping osmotics close to the granule membrane, or by acting as a source of gel swelling. As mentioned earlier, the acrosome is kept compressed and condensed partly by an osmotic deficit which shrinks the granule membrane onto the acrosomal matrix (Green, 1982). We reasoned that if the acrosome was driven to swell without rupturing the acrosomal membrane, then it might reveal osmotic

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behaviour in its matrix which could account for bleb formation. The regions of particular interest, in this regard, would be the two regions, d and e in Fig. 1, which have previously been noted as early points of exocytotic membrane fusion.

In the experiments described here, the acrosome was swollen with ammonium acetate and hypotonic saline solutions. Ammonium acetate produces a much larger swelling than hypotonic saline, and this appears to be due to the much greater decondensation of acrosomal matrix which ammonium acetate causes. This decondensation would increase the number of osmotically active particles and lead to a higher osmotic pressure. It would also uncouple, to some extent, the matrix from the acrosomal membrane, thereby allowing the whole acrosome a greater opportunity to swell. Whatever the causes of the osmotic excess which drives acrosomal swelling, it appears to act, in both ammonium acetate and hypotonic medium, on the secretory granule membrane rather than the matrix itself. In doing so, it should allow any incipient osmotic activity in the matrix to be expressed. Despite relaxation of the intragranular osmotic deficit, and decondensation of much of the matrix, the region e in Fig. 1 remains consistently identifiable and intact, and the region d frequently so. Where the loss of d does occur, it is attributable more to wholesale changes in the matrix occasioned by the ammonium acetate. There is no evidence of membrane blebbing in either solution.

As the acrosome swells in ammonium acetate, part of the outer acrosomal membrane remains attached to the inner acrosomal membrane through the intermediacy of the acrosomal matrix. The consequence of this is that two adjacent areas of outer acrosomal membrane are folded onto themselves, and frequently fuse. This was a wholly unexpected finding and raises some interesting questions. Firstly, it is not clear whether multiple fusions occur, or whether a single fusion pore enlarge into a topographically complex hole. However, the fusions occur in the absence of external calcium and, for the reasons outlined earlier with the stachyose-induced fusions, are likely to be independent of calcium altogether. The cause of the fusion would appear to be the development of a compressive force pushing the membranes together as the acrosome continues to swell. This force presumably overcomes the repulsive forces which normally exist between membranes, and allows the two areas of membrane to come into contact close enough to permit fusion. This suggests that compressive removal of water occurs, and that membrane proteins in the outer acrosomal membrane do not, collectively, occlude the close contact needed for fusion. The size of the compressive forces (if, indeed, they are responsible for fusion), and their relationship to the magnitude of the forces which normally prevent membranes from fusing, remain completely unknown. Swelling of acrosomes in ammonium acetate cannot be a direct mechanism for inducing which occurs during the acrosome reaction, however, partly because the plasma membrane itself is osmotically lifted away from the outer acrosomal membrane, partly because the greater part of the swelling in the normal acrosome reaction takes place after exocytosis. What the results do suggest is that the outer acrosomal membrane does not possess any innate ability to bleb when the osmotic pressure inside the acrosome is allowed to rise, and that, by exclusion, all swelling of the acrosome and its matrix occurs after exocytosis.

When swollen sperm are returned to normal saline, both the acrosome and cytoplasm shrink. In doing so, acrosomes adopt highly varied outlines. Close observation of sperm by light microscopy during shrinkage (Fig. 2F,G) suggest that these new shapes are stable and not transient. Electron micrographs (Figs 6,7) suggest that, just as swelling is due partly to decondensation of the matrix, possibly through 'salting in', so stabilization of shape is due to condensation of acrosomal matrix proteins following loss of intra-acrosomal ammonium acetate, possibly through a 'salting-out' effect. It is common to find plasma membrane within the crevices of these complex outlines, consistent with the view that the cytoplasm remains an osmotically active compartment and shrinks onto the acrosomal membrane before the acrosome itself shrinks substantially (Figs 6,7). This suggests that the cell remains an osmometer, even after swelling. This is consistent with the ability to exclude PI which the majority of sperm show, even when swollen, and their ability after shrinking to undergo an A23187-induced acrosome reaction (Fig. 8). If membrane fusion does require osmotic coupling of matrix and acrosomal membrane, then clearly the mechanism must survive the extensive decondensation of the matrix which ammonium acetate causes. Although this might seem a remote possibility, it cannot entirely be ruled out. The results, as they stand, however, are entirely consistent with the model for exocytotic membrane fusion in which fusion precedes any granule matrix swelling (Green, 1978).

It is now clear that a single common mechanism could account for the main features of exocytosis in those cells whose structure has lent itself to analysis of some aspect of the problem of exocytosis (guinea pig sperm (Green, 1978, 1982), amebocytes (Ornberg and Reese, 1981), mast cells (Breckenridge and Almers, 1987; Zimmerman et al. 1987) and sea urchin eggs (Whitaker and Zimmerberg, 1987; Chandler et al. 1989; Merkle and Chandler, 1989; Almers, 1990)). Exocytotic membrane fusion occurs through some mechanism which is independent of the granule core, possibly through a fusion protein (Almers, 1990). When it occurs, the granule core expands, and the fusion pore enlarges. Expansion and dispersal of the granule contents take place subsequently by a number of mechanisms which are sui generis for each granule type but which include the effects of specific ions (e.g. Ca 2+), ionic strength, colloid osmotic pressure, proteolysis, etc. Some uncertainties still remain nevertheless. For example, it remains unclear whether it is core expansion which dilates fusion pores and renders them more patent. Recent evidence from mast cells has been taken to suggest not (Monck et al. 1991). However, in those experiments, some granule core swelling took place, and as with earlier work on mast cells (Breckenridge and Almers, 1987; Zimmerman et al. 1987), the possibility of local swelling at the fusion site was not considered. In sea urchin eggs, osmoticators of low Mf inhibit both fusion pore dilation and dispersal of the core contents (Merkle and Chandler, 1989). Evidence suggests that the effect of hyperosmotic haline was either to halt granule matrix dispersal or to suppress the cytoskeletal rearrangement which makes the dilation possible (Merkle and Chandler, 1989). Why only osmoticators of low Mf should produce this effect was not explained. Both in this paper (Fig. 5) and elsewhere (Green, 1990), evidence suggests that mechanical coupling of the granule membrane to the granule core occurs, at least in some secretory cells. If that is the case, then failure of a fusion pore to dilate could well follow mechanical stabilization of the granule matrix. This would happen through exclusion of solute in the case of
solute of high Mr, where partial stabilization occurs (Green, 1982; Whitaker and Zimmerberg, 1987; Chandler et al. 1989), or possibly ‘salting out’ of the granule matrix for solutes such as sodium sulphate (Merkle and Chandler, 1989). In other words, fusion pore dilation may well be dependent, in some cells, on local behaviour of the granule matrix at the site of the pore, and is made possible either because the matrix itself expands locally immediately after initial pore formation, or mechanical coupling of granule membrane to granule core is severed, for example, by proteolysis.

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References


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