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Authors

Mrsny, RJ
Meizel, S

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Potassium Ion Influx and Na^+,K^+-ATPase Activity Are Required for the Hamster Sperm Acrosome Reaction

RANDALL J. MRSNY and STANLEY MEIZEL
Department of Human Anatomy, School of Medicine, University of California, Davis, California 95616

ABSTRACT The role of a K^+ ion influx and Na^+,K^+-ATPase activity in the hamster sperm acrosome reaction (AR) was examined, using a range of concentrations of K^+,K^+ ionophores and a Na^+,K^+-ATPase inhibitor. Washed epididymal hamster sperm, capacitated in vitro in an artificial medium containing 2 mM Ca^{2+}, 147 mM Na^+, and 3, 6, 12, 18, or 24 mM K^+, began undergoing the AR after 3 h of incubation. Sperm incubated in low K^+ (0.9 mM) failed to undergo the AR even after 5 h of incubation. Sperm in 0.9 mM K^+ could be induced to undergo the AR when either K^+ (12 mM) alone or K^+ (12 mM) with 0.1 μM nigericin was added after 3.5 h of incubation. The addition of K^+ alone stimulated the AR in 30 min, whereas nigericin plus K^+ stimulated the AR 15 min after addition. Neither nigericin added alone (0.9 mM K^+) nor nigericin plus 12 mM K^+ added to a low Ca^{2+} (0.35 mM) system resulted in acrosome reactions. Valinomycin (1 nM) did not stimulate the AR when added together with K^+ (3–24 mM) to sperm incubated in 0.9 mM K^+ for 3.5 h but markedly decreased sperm motility. Micromolar levels of ouabain blocked the AR when added between t = 0—3 h to sperm incubated with 3–24 mM K^+. Inhibition of AR by the addition of 1 μM ouabain to sperm incubated with 3 mM K^+ was completely reversed by the addition of 0.1 μM nigericin at t = 3.5 h. These results suggest that Na^+,K^+-ATPase activity and the resulting K^+ influx are important for the mammalian sperm AR. Some similarities between requirements for the hamster sperm AR and secretory granule exocytosis are discussed.

The mammalian sperm acrosome, a membrane-bounded organelle in the sperm head, is derived from vesicles formed in the Golgi apparatus, as are primary lysosomes and secretory granules of somatic cells (8). Biochemical analysis of the acrosomal enzyme content has led to the acrosome being called a "specialized" lysosome (1), with the acrosome's pH being acid, like that of the lysosome (21). However, ultrastructural cytochemical studies have also led to the acrosome being described as more similar to a zymogen granule (9), and in fact the acrosome does contain a zymogen of the enzyme acrosin (19).

The mammalian acrosome reaction (AR), a fusion and vesiculation of the outer acrosomal membrane and overlying plasma membrane, is essential for fertilization in vivo and in vitro and requires prior changes in the sperm, collectively called capacitation (3, 20). The molecular mechanisms of these events, including the role of ions, are not yet fully understood (3, 20). However, at least one divalent cation, Ca^{2+}, is required for the AR (42). An increase in the ratio of K^+ to Na^+ in the incubation medium has been shown to stimulate rat fertilization in vitro (37), but whether this effect was on sperm capacitation and/or the AR was not known. The requirement for Ca^{2+} in the stimulus-secretion of zymogen granules (15) and other membrane fusion events (29) is well established. A stimulatory role for K^+ (possibly due to membrane depolarization) has been suggested in the secretion of neurotransmitters, hormones, and enzymes (32).

Capacitation and the AR of hamster sperm can be obtained in vitro by incubating sperm for several hours in a relatively defined medium (22, 27). Here we used in vitro capacitating conditions to demonstrate for the first time that a K^+ influx and Na^+,K^+-ATPase activity are essential for the hamster sperm AR. Part of these results has been published in preliminary form (25, 26).

MATERIALS AND METHODS

Sperm Incubation

Caudal epididymal sperm were obtained from mature male golden hamsters maintained for at least 3 wk on a 14 h light:10 h dark cycle. The sperm were washed with a phosphate-buffered saline (PBS)–sucrose medium as described previously (22), except that no penicillin was present and the PBS was slightly modified to contain the following: NaCl (143.7 mM), KCl (3.09 mM), Na_2HPO_4

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(6.34 mM), KH_2PO_4 (1.47 mM), $CaCl_2 \cdot 2H_2O$ (0.93 mM), and $MgCl_2 \cdot 6H_2O$ (0.49 mM). A 95% motile sperm suspension was prepared by passing the washed sperm through glass bead columns (17). Sperm were diluted with a modified Tyrode's eluting solution containing albumin and metabolites (TAM) as previously described (22), except that the respective concentrations of glucose, lactate, and pyruvate in the TAM were 5 mM, 6.25 mM, and 0.125 mM, before addition to the incubation medium. The final incubation suspension contained 12 mg/ml bovine serum albumin, purified by TCA precipitation and ethanol resolubilization (16, 23), 0.5 mM hypotaurine and 50 μM (−)-epinephrine. No penicillin was used in the medium in these experiments and the final concentration of sperm in the 100-μl (total volume) incubation was 2.63×10^6/ml. Incubations (pH 7.4–7.6) were carried out at 37°C in a humidified 5% CO_2/95% air atmosphere and in 1.5-ml polypropylene tubes (27). In these experiments, all solutions were passed through nucleopore filters (0.45 μm) before use.

Sperm Incubation in Low K^+ or Low Ca^{2+} Media

In low Ca^{2+} incubations, sperm were washed and treated as described above, except that all solutions were Ca^{2+} free (with the exception of the wash media). The final $[Ca^{2+}]$ (extracellular Ca^{2+} concentration) in the incubation suspension using these Ca^{2+}-free solutions was calculated to be ~0.35 mM. The $[K^+]_o$ (extracellular K^+ concentration) of these incubations was 12 mM, added at t = 0 h. For low K^+ incubations, sperm were washed and treated as described above, except that all solutions were K^+ free (potassium salts were replaced with sodium salts to maintain osmolality at 280–290 mOsm/kg H_2O).

Extracellular K^+ Concentration

The $[K^+]_o$ in sperm incubations prepared using K^+-free media was 0.9 mM K^+. This K^+ was assumed to come from the sperm. Addition of 3 mM K^+, for example, produced a $[K^+]_o$ of 3 mM K^+ and not 3.9 mM, suggesting that 0.9 mM $[K^+]_o$ was taken up by the sperm in the same manner in which it was released. All K^+ ion concentrations were determined in sperm-free solutions (sperm were pelleted with a Beckman microfuge; Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) with a Flame Photometer model 343 from Instrumentation Laboratory, Inc. (Lexington, Mass.). Leakage of K^+ from pH electrodes into K^+-free media occurred after even a short duration. Therefore, during the pH adjustment of media, small aliquots were removed for actual pH determination and discarded.

Addition of Ions, Ionophores, and Ouabain

Incubation tubes had 90 or 95 μl as the initial volume of incubation (the volumes were the same for all tubes throughout a particular type of experiment). At t = 0 h or after incubation for either 3 or 3.5 h (t = 3 or 3.5 h), tubes were briefly removed from the incubator and 5 μl of stock ion, and/or ionophore or ouabain solutions were added. After all additions in a particular experiment, the final volume was always 100 μl. Additions were made of compounds dissolved initially in modified Tyrode's (ouabain, K^+, Ca^{2+}) or absolute ethanol (nigericin, valinomycin) and then diluted further in modified Tyrode's of the proper K^+,Ca^{2+} or ethanol content.

Sperm Preparation for Ultrastructural Analysis

Nigericin-treated sperm were analyzed by transmission electron microscopy for the presence of the morphological criteria of the "physiological" AR (3, 9, 20). Hamster sperm were incubated in 3 mM K^+ for 3 h; ouabain (1 μM) was then added and, after 0.5-h further incubation, nigericin (0.1 μM) was added. 25 min after the addition of nigericin, the time at which 50–60% of the sperm are acrosome-reacted, the sperm suspension was centrifuged at 1,500 *g* for 5 min at room temperature and the supernate was discarded. The sperm were resuspended in a tannic acid-glutaraldehyde fixative prepared according to Mizuhira and Futaesaku (24). Sperm were fixed for 1 h at room temperature, collected by centrifugation as described above, and exposed to fresh fixative for 15 min. The fixed sperm were then washed in 0.05 M sodium phosphate buffer, pH 7.4.

The remaining preparation of the sperm was perfórmed by this school's electron microscopic facility. The sperm pellet was resuspended in 7% agar at 44°C for 1 h. The agar was cooled to room temperature and diced into 1-mm cubes which were rinsed in buffer and then water before being stained *en bloc* with 2% aqueous uranyl acetate for 10 min. Samples were dehydrated in a series of graded acetone solutions and embedded in Spur's resin (35). Ultrathin sections were stained in 4% uranyl acetate (5–7 min) and Reynolds' (30) lead citrate (5 min.). Sections were viewed in a Philips EM-400 electron microscope.

Assays of Sperm and Statistical Analysis

The percentage of acrosome reactions in at least 100 strongly motile sperm and the subjective estimation of the percentages of sperm motility and activation (whiplash-like flagellar movement characteristic of capacitated hamster sperm) were determined by phase-contrast microscopy (22). Previously described criteria (40, 41) were used for identification of activation and acrosome reactions. Data were analyzed using a chi-square distribution, after being organized into 2 × 2 contengency tables, and chi-square values were calculated for each pair of data points (6). These values were added together to obtain an overall chi-square value for comparison of each pair of experimental curves. The Yates' correction for continuity was applied to insure that conservative conclusions would be drawn. Statistics were not done for motility or activation results due to the subjective nature of these estimations.

Chemicals Used

Pyruvic acid sodium salt, L-(+)-lactic acid (30%), D-(+)-glucose, L-(−)-epinephrine, glutaraldehyde, valinomycin, and ouabain octahydrate were purchased from Sigma Chemical Co. (St. Louis, Mo.). Sucrose, tannic acid, and trichloroacetic acid were obtained from Mallinckrodt. Hypotaurine was purchased from Calbiochem-Behring Corp., American Hoechst Corp. (San Diego, Calif.). Nigericin sodium salt was obtained from Eli Lilly and Co. (Indianapolis, Ind.). Pentex fraction V BSA was obtained from Miles Laboratories (Elkhart, Ind.).

RESULTS

Sperm motility ranged from 80 to 90% in all experiments and remained constant throughout. The intensity of motility did not appear to be altered from controls by any of the concentrations of experimental compounds or K^+ used except where noted. High percentages (70–90%) of activation, a whiplashlike flagellar movements characteristic of capacitated sperm of several species (20), including hamster (41), were observed in all experiments. Sperm also remained strongly motile for at least 45 min after a high percentage of AR had occurred.

$[K^+]_o$ Requirement for the Acrosome Reaction

Sperm incubated in K^+-low (0.9 mM) media did not undergo the AR (Fig. 1), even after 5 h of incubation. However, AR did occur when K^+ was added to the sperm incubation suspensions at t = 0 h to produce final concentrations of 3, 6, and 24 mM K^+ (Fig. 1). Percentages of AR obtained with 12 and 18 mM K^+ were not statistically different from those of 6 and 24 mM (data not shown). Percentages of AR obtained with 6 mM K^+ were greater than those obtained with 3 mM K^+ at a significant level of <0.01.

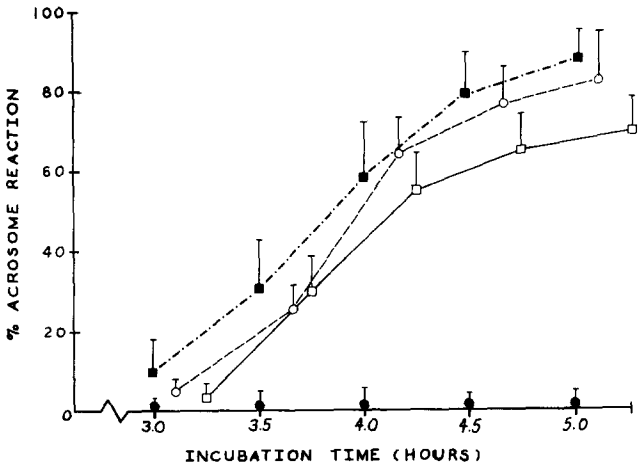

FIGURE 1 The effect of K^+ ion concentration on the hamster sperm AR. Sperm incubation suspensions containing 0.9 mM K^+ were prepared. Tyrode's buffer containing various K^+ concentrations was added at t = 0 h to produce the following final concentrations: 24 mM K^+ (■); 6.0 mM K^+ (○); 3.0 mM K^+ (□); and 0.9 mM K^+ (●); and *n* = 3. Each point is the mean ± SEM of percent sperm with acrosome reactions.

Effect of Ionophores on the Acrosome Reaction

Sperm incubated in 0.9 mM K^+ could also be induced to undergo the AR (Fig. 2) when K^+ (12 mM) was added at t = 3.5 h, either alone or together with 0.1 μM nigericin, a monovalent ionophore known to exchange K^+ or Na^+ for H^+ across membranes (14). Transmission electron microscopy was used to examine the morphology of sperm after acrosome reaction induction by nigericin. Many sperm were observed undergoing organized fusion and vesiculation of sperm head membranes with fusion of the remaining outer acrosomal membrane and plasma membrane at the equatorial segment (Fig. 3), events characteristic of the "true" physiological acrosome reaction (3, 9, 20). Vesiculation and loss of plasma membrane, particularly in the region over the equatorial segment, was observed when sperm were incubated with nigericin for up to an hour beyond the time of maximal stimulation of the AR (Fig. 4).

The lag phase for the increase in AR detected by phase-contrast microscopy was ~30 min when K^+ was added alone, while addition of K^+ with nigericin increased AR in only 15 min (Fig. 2). There was no stimulation of the AR by the addition of nigericin alone in the presence of constant low (0.9 mM) K^+, even though a high concentration at Na^+ (130 mM) was present (Fig. 2). Similar results (data not shown) were obtained when either 3 or 24 mM was used in experiments identical to those of Fig. 2 using 12 mM K^+. In fact, values of AR stimulation by late addition of 3, 12, or 24 mM K^+ were indistinguishable by the methods of statistical analysis employed. In the presence of 12 mM K^+, but only 0.35 mM Ca^{2+}, sperm did not undergo the AR even after addition of 0.1 μM nigericin at t = 3.5 h. However, sperm did undergo the AR in 15 min in the presence of 12 mM K^+ when Ca^{2+} (2 mM final concentration) was added along with 0.1 M nigericin at t = 3.5 h (data not shown).

Valinomycin, an electrogenic K^+ ionophore (2, 18), was also tested (data not shown). The experimental procedures used were identical to those of nigericin (see legend of Fig. 2), except that the final valinomycin concentration was 1 nM. Valinomycin (1 nM) added to sperm incubations containing 0.9 mM K^+ had no effect on the motility, activation, or AR of the sperm. Addition of 1 nM valinomycin together with K^+ (3–24

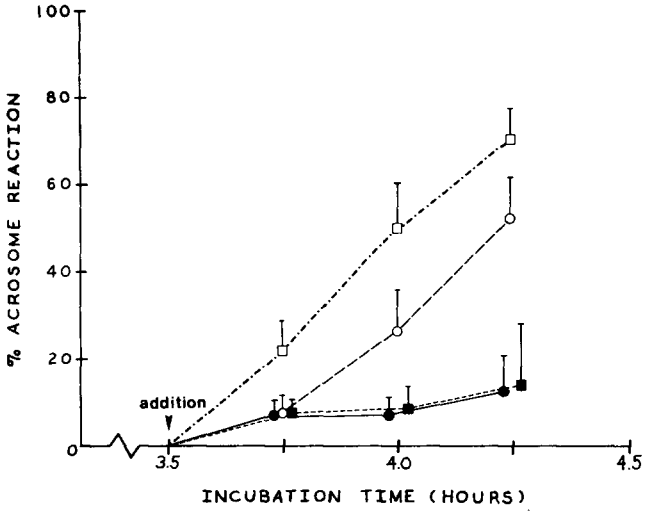

FIGURE 2 The effect of nigericin on the hamster sperm AR. Sperm incubation suspensions containing 0.9 mM K^+ were prepared. Nigericin was added at t = 3.5 h (solubilized in absolute ethanol to a concentration of 1.0 mM and diluted with Tyrode's buffer containing either 240 mM K^+ or no K^+). The final concentration of nigericin was 0.1 μM, of ethanol, 0.01%, plus either 12 mM (□) or 0.9 mM (■) of K^+. As a control, absolute ethanol was diluted with Tyrode's buffer as described above for nigericin and added to incubation suspensions, producing final K^+ concentrations of 12 mM (○) or 0.9 mM (●) and a final ethanol concentration of 0.01%; $n = 4$.

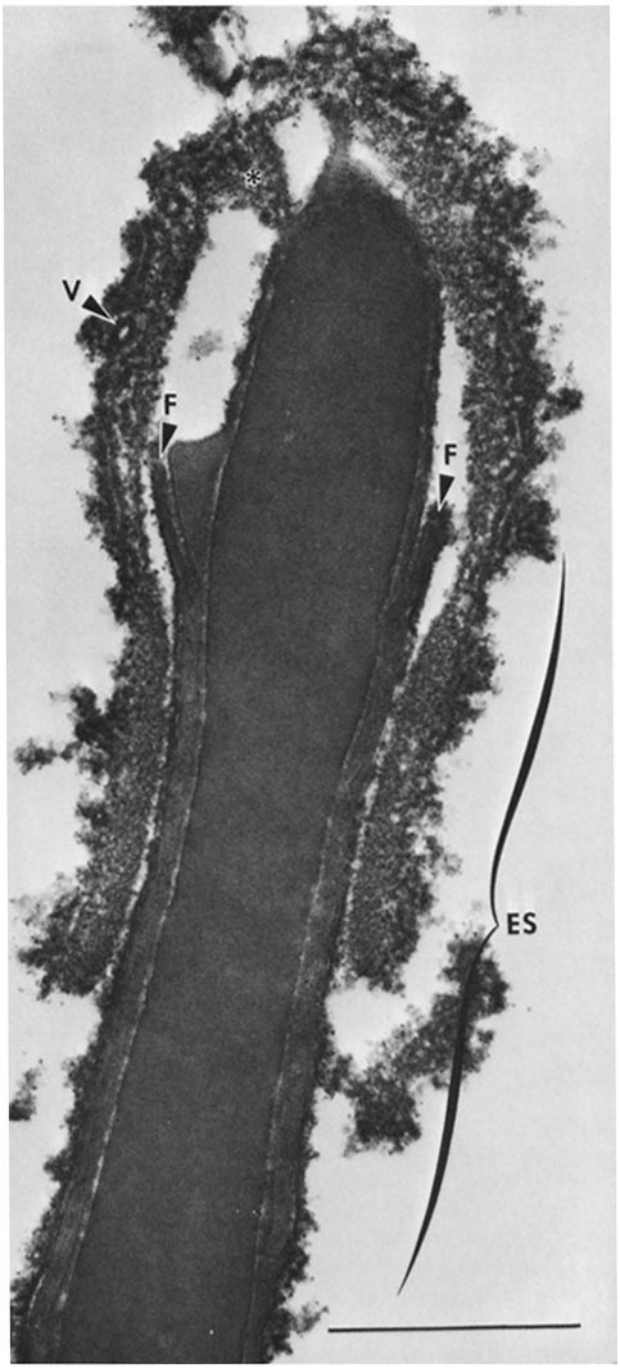

FIGURE 3 An early stage of the hamster sperm acrosome reaction induced by nigericin. Sperm were fixed 25 min after the addition of 0.1 μM nigericin to sperm incubated in the presence of 1 μM ouabain and 3 mM K^+. Vesicles, presumably of plasma membrane fused with outer acrosomal membrane, are present (arrowhead-V), as well as some remaining acrosomal matrix (asterisk). Fusion between remaining outer acrosomal membrane and overlying plasma membrane (arrowhead-F) at the anterior of the equatorial segment region (bracket-*ES*) demonstrates continuity of these membranes obligatory for the "true" physiological acrosome reaction. Bar, 0.5 μm. × 66,000.

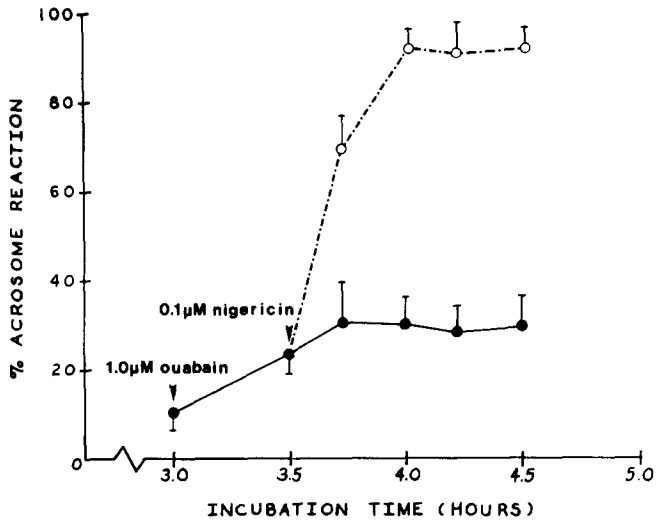

FIGURE 4 Reversal of ouabain inhibition of the AR by nigericin addition. Sperm incubation suspensions containing 3 mM K^+ were prepared. Ouabain was diluted with Tyrode's solution containing 3 mM K^+ and added to the sperm incubation suspension at t = 3.0 h to produce a final concentration of 1.0 μM. Nigericin stock solution was prepared as described in the legend for Fig. 2 except the Tyrode's solution contained 3.0 mM K^+. Nigericin was added at t = 3.5 h to produce a final concentration of 0.1 μM (○). Control additions (●) consisted of Tyrode's solution containing 3.0 mM K^+ and an amount of ethanol (0.01%) equivalent to that in the nigericin additions. $n = 4$.

mM) retarded sperm motility and activation in a dose-dependent fashion as $[K^+]_o$ was increased. There was no stimulation of AR among those sperm remaining strongly motile (the only sperm examined for AR). Higher concentrations of valinomycin (5 μM) inhibited sperm motility in the absence or presence of K^+ concentrations >0.9 mM.

Ouabain Inhibition of the Acrosome Reaction

Ouabain, the classical Na^+,K^+-ATPase inhibitor (10), blocked the AR when added at micromolar concentrations at t = 0 h and t = 2.5 h (data not shown), or t = 3 h (Fig. 4). Addition of ouabain to sperm incubated for >3 h (data not shown) resulted in only slight inhibition of AR (e.g., 10% inhibition). Concentrations of ouabain >5 μM negatively affected both sperm motility and activation. In these experiments, the addition of 1 μM ouabain to sperm incubated for 3 h completely inhibited the AR normally stimulated by 3 mM K^+. This inhibition was markedly reversed in 15 min by the addition of 0.1 μM nigericin at t = 3.5 h (Fig. 4).

DISCUSSION

This report presents evidence that suggests that some component or components of the membrane fusion and vesiculation in hamster sperm AR in vitro requires an influx of external K^+, a condition previously shown for secretion responses (i.e., exocytosis) in other cells (15). In these experiments, a $[K^+]_o$ of 0.9 mM did not stimulate the AR, but K^+ concentrations from 3 to 24 mM added at t = 0 h did stimulate over a time period of 3 to 5 h after addition (Fig. 1). Hamster sperm AR were stimulated more by 6 mM than by 3 mM $[K^+]_o$ added at t = 0 h, while higher concentrations of K^+ did not stimulate the AR above values obtained from the addition of 6 mM (Fig. 1). However, addition of K^+ at t = 3.5 h of incubation to produce final concentrations of 12 mM (Fig. 2), or 3 or 24 mM (data not shown), caused stimulation of the AR to the same extent at 30–45 min after addition. To explain these differences between the effects of various $[K^+]_o$ added at either t = 0 or t = 3.5 h, we suggest that higher $[K^+]_o$ may have a role in capacitation as well as the AR of hamster sperm. In this regard, it must be pointed out that in both instances (addition of K^+ at t = 0 or 3.5 h) capacitation and stimulation of the sperm AR were not synchronous. While differences in stimulation of the AR by 3 and 6 mM K^+ added at t = 0 were observed over a 2-h period (Fig. 1), the shorter period of assaying (30–45 min after addition) sperm with K^+ added at t = 3.5 h (Fig. 2) may not have been a long enough period of time to allow detection of differences in capacitation and stimulation of AR induced by various K^+ concentrations (i.e., < 0.9 mM).

Nigericin, a monovalent ionophore known to exchange K^+ or Na^+ or H^+ across membranes (14), showed K^+ selectivity over Na^+ in stimulating the AR (Fig. 2). This stimulation, as with the exocytosis of somatic cell secretion granules (31), also required the presence of Ca^{2+}. Nigericin, in the presence of K^+, resulted in AR only 15 min after addition (Fig. 2), a result which is within the time frame of 10–20 min previously described for the hamster sperm AR (20, 36). Ultrastructural analysis of sperm after AR induction by nigericin revealed that many sperm displayed the morphological criteria characteristic of "true" physiological AR, including fusion of the remaining outer acrosomal membrane and plasma membrane at the equatorial segment (Fig. 3). The vesiculation of plasma membrane at the equatorial segment, observed with periods of exposure to nigericin beyond the time of maximal AR might be indicative of its labile nature. That region of plasma membrane has been described as the site of initial membrane fusion between the sperm and egg plasma membranes during mammalian fertilization (3).

Valinomycin, an electrogenic K^+ ionophore (2), decreased the number of motile sperm (to 30–40%), the intensity of motility, and the percentage of activated sperm. No stimulation of the AR was observed by the addition of valinomycin, which is capable of inducing both transmembrane K^+ movement down the electrochemical gradient for that ion and depolarization of membrane potential (2, 14). Membrane depolarization has been suggested as a possible trigger (7) or as one event, but not a critical step (32), in the stimulus-secretion coupling of the exocrine pancreas. Depolarization of membrane potential could account for the deleterious effects of valinomycin on hamster sperm because normal membrane potential is important for mammalian sperm motility (18). It is also possible that those hamster sperm which might have been stimulated by valinomycin to undergo the AR could have become immotile immediately following the loss of the acrosome and would not have been detected in our assay of only motile sperm. However, the inability of valinomycin to stimulate the AR in the remaining 30–40% motile cells does not support this idea. These results could also be explained by the possibility that a K^+/H^+ exchange (such as would be induced by nigericin) is required and not simply a movement of K^+ and loss of membrane potential (as would be induced by valinomycin). This putative exchange, possibly across the acrosomal membrane, could act to increase the pH of the acidic acrosome (21).

It was noted in our discussion that t = 0 h additions of various $[K^+]_o$ showed dose-dependent stimulation of the AR (Fig. 1) with maximal stimulation at K^+ of ≥6 mM. These results suggest the involvement of a K^+ transport mechanism,

with a maximum velocity, in the stimulation of the hamster sperm AR. Interestingly, the K^+ transport of the plasma membrane (Na^+,K^+–ATPase) has been shown to be saturated by [K^+] between 5 and 10 mM (10). We also noted previously that later addition of K^+ required 30 min to stimulate the AR, while late addition of K^+ plus nigericin stimulated in only 15 min (Fig. 2). This difference can be explained by the ability of nigericin to by-pass the Na^+,K^+–ATPase-regulated K^+ movement, thus allowing for a more rapid rate of K^+ influx and causing the faster stimulation of the AR. Taken together, the results of these experiments suggest a role for Na^+,K^+–ATPase in the capacitation and/or the AR of hamster sperm.

Na^+,K^+–ATPase activity has been demonstrated in mammalian sperm (38). The fact that ouabain, a potent and specific Na^+,K^+–ATPase inhibitor (10), inhibited the hamster sperm AR (Fig. 4) certainly supports the involvement of that enzyme. Ouabain has been reported to decrease intracellular [K^+] of bull sperm (18) and inhibit pancreatic zymogen secretion (15). While previous studies have shown that 5 μM ouabain saturates most of the transport sites in bull sperm at a concentration of 5×10^8/ml (28), our experiments used 1 μM ouabain at a sperm concentration of 2.63×10^6/ml. Therefore, our ouabain concentration per sperm cell was 38 times higher than those levels used with bull sperm, suggesting that 1 μM ouabain is inhibiting most or all of the Na^+,K^+–ATPase of the hamster sperm. Higher concentrations of ouabain produced inhibition of both motility and activation of hamster sperm. Ouabain, at similar high concentrations, has been shown to inhibit bull sperm motility, apparently due to a loss of membrane potential (18). It is of interest that ouabain added to hamster sperm previously incubated for >3.0 with 3 mM K^+ did not inhibit the AR as much as earlier additions (data not shown). This may have been due to a time requirement for ouabain binding at 1 μM or to a sufficient intracellular K^+ concentration having already accumulated during capacitation.

In our report, the fact that nigericin addition reversed ouabain inhibition (Fig. 4) strongly suggests that a K^+ influx is the important step blocked by ouabain. We cannot rule out the possibility that nigericin may produce ionphore effects at both the plasma membrane and the outer acrosomal membrane but assume that its initial action for K^+ influx occurs at the plasma membrane. These assumptions are based on the following: the AR was inhibited by ouabain which is known to bind to external plasma membrane Na^+,K^+–ATPase sites in all cells; nigericin overcomes the inhibition of AR produced by ouabain; and localization of Na^+,K^+–ATPase on the outer acrosomal membrane has not yet been demonstrated. Another explanation of our results might be that the lack of the AR in low K^+ is due to a requirement of K^+ in some energy-producing step essential for the AR, but this explanation seems unlikely since the motility and activation (which also require energy) of the hamster sperm were unaffected when incubated in 0.9 mM K^+ or when 1.0 μM ouabain was added.

Cytochemical studies have demonstrated ouabain-sensitive ATPase activity in the periacrosomal plasma membrane of capacitated, but not of noncapacitated, rabbit sperm (11). Surprisingly, the possibility that this rabbit sperm ATPase activity was a Na^+,K^+–ATPase was not discussed. A stimulatory effect of increased K^+/Na^+ ratios on fertilization rates of rat gametes in vitro has been observed, but whether the effect was on acrosome reaction or capacitation was not determined (35). Our results suggest that K^+ may be involved in both capacitation and the AR of hamster sperm.

The effect of K^+ movements on the AR of invertebrate sperm appear to be quite different than the effect on the mammalian sperm AR reported here. The sea urchin sperm was inhibited when $[K^+]_0$ was increased from 10 mM up to 20 mM and induction of the AR by 55 μM nigericin occurred with either Na^+ or K^+ as the predominent external monovalent cation (31). It was further suggested that a Na^+–H^+ exchange precedes a K^+ efflux in the sea urchin sperm AR (34). Though the hamster sperm AR appears to require a K^+ specific influx, the possibility of a K^+ influx followed by a very rapid K^+ efflux cannot be ruled out by our data. However, in this regard, ouabain, which should promote K^+ efflux (10, 18), inhibited rather than stimulated the hamster sperm AR.

The oviduct is the in vivo site of mammalian sperm capacitation and AR (3, 20). The levels of K^+ found in mammalian oviductal fluid are several times higher than that of blood serum (e.g., an average of 21.1 mM K^+ for human oviductal fluid while human serum contains 3.7–5.0 mM K^+ [4]). Perhaps the higher [K^+] found in the oviduct is in some way necessary for the AR and/or capacitation in vivo, but at least under our in vitro conditions such high levels are not required for the hamster sperm AR.

The results presented emphasize some similarities between events of secretory granule exocytosis and the mammalian sperm AR. Both require the obligatory presence of Ca^{2+}, appear to be stimulated by a K^+ influx and appear to require active Na^+,K^+–ATPase.

Given our results, it is of interest that: (*a*) catecholamines, which stimulate the hamster sperm AR *in vitro* by hormonal mechanisms and possibly other mechanisms, such as chelation (5, 23), can also stimulate Na^+,K^+–ATPase activity of several other cell types by chelation (12, 13) or receptor-mediated mechanisms (33); and (*b*) taurine, which can stimulate hamster sperm motility, capacitation and AR in vitro (22, 27) has been shown to be involved in the maintenance of [K^+] in heart cells (39). Possible interrelationships between these molecules and the effect of K^+ on the AR are currently under investigation in this laboratory, as are possible mechanisms of K^+ action such as cotransport of H^+.

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