

Intracellular Sodium Activity in the Sea Urchin Egg during Fertilization

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ABSTRACT Fertilization of the sea urchin egg triggers a sequence of events that are necessary for metabolic derepression and stimulation of proliferation. Changes in intracellular Ca^{2+} and H^+ activities regulate the sequence of events. Intracellular sodium activity is important in the regulation of the intracellular activities of these ions and may directly regulate metabolic events. Using Na^+ -sensitive microelectrodes we continuously measured the intracellular Na^+ activity during fertilization. The results show an increase in intracellular sodium activity mediated by two pathways of Na^+ entry: Na^+ permeability increase during the fertilization potential and initiation of Na^+ - H^+ exchange activity. Intracellular Na^+ activity returned to unfertilized levels by 20 min after fertilization. This decrease was inhibited by ouabain, which suggests the activation of Na^+ , K^+ ATPase during fertilization.

Fertilization of the sea urchin egg initiates a sequence of morphologic and biochemical events necessary for its conversion from a quiescent to a proliferous state. An increase in intracellular pH is a major event that regulates the biosynthetic events during fertilization (48). The postulated ionic mechanism for cytoplasmic alkalinization is activation of an exchange between extracellular Na^+ and intracellular H^+ (16). Acid release during fertilization has been reported by a number of investigators (38). The transport of H^+ equivalents does not require K^+ , Ca^{2+} , or Mg^{2+} (26), but does require Na^+ (16, 22). The increase in intracellular pH during fertilization is also Na^+ dependent (16, 40). Further evidence of a Na^+ - H^+ exchange mechanism is the sensitivity of both acid efflux and cytoplasmic alkalinization to amiloride (16, 40). Although acid production and the increase in internal pH during fertilization are both Na^+ dependent and amiloride sensitive, acid release and cytoplasmic alkalinization may not be directly coupled in a 1:1, simple mechanism for the following reasons. (a) The rate of acid production is linearly dependent on external Na^+ concentration, whereas the rate of cytoplasmic alkalinization is independent of Na^+ concentration above the minimal concentration necessary for egg activation (16, 40). (b) Eggs activated with 10 mM NH_4Cl and then washed in fresh seawater release acid upon fertilization (27). Fertilization of ammonia-pulsed eggs does not induce further cytoplasmic alkalinization (37). (c) Acid efflux initiates 30–60-s after fertilization and is completed by 2 to 4 min (2, 22, 47). The rise in intracellular pH starts 60 to 90 s after fertilization and is completed by 6 to 8 min (15, 46). (d) Amiloride at 0.1 mM

in 40 mM Na^+ artificial seawater (ASW)¹ blocked acid release (16); however, at similar concentrations, cytoplasmic alkalinization was unchanged (40). An alternative proposal to link acid production with cytoplasmic alkalinization is an allosteric role for Na^+ . The binding of Na^+ to sites on the plasma membrane is supposed to be regulatory and required for activation of the mechanism directly responsible for the rise in internal pH (40). Since the internal buffering power of the egg is nearly 40 mEq/pH unit (29), the kinetics of acid production might appear to be faster than the kinetics of intracellular alkalinization. One method to resolve the uncertainty is the direct measure of Na^+ activity during fertilization.

A critical role for ion movements in the control of mammalian cell metabolism and proliferation is becoming increasingly evident. Among the earliest events in growth factor stimulation of cellular proliferation is depolarization of the plasma membrane, due to an increase in Na^+ permeability (13, 20, 31). This increase in Na^+ influx may lead to increased cellular Na^+ content (34, 42), which may be of mitogenic importance (8, 9). In addition, the increase in Na^+ influx may be closely linked to activation of the Na^+ , K^+ ATPase (17, 35, 42). More recently a second pathway of Na^+ entry has been proposed, that of the Na^+ - H^+ exchange (21, 25, 32, 36). Although these results suggest the importance of changes in intracellular Na^+ activity during proliferation, no direct measurement of intracellular Na^+ activity during cellular activa-

¹ Abbreviations used in this paper: ASW, artificial seawater; a_{Na^+} , intracellular Na^+ activity; E_m , membrane potential.

tion has been reported.

A similar sequence of ion movements has been documented during fertilization of the sea urchin egg. The earliest measurable response to fertilization is membrane depolarization or the fertilization potential (12), which results in part from an increase in Na^+ permeability of the egg membrane (14, 44). The resultant Na^+ influx elevates the Na^+ content by 20 to 30% (4, 28) and activation of Na^+ , K^+ ATPase during fertilization has been reported (5, 11). Furthermore, a fraction of the Na^+ entry may be coupled to H^+ efflux via Na^+ - H^+ exchange (11, 16, 29). In this study we measured intracellular Na^+ activity (a_{Na}^i) with Na^+ -sensitive microelectrodes during fertilization. We found an elevation of Na^+ activity by both Na^+ influx during the fertilization potential and activation of Na^+ - H^+ exchange. We present further evidence for a metabolic energy dependent, internal pH regulator in fertilized eggs. By 20 min after fertilization, Na^+ activity returned to unfertilized levels by an ouabain-sensitive mechanism.

MATERIALS AND METHODS

Gametes and Solutions: *Lytechinus pictus* were purchased from Pacific Bio-Marine Laboratories Inc. (Venice, CA) and maintained in Instant Ocean culture systems (Aquarium Systems, Mentor, OH) with biweekly feedings of the *Macrocyctis* algae. Eggs and sperm were obtained by injection of 0.5 M KCl into the coelomic cavity. The sperm were collected dry and stored at 4°C. The jelly coats were removed from the eggs by passage of the eggs through fine mesh silk, and then the eggs were washed twice in ASW. The deejellied eggs were maintained at 16 to 18°C and constantly stirred at 60 rpm. All experiments used eggs within 4 h of shedding and were done in ASW of the following composition (millimolar): NaCl, 470; KCl, 10; CaCl_2 , 11; MgSO_4 , 29; MgCl_2 , 27; NaHCO_3 , 2.5; pH 8, with 0.1 M NaOH or 0.1 M HCl. Low Na^+ (25 mM) ASW had the following composition (millimolar): NaCl, 25; choline Cl, 445; KCl, 7.5; CaCl_2 , 11; MgSO_4 , 29; MgCl_2 , 27; KHCO_3 , 2.5; pH 8 with 0.1 M KOH or 0.1 M HCl. The osmolality of the stock choline Cl (Sigma Chemical Co., St. Louis, MO) was determined with a freezing point osmometer (model 3W; Advanced Instruments, Inc., Needham Heights, MA). Ouabain (Sigma Chemical Co.) and amiloride (Merck, Sharp and Dohme Research Laboratory, Rahway, NJ) were dissolved in 25 mM Na^+ ASW at 1 mM and 0.5 mM, respectively. In the experiments reported here, the solutions were either used straight or diluted with the bath solution 1:1.

Fabrication of Microelectrodes: Conventional microelectrodes for recording membrane potentials were made from 1.5-mm o.d. omega-dot tubing (Glass Co. of America, Inc., Bargaintown, NJ) and filled directly with 3 M KCl. The tip resistances of these electrodes in ASW ranged from 25 to 60 M Ω . Na^+ -sensitive microelectrodes were constructed by the use of techniques similar to those reported by others (30). Pulled pipettes were preheated for 2 h at 200°C in a drying oven, exposed to hexamethyldisilazane (Sigma Chemical Co.) vapor for 20 to 40 min, and baked for 30 min more after the vapor was evacuated. The electrodes were allowed to cool in a desiccator and backfilled with 0.2 μl of Na^+ exchanger (W-P Instruments, Inc., New Haven, CT). The resin-filled electrodes were then backfilled with 0.5 M NaCl reference solution. To minimize the exchanger column length and contact exchanger with reference solution, a second micropipette with a long tip taper was pulled, broken below the shoulder, and inserted into the back of the Na^+ electrode (24). The tip of the insert was positioned to 100 to 200 μm of the tip of the outer microelectrode. The length of the Na^+ exchanger was thus reduced, thus lowering the electrode resistance (5×10^{10} to 2×10^{11} Ω) and greatly improving the electrode performance.

The Na^+ -selective microelectrodes were calibrated in pure NaCl solutions (10 to 500 mM) and in solutions consisting of 10 to 100 mM NaCl with 220 mM KCl. The slopes of the electrodes used in this report varied from 56 to 61 mV/decade. The selectivity of the electrodes was measured by the separate solution method (19) in 0.1 M NaCl, 0.1 M KCl, and 0.1 M choline Cl. The selectivity ratios were 32 to 50 for Na^+ over K^+ , which agreed with those determined by the fixed interference method (19). The selectivity ratios were 250 to 440 for Na^+ over choline. Even with an intracellular K^+ concentration of 220 mM (43), the Na^+ -sensitive microelectrodes were sufficiently sensitive to record 0.1 to 1 mM changes in Na^+ activity in the ranges reported here.

Measurement of Intracellular Na^+ Activities: Eggs were held on poly(lysine)-coated plastic petri dishes (Falcon 1008; Falcon Labware Div.,

Becton-Dickinson & Co., Oxnard, CA). The membrane potential was recorded as the potential difference between the 3 M KCl-filled conventional microelectrode and a 3 M KCl-agar bridge connected by an Ag-AgCl wire to ground with a Biodyne AM-4 preamplifier (Biodyne Electronics, Santa Monica, CA). The Na^+ -sensitive microelectrode was connected by an Ag-AgCl wire to a FD223 electrometer (W-P Instruments, Inc.). The criteria for successful penetration of sea urchin eggs by both microelectrodes have been detailed elsewhere (37). Impalement of eggs with conventional microelectrodes was made by bringing the electrode against the egg membrane, then briefly overturning the negative capacitance compensation. This method of impalement does not work with ion-selective microelectrodes, whose entry into the egg requires a sharp mechanical tapping of the manipulator. To determine that both electrodes are implanted in the egg with minimal damage, current pulses are passed through the KCl electrode periodically and the corresponding voltage deflections are monitored by the ion-selective electrode. Excessive membrane damage or vesiculation at the electrode tips results in a loss of electrical coupling. In some cases, partial egg activation occurred. This could be detected visually by a localized elevation of the fertilization envelope and poor electrical coupling. When partial egg activation occurred, the experiment was terminated. The outputs of the two amplifiers were displayed on a Tektronix 5111 storage oscilloscope (Tektronix, Inc., Beaverton, OR) and stored on magnetic tape with an instrumentation cassette recorder (model C4; A. R. Vetter Co., Rebersburg, PA). Pen recordings were made with a differential chart recorder (model 3314, Soltec Corp., Sun Valley, CA).

Ionic activities of calibrating solutions and ASW were determined from activity coefficients published by Conway (10). The Na^+ activity coefficient in ASW was estimated at 0.68. In all experiments, the Na^+ -sensitive electrode was calibrated both before and after impalement of the egg. Changes in base line or slope were $< \pm 3$ mV for the experiments reported. The intracellular Na^+ electrode potential was electronically corrected for the simultaneously measured membrane potential (E_m). The a_{Na}^i was determined by interpolation of the corrected potential with the calibration curve obtained from varying NaCl with a constant 220 mM KCl (23). All experimental data are expressed as mean \pm standard deviation. Statistical comparisons were carried out by conventional paired-data analysis.

Acid Release during Fertilization: Acid production by sea urchin eggs during fertilization was followed by using a pH-stat system consisting of a Radiometer (Copenhagen) pH meter (PHM 82), titrator (TTT 80), and autoburette (ABU 12). 2-ml aliquots of 0.5% ovicrits in ASW were maintained in suspension with a magnetic "flea." The pH of the egg suspension was allowed to stabilize at 8.00. After insemination with 5 μl of dry sperm, the decrease in the ASW pH was titrated with the addition of 1 mM NaOH. A chart recorder (Radiometer SBR3) plotted the delivery of base as a function of time. The largest pH deviation during clamping to 8.00 was 0.03 pH unit. The calculated acid efflux values were corrected for acid release by the sperm and expressed as mM by: molar acid release from eggs = (volume of base added \times molarity of base/egg volume determined by ovicrit. Acid release by sperm activation was measured by the addition of 5 μl dry sperm to ASW without the eggs.

RESULTS

Intracellular Na^+ Activity during Fertilization in ASW

Unfertilized eggs of *Lytechinus pictus* were placed in ASW and penetrated with both a Na^+ -sensitive microelectrode and a conventional microelectrode. The measured a_{Na}^i was well below electrochemical equilibrium. From seven recordings in ASW, we calculated a mean a_{Na}^i of 23.7 ± 7 mM (range 14 to 34 mM) and a mean sodium equilibrium potential of +66 mV. The E_m of these doubly impaled unfertilized eggs was -10 ± 2.2 mV, which is similar to values previously reported (38, 39). Fig. 1 shows a typical response to fertilization. In this example, a_{Na}^i of the unfertilized egg stabilized at 25.5 mM and began to rise soon after the elevation of the fertilization potential. The apparent fall of a_{Na}^i at $t = 0$ is probably due to the difference in voltage response times of the ion-selective microelectrode and the conventional microelectrode. When the rise time of the fertilization potential was spontaneously lower, no initial decrease in a_{Na}^i was evident. By 12 to 14 min after fertilization, the a_{Na}^i stabilized at a new elevated level. In Fig. 1, the peak a_{Na}^i was 47 mM and from five fertilization

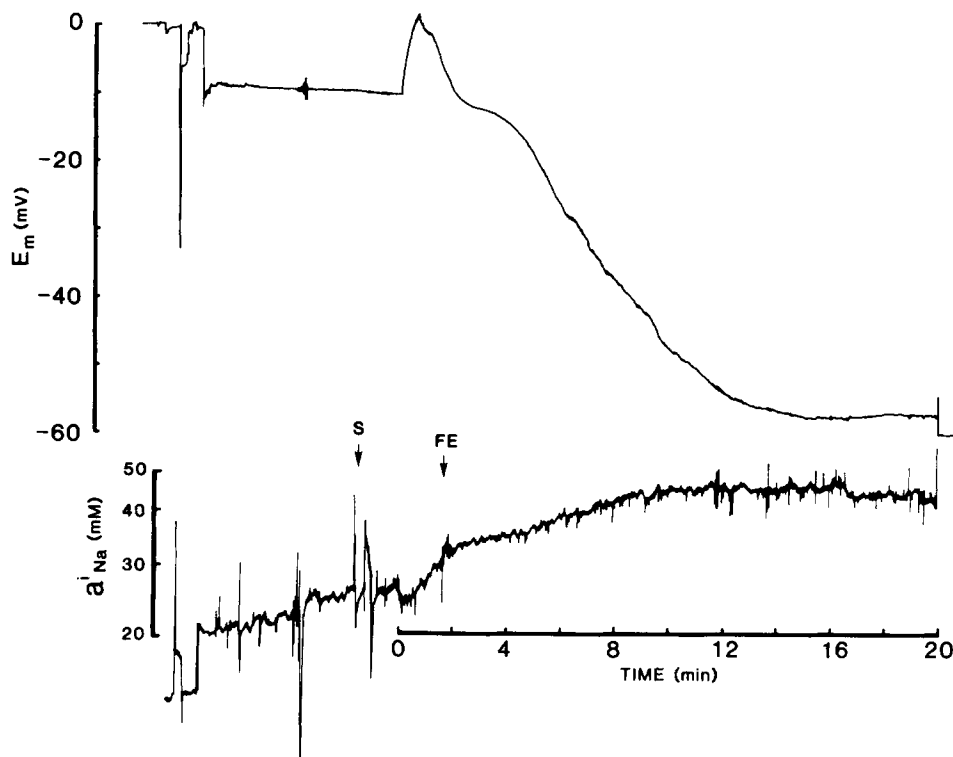


FIGURE 1. Changes in E_m and a_{Na}^i during fertilization in ASW. Impalement with a Na^+ -sensitive occurred before the beginning of this record. A conventional microelectrode was impaled into the same egg by over compensation of the negative capacitance. The first attempt was unsuccessful. Due to the high tip resistance (5×10^{10} to $2 \times 10^{11} \Omega$) of Na^+ -sensitive microelectrodes, slight movements of the microelectrodes induced sudden voltage deflections. Most of these voltage shifts occurred when the egg and electrodes were viewed through the dissecting scope. Upon impalement of the egg with the conventional microelectrode, a_{Na}^i was 20 mM. Over the next few minutes, a_{Na}^i gradually rose and stabilized at 25.5 mM. This rise in a_{Na}^i after placement of both electrodes was probably a result of leakage conductance. A drop of sperm suspension was added (S) to the edge of the dish. Fertilization was assumed to have initiated

ated at the onset of the fertilization potential ($t = 0$). The initial apparent drop in a_{Na}^i during fertilization was probably an artifact of the difference in voltage response times of the two electrodes. Elevation of the fertilization envelope (FE) was used as a visual marker for calcium release (46). When the ion-selective microelectrode was withdrawn ($t = 20$ min), E_m became more negative with the decrease in leakage conductance. In all experiments reported here, the leakage conductance did not abolish membrane hyperpolarization during fertilization.

recordings, a mean peak value of 42 ± 12.9 mM was measured. Subsequently, a_{Na}^i was observed to decrease slowly. During this time the zygote membrane hyperpolarized due to an increased K^+ conductance (44, 45). Although we did not simultaneously measure intracellular pH, increased K^+ conductance is known to depend upon cytoplasmic alkalization (41) and thus serves as an indicator of the rise in internal pH in these experiments.

After fertilization (Table I), a_{Na}^i rose 2 mM in the first 90 s and 7 mM during the first 6 min. The latter paired values were significantly greater ($P < 0.01$) than the unfertilized level. Previous measurements of intracellular pH showed that alkalization occurred between 90 s and 6 min after fertilization (46). Before and during the initial period of alkalization is the fertilization potential, a period of increased Na^+ permeability (44). Thus the increase in a_{Na}^i during fertilization may occur by Na^+ influx during the fertilization potential, as well as during activation of the Na^+ - H^+ exchange. Since cytoplasmic alkalization is normal in 25 mM Na^+ ASW but the Na^+ flux during fertilization is near zero (40), the amount of a_{Na}^i increase due to activation of Na^+ - H^+ exchange may be measured with minimal interference of Na^+ influx during the fertilization potential in 25 mM Na^+ ASW condition.

a_{Na}^i during Fertilization in 25 mM Na^+ ASW

The Na^+ content of ASW was reduced to 25 mM by substitution with choline. From 18 recordings of unfertilized eggs in the low Na^+ ASW, a mean a_{Na}^i of 13.5 ± 8.1 mM (range of 5.5 to 21.5 mM) and a sodium equilibrium potential of +5.8 mV were calculated. The mean E_m of these eggs was

TABLE I. a_{Na}^i during Fertilization

	ASW	25 mM Na^+ ASW
		mM*
Unfertilized	23.3 ± 7.6 (n = 6)	12.0 ± 4.3 (n = 6)
Post-fertilization		
90 s†	25.9 ± 7.8	12.6 ± 3.9
6 min	30.6 ± 10.1	14.7 ± 4.3

* Values are the mean \pm SD of six embryos continuously monitored during fertilization either in ASW or in 25 mM Na^+ ASW.

† Time from start of fertilization was determined from $t = 0$ (onset of the fertilization potential).

-21.8 ± 8.4 mV. The reduction in unfertilized a_{Na}^i was probably due to the decrease in Na^+ entry during electrode impalements with the near 20-fold reduction of ASW Na^+ content. In six successful fertilization recordings (Table I), we observed no significant rise in a_{Na}^i during the first 90 s, but we measured a significant ($P < 0.01$) rise of almost 3 mM by 6 min post fertilization. Since Na^+ influx was greatly reduced in 25 mM Na^+ ASW, this rise in a_{Na}^i was probably a result of activation of Na^+ - H^+ exchange.

Fig. 2 shows a typical fertilization recording in 25 mM Na^+ ASW. The stable a_{Na}^i of this unfertilized egg was 9.5 mM and remained constant until 90 s after fertilization. An inverted fertilization potential was often seen with the reduction of the Na^+ chemical gradient. By 5 min after fertilization, a_{Na}^i had reached a peak value of 11.2 mM. The a_{Na}^i began to decline by 6 min after fertilization, such that by 16 to 20 min, a_{Na}^i had returned to the unfertilized level. During this time the membrane hyperpolarized, which indicated that the rise in intracellular pH had occurred. The stable mean a_{Na}^i of zygotes

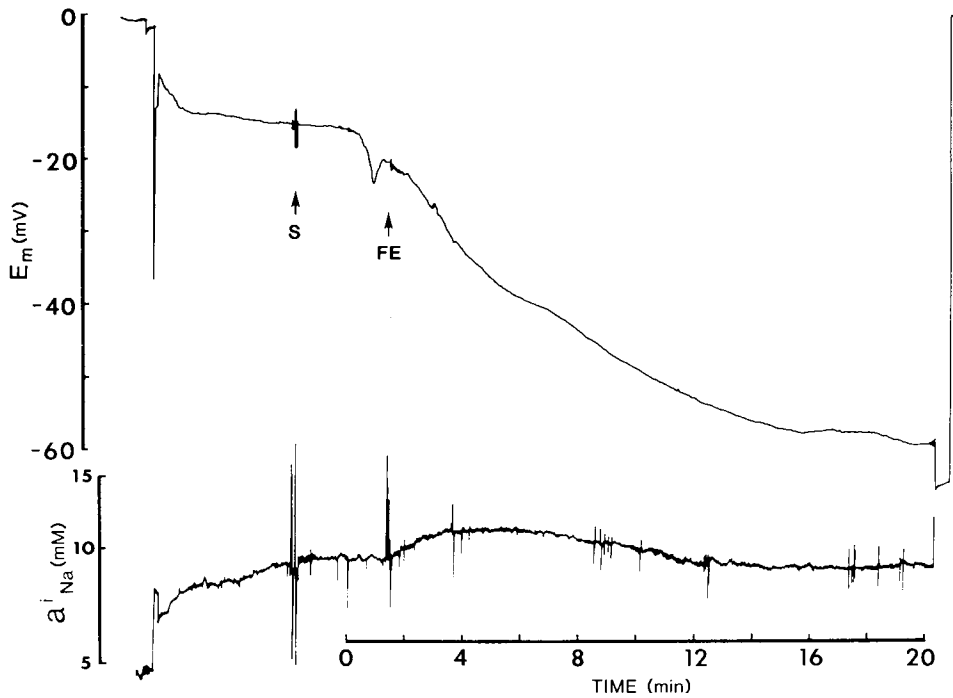


FIGURE 2 Changes in E_m and a_{Na}^i during fertilization in 25 mM Na^+ ASW. The E_m and a_{Na}^i in unfertilized eggs were more negative and lower, respectively, than those measured in ASW. *S*, addition of sperm suspension in 25 mM Na^+ ASW; *FE*, time of fertilization envelope elevation. Note the delay in the rise of a_{Na}^i upon fertilization and its return to unfertilized level by 12 min.

in low Na^+ ASW by 15 min after fertilization was 11.0 ± 4.5 mM.

Acid Release during Fertilization

In many cases, comparisons between acid release, ^{22}Na flux, and cytoplasmic alkalization are made with recordings from different sea urchin species. To alleviate this problem and make comparisons more meaningful, we measured the acid released during fertilization of eggs from the same batches used for measuring a_{Na}^i . In nine experiments, the mean amount of acid released in one minute intervals and the total release were plotted, as shown in Fig. 3; one such continuous record of acid efflux is shown in the inset. Less than 2% of the total acid release occurred during the first minute, with 28, 30, and 20% of the efflux occurring during minutes 2, 3, and 4, respectively. The time course of acid efflux closely matched the time course of a_{Na}^i increase measured in 25 mM Na^+ ASW (Fig. 2). During the interval of cytoplasmic alkalization (90 s to 6 min), 95% of the acid efflux occurred. In nine experiments, the mean acid efflux was 4.45 ± 1.85 mM. Since 20% of this acid release is Na^+ independent and correlates with the cortical granule discharge (16, 22), the calculated Na^+ dependent acid release is ~ 3.6 mM. The mean a_{Na}^i increase during fertilization in 25 mM Na^+ ASW was 2.7 mM (Table I). When 0.68 is used as the activity coefficient of the cytoplasm, this is equivalent to an increase of 3.9 mM of Na^+ , which suggests a 1:1 stoichiometry of the Na^+ - H^+ exchange.

Effects of Ouabain and Amiloride

Activation of the Na^+ , K^+ ATPase during sea urchin fertilization was first suggested by Chambers (4, 5) and has since been studied in greater detail (6, 7, 11). The eventual return of a_{Na}^i to the unfertilized level in zygotes suggested the activation of Na^+ , K^+ pumping during fertilization. As seen in Fig. 4, the addition of 0.5 mM ouabain to 25 mM Na^+ ASW did not appreciably affect a_{Na}^i and E_m of the unfertilized egg, the rise in a_{Na}^i during fertilization, or membrane hyperpolar-

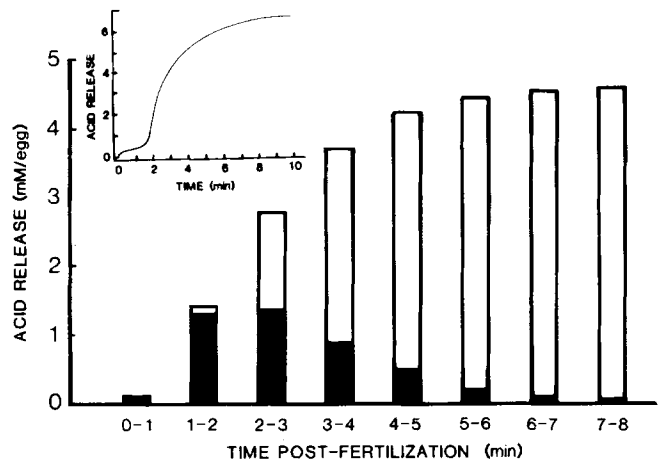


FIGURE 3 Acid efflux during fertilization in ASW. The mean amounts of acid released during each minute from nine experiments are plotted as solid bars. The mean accumulated acid efflux is plotted as open bars. *Inset*: A typical acid release plot. At $t = 0$, 5 μ l of dry sperm was added to the 2 ml of 0.5% ovicrit suspension. The nearly immediate release of acid was associated with sperm activation.

ization. In three experiments, the time course of a_{Na}^i increase during fertilization in ouabain was similar to that in the absence of ouabain. However, the return of a_{Na}^i to the unfertilized level was blocked (Fig. 4), which strongly supports the idea of Na^+ , K^+ ATPase activation during fertilization. Since the membrane hyperpolarized during fertilization in ouabain, inhibition of the Na^+ , K^+ ATPase activation did not affect cytoplasmic alkalization. Furthermore, we observed normal development through the blastula stage in 0.5 mM ouabain, which is in agreement with the observations of Chambers (5).

As a further test of Na^+ - H^+ exchange activation during fertilization, the effect of amiloride (3) was examined. Previous intracellular pH measurements have shown a reduction in the rate of cytoplasmic alkalization with 0.25 to 0.5 mM

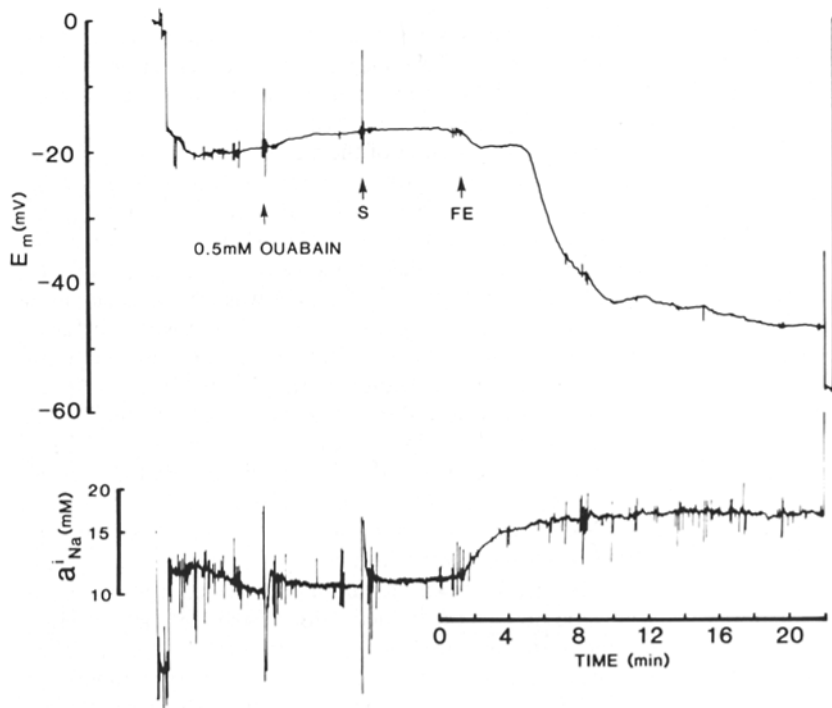


FIGURE 4 Effect of 0.5 mM ouabain on E_m and a_{Na}^i during fertilization in 25 mM Na^+ ASW. Ouabain was added almost 10 min before the start of fertilization by 1:1 dilution of a fresh 1 mM stock in 25 mM Na^+ ASW with the bath. Although ouabain had no significant effect on a_{Na}^i in unfertilized eggs or on the rise in a_{Na}^i during fertilization, a_{Na}^i did not return to unfertilized levels. This embryo was observed to divide for several rounds. For reason unknown, the apparent leakage conductance of the membrane in the presence of ouabain with the withdraw of the Na^+ -sensitive microelectrode was greater than in other conditions.

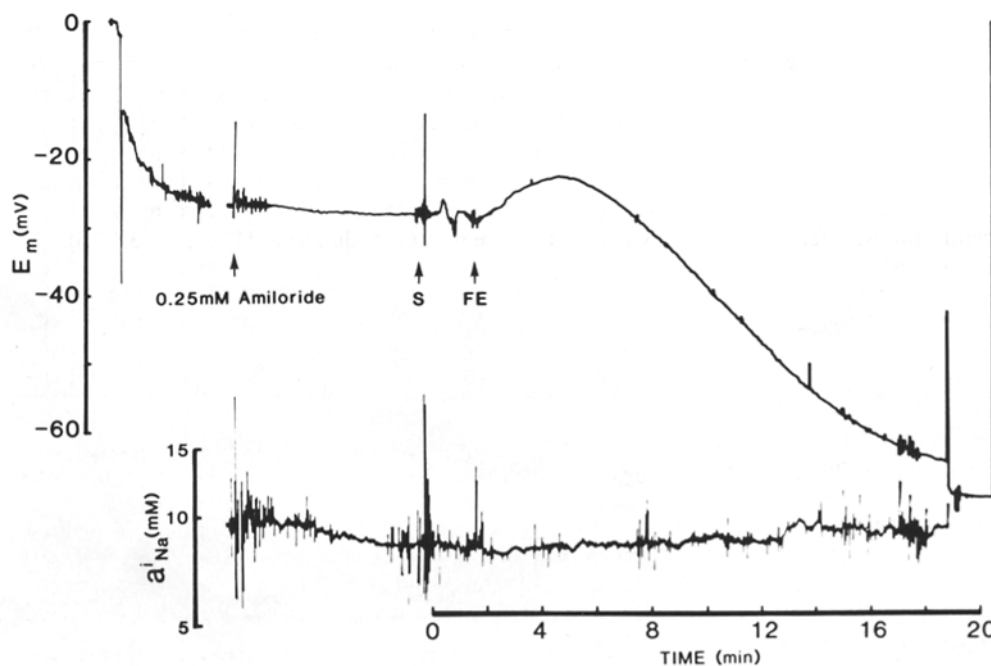


FIGURE 5 Effect of 0.25 mM amiloride on E_m and a_{Na}^i during fertilization in 25 mM Na^+ ASW. There was a break of ~5 min in the record before amiloride was added. The addition of amiloride often caused a decrease in a_{Na}^i in the unfertilized egg. In this case, sperm addition (S) was rapidly followed by the onset of fertilization and fertilization envelope elevation (FE). In this and all other amiloride recordings, no significant change in a_{Na}^i with fertilization was observed, despite the hyperpolarization of the membrane. This embryo was also observed to divide for several rounds.

amiloride in 25 mM Na^+ ASW and an irreversible block at 1 mM amiloride concentration (40). Others have shown inhibition of Na^+ influx and acid release with lower (0.1 mM) amiloride treatment during fertilization (16). As seen in Fig. 5, the addition of 0.25 mM amiloride in 25 mM Na^+ ASW may cause a slight drop in a_{Na}^i in unfertilized eggs. In six experiments with 0.25 or 0.5 mM amiloride, the mean a_{Na}^i in the unfertilized eggs was 11.4 ± 4.6 mM and ranged from 5.7 to 17.5 mM. In all cases with amiloride treatment, fertilization did not result in a significant rise in a_{Na}^i (Fig. 5), which suggested that the increase in a_{Na}^i normally observed was a result of activation of Na^+ - H^+ exchange. At 0.25 mM amiloride concentration, we observed membrane hyperpolarization in all cases (Fig. 5), and at 0.5 mM amiloride, membrane

hyperpolarization was more delayed but did develop. These results agree with earlier observations of delayed cytoplasmic alkalization (40). The lack of a_{Na}^i increase is in apparent contradiction with the presumed rise in internal pH. However, amiloride at these concentrations may only reduce the rate, and not abolish Na^+ - H^+ exchange. Hence, the expected reduced rate of a_{Na}^i increase may be countered by the activity of Na^+ , K^+ ATPase. Alternatively, these levels of amiloride may have blocked Na^+ - H^+ exchange but not the activation of the metabolic energy-dependent, intracellular pH regulator of fertilized eggs (29, 37, 41).

DISCUSSION

Using Na^+ -sensitive microelectrodes, we measured a_{Na}^i during

fertilization of the *Lytechinus pictus* egg. If we assume an activity coefficient for the ooplasm that is similar to the activity coefficient of 0.68 for ASW, our measured Na^+ concentration in the unfertilized egg in ASW is ~ 35 mM, which is within the range of reported values of 22 to 52 mM (4, 28, 33). It appears that the resting Na^+ concentration of the unfertilized egg in ASW is in reality lower than 35 mM. We base this conclusion on the following. (a) Impalement of the egg with microelectrodes introduces a small non-selective leakage (38). (b) In all cases we observed a gradual elevation of the a_{Na}^i after electrode penetration. Normally the Na^+ -selective microelectrode was first placed inside the egg. After achieving a stable ion-selective electrode voltage, the egg was impaled with the conventional microelectrode. The a_{Na}^i was observed to rise over minutes to a higher stable value than the initial activity. (c) The measured Na^+ concentration in unfertilized eggs in 25 mM Na^+ ASW is ~ 20 mM. The reduction in Na^+ concentration resulted from a lower inward Na^+ driving force and decreased amount of Na^+ influx upon electrode penetrations. A similar drop in Na^+ content of unfertilized *Paracentrotus lividus* eggs between seawater and Na^+ -free ASW has been reported (29). (d) The a_{Na}^i of zygotes in ASW remained above the unfertilized level, whereas the zygotes in 25 mM Na^+ ASW returned to unfertilized level. This difference suggests a continued influx of Na^+ through the leakage conductance.

Fertilization of the sea urchin egg in ASW resulted in an almost 77% increase in a_{Na}^i . Most of this increase appears to be a result of increased Na^+ permeability with fertilization. A 20-fold reduction of external Na^+ concentration reduced the inward Na^+ driving force and the resulting increase in a_{Na}^i during fertilization to 22% (Table I). This value is similar to the measured increase in Na^+ concentration with fertilization (4, 7), which suggests that intracellular Na^+ is not compartmentalized. The rise in a_{Na}^i was paralleled by acid efflux, which implies that the Na^+ influx is by Na^+ - H^+ exchange. We calculate the stoichiometry of this exchange to be 1:1, a value consistent with tracer flux determinations (11, 29).

Although a_{Na}^i of fertilized eggs in ASW remained elevated above unfertilized levels, the a_{Na}^i of fertilized eggs in 25 mM Na^+ ASW returned to unfertilized levels by 20 min after fertilization. Since Na^+ content of fertilized eggs measured by flame photometry returned to unfertilized level by 20 min (28) and there was no evidence for compartmentalization of Na^+ , it appears that the permanently elevated a_{Na}^i observed here of zygotes in ASW was a consequence of a microelectrode impalement artifact, i.e., of continued entry of Na^+ through the leakage conductance. The slight decrease in a_{Na}^i in ASW and the full return to unfertilized level in 25 mM Na^+ ASW appear to be consequences of activation of Na^+ , K^+ ATPase during fertilization. Initially reported by Chambers (4, 5), the Na^+ , K^+ ATPase in sea urchin eggs has recently been characterized (6, 7). The activity of Na^+ , K^+ ATPase of unfertilized egg cortices increased nearly 10-fold with an increase in pH between 6.7 and 7.7 (6). Since the rise in internal pH is from 6.9 to 7.3 (39) and a consequence of Na^+ - H^+ exchange, the activation of the Na^+ , K^+ ATPase may at most truncate the rise in a_{Na}^i near its completion. Since the presence of 0.5 mM ouabain in 25 mM Na^+ ASW had no appreciable effect on a_{Na}^i in the unfertilized egg, the Na^+ , K^+ ATPase is probably inactive (6) and has no noticeable effect upon the rise of a_{Na}^i during fertilization. But the return to the unfertilized level was blocked. It is interesting that the development of sea

urchins in ouabain remained normal in appearance through the blastula stage. This suggests that the regulatory role of Na^+ , if any, is its influx or increased intracellular activity, rather than its efflux or decreased activity.

Further substantiation of the elevation of a_{Na}^i as a result of Na^+ - H^+ exchange was its inhibition by amiloride. Although 0.1 mM amiloride blocked acid efflux (16), 1 mM amiloride was required to block cytoplasmic alkalinization (40). The data presented here suggest a possible explanation for this discrepancy. Amiloride of 0.25 mM was found to block the rise in a_{Na}^i , which would agree more closely with the earlier reported inhibition of acid efflux. Since membrane hyperpolarization during fertilization requires cytoplasmic alkalinization (41), the observed membrane hyperpolarization during fertilization in amiloride agrees with earlier observations of cytoplasmic alkalinization during fertilization in low concentrations of amiloride (40). A second ionic mechanism, which is directly dependent upon metabolic energy, has been suggested for regulation of intracellular pH in zygotes (29, 37, 41). The rise in intracellular pH at low concentrations of amiloride may be due to activation of the metabolic energy-dependent intracellular pH regulator (29, 37, 41). The alternative possibility of Na^+ , K^+ ATPase activity countering a decreased rate of Na^+ - H^+ exchange is unlikely because of the pH sensitivity of Na^+ , K^+ ATPase (6) and the observed suppression of ^{86}Rb uptake during fertilization in low Na^+ ASW containing 0.1 mM amiloride (7). Amiloride may directly inhibit the Na^+ , K^+ ATPase (43). Currently, we have no information about the ionic basis of the metabolic energy requiring pH regulator. However, our observations of inhibition of the a_{Na}^i rise with low concentrations of amiloride is in agreement with the blocking concentration of amiloride on Na^+ - H^+ exchange in mammalian cells (1, 18, 21, 32, 36).

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