

Block of Inward Rectification by Intracellular H^+ in Immature Oocytes of the Starfish *Mediaster aequalis*

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ABSTRACT Intracellular pH was recorded in immature starfish oocytes using pH-sensitive microelectrodes, and inwardly rectifying potassium currents were measured under voltage clamp. When the intracellular pH was lowered using acetate-buffered artificial sea water from the normal value of 7.09 to 5.9, inward rectification was completely blocked. The relationship between inward rectification and internal pH between 7.09 and 5.9 could be fit by a titration curve for the binding of three H ions to a site with a pK of 6.26 to block the channel. The H^+ block showed no voltage dependence, and the activation kinetics of the inwardly rectifying currents were not affected by the changes in internal pH.

INTRODUCTION

The immature oocyte of the starfish shows a potassium conductance that is activated by hyperpolarization of the membrane from the resting potential (see Hagiwara and Takahashi, 1974). This has been termed anomalous, or inward, rectification, and its properties in oocytes are similar to those of anomalous rectification in vertebrate skeletal muscle (Katz, 1949; Adrian, 1969; Adrian et al., 1970; Standen and Stanfield, 1978).

The inwardly rectifying potassium currents in starfish oocytes are sensitive to a variety of ions in both the intracellular and extracellular solutions. External Ba^{2+} , Cs^+ , Rb^+ , and Tl^+ block the channel (see Hagiwara et al., 1978, for review). Intracellular Na^+ is required for the channel to be conductive, even though Na^+ itself is not permeant (Hagiwara and Yoshii, 1979). In addition, the channel binds external, but not internal, K^+ in such a way as to yield an apparent dependence of the conductance on the difference between the membrane potential, V_m , and the potassium equilibrium potential, V_K , rather than on V_m alone when the extracellular K^+ concentration is changed (Hagiwara and Yoshii, 1979).

In the present experiments we have combined the voltage-clamp technique with direct microelectrode measurements of intracellular pH (pH_i) to investigate the effect of changes in pH_i on inward rectification in the starfish

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oocyte. There has been a preliminary report that intracellular H^+ blocks inward rectification in skeletal muscle, but no measurements were made to indicate the internal pH range over which the block occurred (Blatz, 1980). Relatively few studies have been done on the effects of intracellular pH on specific ionic conductances. Low internal pH blocks delayed outward potassium currents in the squid giant axon (Wanke et al., 1979), and in the same preparation high internal pH removes Na^+ current inactivation (Brodwick and Eaton, 1978). In frog skeletal muscle, decreased pH_i blocks sodium current inactivation (Nonner et al., 1980). In crayfish slow muscle fibers, decreasing the intracellular pH blocks delayed rectification, and as a result, greatly increases the amplitude of Ca^{2+} -dependent action potentials (Moody, 1980).

The sensitivity of inward rectification to changes in pH_i should indicate the importance of titratable groups near the inner membrane surface in the functioning of this channel and thus yield some information about the channel structure. The effects of pH_i in oocytes may in addition be of some physiological importance, since changes in pH_i are known to occur in various oocytes during maturation and at fertilization (Shen and Steinhardt, 1978; Lee and Steinhardt, 1981).

We report here that decreased intracellular pH blocks inward rectification in starfish oocytes. The block occurs over a fairly narrow range of pH_i and within 1 unit of the resting value, and is fully reversible. The shape of the relation between conductance and pH_i is consistent with the protonation of a titratable group to block the channel.

Some of these results have been reported in abstract form (Moody and Hagiwara, 1981).

METHODS

Starfish (*Mediaster aequalis*) were collected by a local supplier between December 1980 and March 1981. They were maintained at 8–12°C in an artificial sea water system. The collection of immature eggs and general experimental procedures have been described previously (Hagiwara et al., 1975).

Solutions

Standard artificial sea water (ASW), in which eggs were maintained before the experiment and in which microelectrode impalements were done, had the following composition: 460 mM NaCl; 10 mM KCl; 10 mM $CaCl_2$; 50 mM $MgCl_2$; 10 mM HEPES. The pH was adjusted to 7.8 with NaOH. All voltage-clamp experiments were done in 25 K^+ ASW (containing 15 mM additional KCl, replacing NaCl, with other components unchanged), with which the cell was superfused after recovery from penetration (see Fig. 1).

The intracellular pH was lowered by decreasing the external pH in the presence of the membrane-permeant weak acid, acetate. In initial experiments designed to demonstrate the block of inward rectification by internal H^+ (e.g., Fig. 2), we used 25 K^+ ASW pH 5.0, buffered with 10 mM acetate, substituted for chloride. Control experiments for low external pH were done in 25 K^+ ASW with 20 mM biphthalate, a membrane-impermeant buffer, substituted for Cl^- . The higher concentration of biphthalate was used to equalize the buffering power of the two low pH solutions (see below). Complete titration curves (e.g., Figs. 3 and 4) were obtained in ASW of the

following composition: 385 mM NaCl; 15 mM KCl; 10 mM CaCl₂; 50 mM MgCl₂; 10 mM potassium biphthalate; 10 mM sodium acetate; 10 mM HEPES; 10 mM Tris maleate; 10 mM glycine. The pH of this solution was brought to 7.8 with NaOH, and then to various values between 7.8 and 5.0 (see Fig. 3) with HCl. This procedure was used so that the external Na⁺ activity would not decrease with external pH, and thus possibly contaminate the titration curve by lowering the internal Na⁺ activity (Thomas, 1972) and reducing inward rectifier currents (Hagiwara and Yoshii, 1979).

The preparation was continuously superfused throughout the experiment. The exchange of time of the solution in the experimental chamber was ~1 min. All experiments were done at room temperature.

Microelectrodes

In each experiment, the oocyte was impaled with three microelectrodes in order to measure intracellular pH and deliver voltage-clamp pulses. The two conventional microelectrodes were filled with 3 M KCl and were broken to resistances of <5 MΩ. Recessed-tip, pH-sensitive microelectrodes were constructed according to Thomas (1978) with two modifications: (a) N₂ pressure of 1,000–1,200 lbs/in² (50–70 bar) was used to make the glass-to-glass seal; and (b) the outer electrode was pulled using a Brown-Flaming electrode puller (Sutter Instrument Co., San Francisco, Calif.). The latter modification allowed pipettes to be pulled with short shanks, steep terminal tapers, and fine tips, a combination much more difficult to achieve with a conventional electrode puller. The pH electrodes had tips of <1 μm in diameter, slopes of 57–59 mV/pH unit, and 90% response times of <1 min. Na⁺-sensitive microelectrodes were constructed according to Thomas (1978). The same type of outer pipette was used as for the pH electrodes. Na⁺ electrodes had slopes of 56–58 mV for a 10-fold change in Na⁺ concentration (K⁺ substitution) and 90% response times of ≤ 1 min. Ion-sensitive electrodes were calibrated before and after each experiment to insure that neither the DC signal nor the response amplitude had changed by more than a few millivolts during the experiment.

Electronic Arrangements

The method for the two-electrode voltage clamp was similar to that previously described (Hagiwara et al., 1975). The membrane potential was recorded differentially between one of the KCl-filled intracellular electrodes and the extracellular electrode. The membrane current was recorded as the voltage drop across a 100-kΩ resistor inserted between the output of the feedback amplifier and the intracellular current electrode. The rise time of the voltage steps was ~2 ms, which was sufficiently fast for the present experiments.

The pH electrode signal was recorded with a varactor diode amplifier (311J; Analog Devices, Inc., Norwood, Mass.). The difference signal between the pH microelectrode and the membrane potential electrode was displayed on one channel of a slow speed chart recorder. The difference signal between the membrane potential electrode and an extracellular microelectrode (<1 MΩ) was displayed on a second channel. Figs. 1–3 and 5 are direct photographs of the chart records.

RESULTS

Normal Intracellular pH of Immature Oocytes

Fig. 1 shows the beginning of a typical experiment in which the pH_i was recorded and inwardly rectifying currents were measured under voltage-clamp

conditions in a single oocyte. At the beginning of the experiment, the pH microelectrode was calibrated by exposure to pH 6.8 (MES-buffered) ASW. The current-passing microelectrode was inserted into the cell first (not shown). Next, the voltage microelectrode was inserted and a stable resting potential of -71 mV was recorded. Finally, the pH microelectrode was inserted, causing a 4-mV depolarization of the cell.

During the first 30 min of the recording, the cell showed a fairly pronounced acid injury. The pH_i decreased by 0.15 unit during the first 10 min after impalement and then slowly recovered. (At the peak of this injury, an action

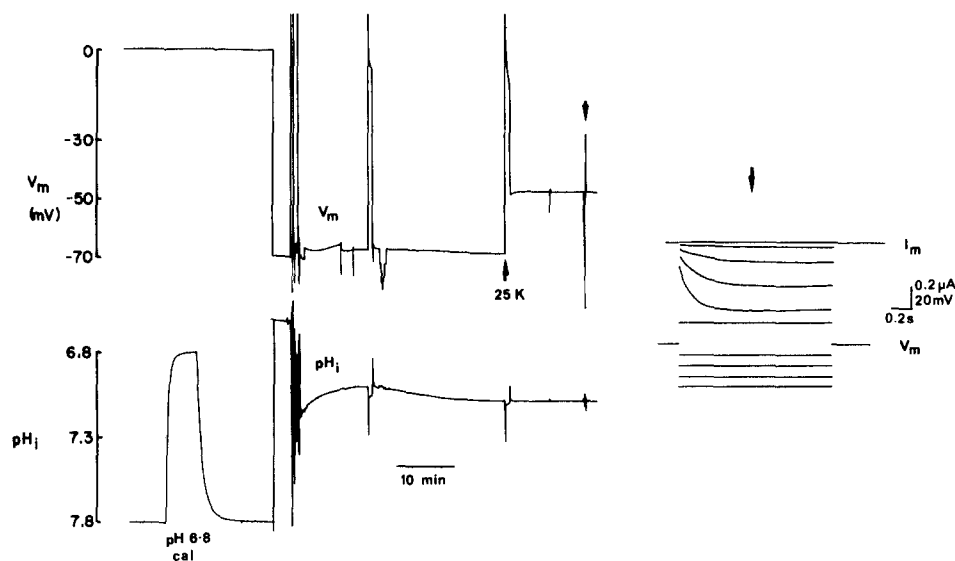


FIGURE 1. Recording of pH_i , V_m , and inward rectifier currents in the starfish oocyte. The pH microelectrode was first calibrated by exposure to pH 6.8 ASW. Next, the V_m electrode was inserted and the pH_i trace was deflected upward by 70 mV at this point because the pH_i record is the difference signal between the pH and V_m electrodes. After six attempts, the pH electrode was inserted into the cell. About 12 min after impalement, an action potential was elicited by a current pulse (see text). After 35 min, when pH_i and V_m had stabilized, the external solution was changed to 25 K^+ ASW, triggering an action potential and causing a 19-mV depolarization. About 14 min later (arrow), a series of voltage-clamp pulses was delivered, and the associated inward rectifier currents are shown in the right-hand part of the figure. In this and all subsequent figures, inward membrane currents are shown as downward deflections of the current records. In subsequent figures, the V_m pulses are omitted from the records.

potential was elicited by injected current to check the condition of the oocyte and the quality of the penetration; see below.) The pH_i stabilized at a value of 7.09, and V_m stabilized at -70 mV.

Average values for pH_i and V_m in these experiments were 7.09 ± 0.08 and -71.3 ± 1.6 mV, respectively (mean \pm SD; $n = 14$). These immature oocytes, therefore, maintain pH_i at a value that is on the average 0.50 unit more

alkaline than predicted from an equilibrium distribution of H^+ across the membrane. Both the absolute pH_i value and its value relative to equilibrium in these cells are in close agreement with microelectrode measurements of pH_i in a variety of neurons and muscle fibers (Thomas, 1974; Aickin and Thomas, 1977; Ellis and Thomas, 1976; Boron and de Weer, 1976; Boron, 1977; Moody, 1980 and 1981). It seems likely that the immature starfish oocyte and these other cells share similar mechanisms of active H^+ extrusion to maintain their relatively alkaline pH_i values (see Roos and Boron, 1981, for a review of such mechanisms).

After pH_i and V_m had stabilized (Fig. 1), the K^+ concentration of the Ringer's solution was increased from 10 to 25 mM, the concentration at which inward rectification currents were measured. This caused the egg membrane to depolarize from -70 to -49 mV and triggered an action potential. Comparing the pH_i trace before and after this action potential, it is apparent that the 21-mV depolarization caused by the increased K^+ concentration was not registered on the pH_i trace, and thus that the rejection of membrane potential changes by the differential pH_i recording was adequate.

The transients recorded on the pH_i trace during the rising and falling phases of the spikes in Fig. 1 result from the filtering of the pH_i electrode signal before the V_m signal is subtracted. However, the plateau phase of the first action potential (elicited by a current pulse 12 min after impalement) does not appear to have been well rejected from the pH_i record. It is possible that during the first 20–30 min after some penetrations, a gradual resealing of the membrane occurs at the sites of impalement, and that before this process is complete, the pH and V_m electrodes do not record identical membrane potential changes.

Cells were voltage-clamped at a holding potential equal to the resting potential in 25 K^+ ASW (-50 ± 1.2 mV). Fig. 1 shows the currents recorded in this cell for clamp steps ranging from $+20$ to -40 mV from the holding potential. The inwardly rectifying currents are apparent during the hyperpolarizing voltage steps (a current-voltage relation for the oocyte at normal pH_i is shown in Fig. 4 B). The rectification has both instantaneous and time-dependent components. The time-dependent component of the conductance increase follows first-order kinetics, with time constants of 0.1–0.4 s in this potential range (see Hagiwara et al., 1976, for a full description of these currents).

Effect of Low pH_i

The intracellular pH was decreased by exposing the oocytes to low-pH ASW buffered with 10 mM acetate. The undissociated form of the buffer (acetic acid, $pK = 4.75$) crosses the membrane readily and dissociates to liberate H^+ intracellularly, thus lowering the pH_i (see Green, 1949; Sharp and Thomas, 1981). To control for the effects of low external pH, the membrane-impermeant buffer biphthalate was used. Since the pH_i was recorded in each experiment, we were able to confirm that the buffers had their intended effects on pH_i .

Fig. 2 shows an experiment comparing the effects of acetate- and biphthal-

ate-buffered ASW, pH 5.0, on internal pH and the inwardly rectifying currents. The record begins 20 min after impalement with the cell showing a stable intracellular pH of 7.08. The inward rectifier currents recorded at this time are shown in panel 1 at the bottom of the figure. After recording the currents, the cell was exposed to pH 5.0 ASW (biphthalate) for 13 min. This had virtually no effect on either the pH_i or inward rectification (panel 2).

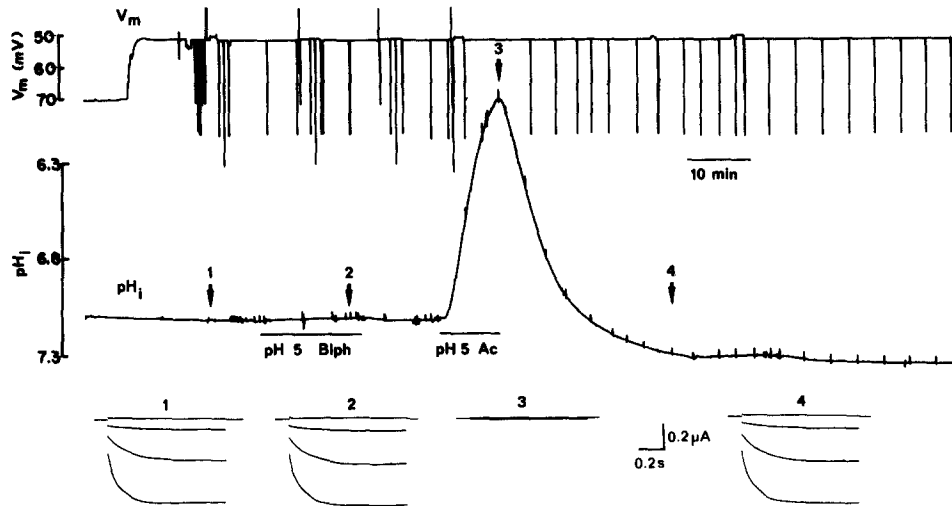


FIGURE 2. Experiment showing the differential effects of pH 5.0 (biphthalate) and pH 5.0 (acetate) ASW on pH_i and inward rectification. The records begin 20 min after penetration. The upper trace shows the membrane potential, the middle trace shows the pH_i , and the bottom trace shows membrane currents recorded at the four times indicated for voltage-clamp pulses of amplitudes +10, -10, -20, and -30 mV, from $V_{hold} = -50$ mV. After changing to 25 K^+ ASW, inward rectifier currents were recorded at normal pH_i (1). The cell was then exposed to pH 5.0 (biphthalate) ASW, which caused the pH_i to decrease by at most 0.01 unit, and had no effect on inward rectifier currents (2). The cell was then returned to normal ASW for 13 min. Next, the cell was exposed to pH 5.0 (acetate) ASW, which caused the pH_i to decrease to 5.84, and reduced the inward rectifier currents to $\sim 2\%$ of control (3). Upon return to pH 7.8 ASW, the pH_i increased to 7.26 and the inward rectifier currents returned to their control amplitude (4). The repeated single-clamp pulses that appear on the V_m trace and as artifacts on the pH_i trace were used during the experiment to monitor the time-course of the changes in current amplitude. Immediately after trace 4 on the pH_i record, a slight slowing of the pH_i recovery rate is apparent; during these 10 min, the cell was exposed to pH 4.0 (biphthalate) ASW (see Results on the effects of external pH).

After returning to normal ASW for a brief period, the cell was exposed to pH 5.0 ASW (acetate). This caused the pH_i to decrease rapidly. At $pH_i = 5.84$, the inward K^+ currents were reduced to $<2\%$ of their control value (panel 3). Upon return to normal ASW, the pH_i returned to somewhat above its value

at the beginning of the experiment. At $\text{pH}_i = 7.26$, a series of voltage-clamp pulses was delivered and the currents had returned to 97% of their control value (panel 4).

The experiment shown in Fig. 2 demonstrates that low pH_i reversibly eliminates inwardly rectifying K^+ currents in starfish oocytes. In six experiments, pH 5.0 (acetate) ASW caused the pH_i to decrease to 5.75–6.0, and reduced inward rectification to 2–10% of control. pH 5.0 (bipthalate) ASW caused the pH_i to decrease by only 0.01–0.12 unit, and reduced the inward K^+ currents to 88–100% of control. In bipthalate, the amount of inhibition of inward rectification was not correlated with the size of the pH_i change. The effect of low-pH bipthalate ASW appears to be a direct effect of external pH on the currents (see below). It is unlikely that the different effects of bipthalate- and acetate-buffered solutions are caused by differences in their abilities to change the pH in a highly buffered extracellular compartment, since the buffering power of 20 mM bipthalate is ~ 1.6 times that of 10 mM acetate at pH 5.0 (Dawson et al., 1969). (Earlier measurements of a pK of 5.3–5.4 for the block of inward rectification by external H^+ [Hagiwara et al., 1978] were incorrect. All buffers in these experiments contained the permanent weak acid acetate, and thus the effects were those of internal, not external, H^+ .)

Titration Curve for the Effect of pH_i

In three cells, currents were recorded at several values of pH_i between the extremes shown in Fig. 2 in order to obtain a titration curve for the pH_i effect. Using 10 mM acetate ASW (see Methods for complete buffer composition of this solution), the external pH was decreased in three steps from 7.8 to 5.0. When pH_i stabilized after each solution change, voltage-clamp pulses were delivered, and the inwardly rectifying currents were recorded. Fig. 3 illustrates one such experiment. The record begins ~ 1 h after impalement. The pH_i at this time was 7.23, slightly elevated due to a previous exposure to acetate. The currents at this pH_i are shown in the inset to the lower left of the figure. The cell was then exposed to pH 6.0 (acetate) ASW for 18 min. When the pH_i had stabilized at 6.52, the inward rectifier currents were recorded and were found to have decreased to 88% of control. Next, the cell was exposed to pH 5.5 (acetate) ASW, which caused the pH_i to decrease to 6.22 and the currents to decrease to 50% of control. The pH_i was then decreased to 5.97 by exposing the cell to pH 5.0 (acetate) ASW, and the currents were decreased to 8% of control. Finally, the entire set of solution changes was reversed, and the pH_i returned, in the three steps, to within 0.01 unit of its initial value. The inward rectifier currents similarly returned stepwise to within 6% of their initial value as the pH_i increased.

This experiment demonstrates a reversible, monotonic relation between the pH_i and the amplitude of inwardly rectifying currents. The results of this type of experiment were very consistent among cells. A titration curve was obtained by pooling the data from several cells, including three such as in Fig. 3, in which currents were measured at four different values of pH_i . These data are plotted in Fig. 4 A. The slope conductance at $V_m = -90$ mV (pulse amplitude

= -40 mV), normalized to its value at the resting pH_i , is plotted vs. pH_i . The solid line is drawn according to the equation

$$g/g_{\text{max}} = K^3 / (K^3 + [H^+]_i^3)$$

where g_{max} is the slope conductance at resting pH_i , and $K = 10^{-6.26}$. This equation assumes that three H ions bind to a site (or identical sites) with a $\text{p}K$

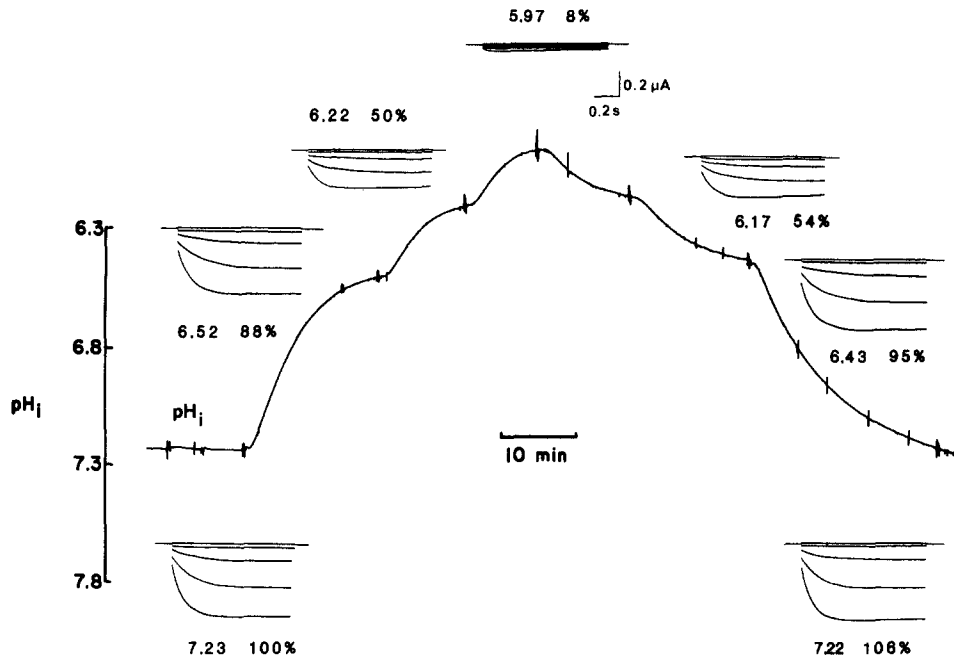


FIGURE 3. Experiment designed to measure the amplitude of inward rectifier currents at several different pH_i values. The record begins ~ 1 h after impalement, in 25 K^+ ASW, with the pH_i somewhat elevated by a brief exposure to acetate about 30 min earlier. The cell is exposed to seven solutions, each containing 10 mM acetate, of the following pH values: 7.8, 6.0, 5.5, 5.0, 5.5, 6.0, and 7.8. After the pH_i had reached a steady-state value in each solution, inward rectifier currents were recorded. The currents are displayed clockwise around the pH_i record, beginning at the lower left, and each is marked with the pH_i value at which it was taken, and the current amplitude is expressed as a percentage of that at $\text{pH}_i = 7.23$. Calibrations apply to all current records. Currents are shown for voltage-clamp pulses of amplitudes +10, -10, -20, -30, and -40 mV from $V_{\text{hold}} = -50$ mV.

of 6.26 to block the channel. Although an exponent of 3 provides the best overall fit to the data, the points in Fig. 4 A show a consistent pattern of deviation from the theoretical curve. The points below $\text{pH}_i = 6.4$ are better fit by a fourth-power relation with $\text{p}K = 6.2$. The relation above $\text{pH}_i = 6.4$ is too gradual for its steepness at lower values of pH_i . This may indicate that the several H^+ -binding sites have closely spaced but different $\text{p}K$ values, or that

changes in other intracellular ions that affect inward rectification occur when pH_i changes are imposed on the cell (see below).

The effects of increased pH_i were not examined in detail in these experiments. However, in three experiments—the one in Fig. 2, for example—the pH_i increased beyond its normal value after the change from pH 5.0 (acetate) to pH 7.8 ASW. In the cell of Fig. 2, the currents recovered to their control value upon washout of pH 5.0 (acetate) ASW as the pH_i increased from its low value of 5.84 to the normal 7.08. There was no further change in current amplitude as the pH_i increased further to 7.26. In two other cells, the overshoot of pH_i upon washout of pH 5.0 (acetate) ASW was greater, to 7.56–7.57. In these cells, the inward rectifier currents first recovered to $\sim 95\%$ of control as the pH_i passed through the normal value, and then decreased in amplitude again as the pH_i increased to 7.56–7.57. This indicates that the normal pH_i of

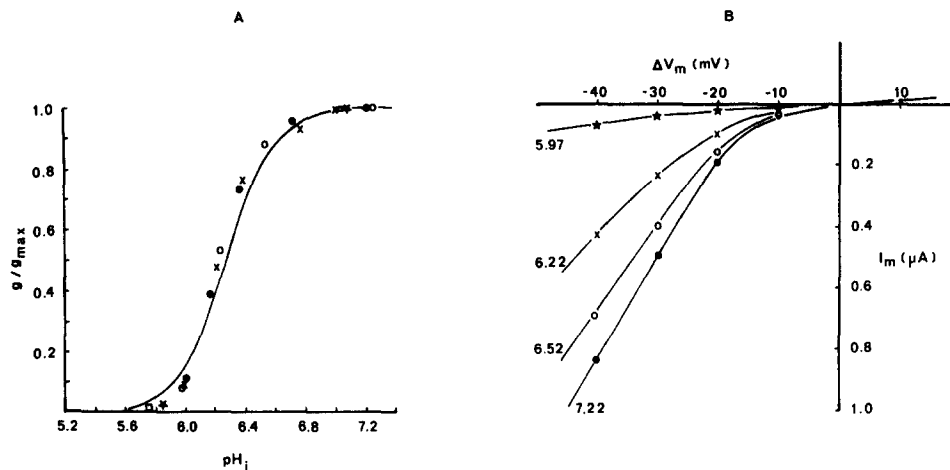


FIGURE 4. (A) Titration curve for the effect of pH_i on inward rectifier currents. Normalized slope conductance at a clamp pulse amplitude of -40 mV is plotted vs. pH_i . The results of five experiments are plotted. (B) $I-V$ relations from the experiment of Fig. 3. Each curve is labeled with the pH_i value at which it was taken.

the immature oocyte may be an optimum value for the conductance of the inward rectifier. In addition, the fact that the currents do not increase above what we have designated the maximum level in Fig. 4 as the pH_i is made more alkaline justifies the normalization procedure used to construct the titration curve in Fig. 4.

Current-Voltage Relations at Different Values of pH_i

Fig. 4 B shows steady-state $I-V$ relations at different values of pH_i , obtained in the experiment of Fig. 3. There is no evidence of a membrane potential dependence of the H^+ block. The fractional inhibition of the membrane current at low pH_i was not significantly different for clamp pulse amplitudes of -20 , -30 , or -40 mV in any of the experiments. Were the H^+ binding site

associated with the channel in the membrane electrical field, one would expect to see a relief of the H^+ block for large hyperpolarizations. We estimate that we could have detected the voltage dependence associated with an H^+ binding site that sensed 0.1 or more of the applied membrane field.

(We have assumed that the entry of H ions to a binding site within the membrane field would have been rapid compared with our pulse durations [typically 1.4 s]. In the case of Ba^{+2} , which shows the most slowly developing voltage-dependent block of inward rectification [Hagiwara et al., 1978], the time constant of entry to the binding site is ~ 1 s in the potential range examined here. This would have been easily detected in the present experiments. One would expect H^+ to diffuse more rapidly into the channel than Ba^{2+} , and in fact Woodhull [1973] estimated that the voltage-dependent H^+ block of Na^+ currents in myelinated nerve occurred in 20 μs or less.)

The current records taken at various values of pH_i (Fig. 3) are consistent with a simple block of the channel by H^+ . The instantaneous and time-dependent components of inward rectification are equally affected by pH_i . Semi-logarithmic plots of the time-dependent phase of the conductance increase revealed no change in activation kinetics caused by decreased pH_i . However, if the activation kinetics of the current were only altered at pH_i values well below the pK for the blocking effect, the effect probably would not have been detected because of the small amplitude of the currents.

Changes in Intracellular Na^+ Concentration during pH_i Changes

Inward rectification in starfish eggs is sensitive to the intracellular concentration of Na^+ ($[Na^+]_i$) such that reducing $[Na^+]_i$ decreases the conductance (Hagiwara and Yoshii, 1979). We wanted to eliminate the possibility that a decrease in $[Na^+]_i$ caused by low pH_i might explain the H^+ block of the inwardly rectifying currents. This might occur at low values of external pH if the inward driving force on H^+ were sufficient to drive Na^+-H^+ exchange in reverse (Thomas, 1980), causing the efflux of Na^+ from the cell. We therefore recorded $[Na^+]_i$ using Na^+ -sensitive microelectrodes in five eggs while the pH_i was lowered with acetate-buffered ASW as in Figs. 2 and 3.

Fig. 5 illustrates one such experiment. After insertion of conventional and Na^+ -sensitive microelectrodes, a period of 20 min was allowed so that a stable record of the normal $[Na^+]_i$ could be obtained. In this experiment, that value was 24.3 mM; the average resting $[Na^+]_i$ could be obtained. In this experiment, that value was 24.3 mM; the average resting $[Na^+]_i$ in the five experiments was 21 ± 5 mM. Next, the cell was exposed sequentially to acetate-buffered ASW at pH values to 6.0, 5.2, and 5.0—essentially the same series of solutions used to determine the titration curve shown in Figs. 3 and 4 A. About 4 min after the change to pH 6.0 (acetate) ASW, $[Na^+]_i$ began to increase and reached a value of 32 mM after 12 min. The external pH was then lowered to 5.2, and $[Na^+]_i$ continued to increase, reaching 38 mM after 10 min. The subsequent decrease in extracellular pH to 5.0 had no further effect on $[Na^+]_i$ during 6 min of exposure.

These results show that the block of inward rectification at low pH_i is not

caused by a decrease in $[Na^+]_i$. In fact, the increase in $[Na^+]_i$ that occurs when the pH_i is lowered has probably caused us to slightly underestimate the sensitivity of inward rectification to small changes in pH_i near the resting level (see below).

In Fig. 5, when the external pH was returned to 7.8, $[Na^+]_i$ did not return towards its resting value. In other immature starfish oocytes, the Na^+-K^+

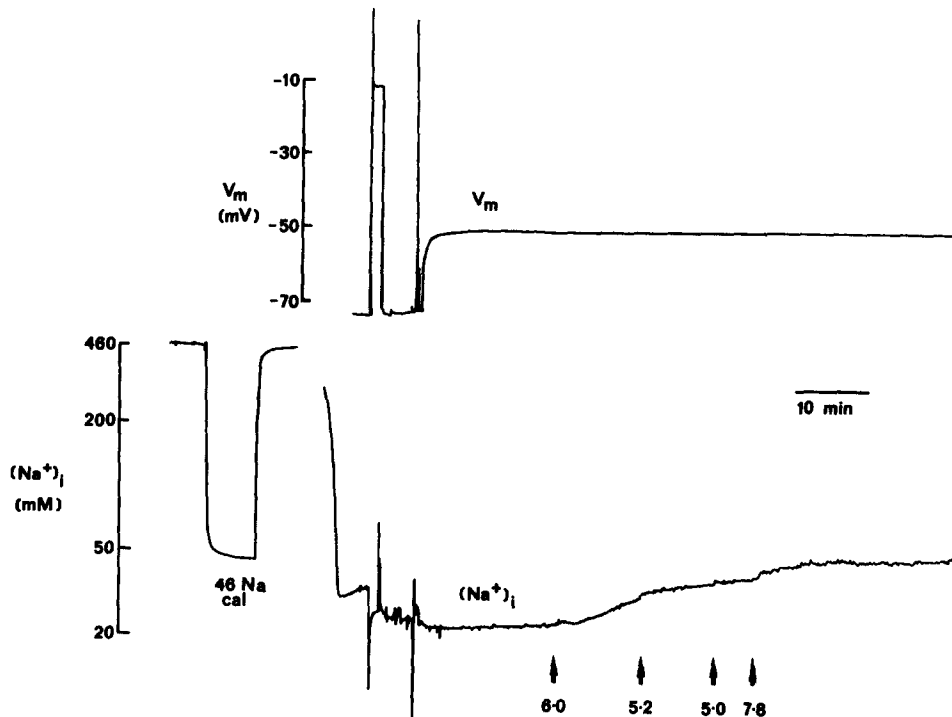


FIGURE 5. Experiment recording $[Na^+]_i$ during decreased pH_i . First, the Na^+ -sensitive microelectrode was calibrated by exposure to 46 mM Na^+ ASW (K^+ substitution). Then the V_m electrode was inserted into the cell (not shown). The Na^+ was inserted next; the $[Na^+]_i$ trace is interrupted at this point, since after the V_m electrode was inserted, the $[Na^+]_i$ trace goes upward offscale. The $[Na^+]_i$ record resumes 2 min after penetration. After the addition of 25 K^+ ASW and the stabilization of the $[Na^+]_i$ trace, the cell was exposed to a series of ASW (acetate) solutions of pH values indicated below the $[Na^+]_i$ record. V_m was clamped at the zero current potential in 25 K^+ ASW throughout the pH changes.

pump appears to be rather unresponsive to increases in $[Na^+]_i$, as compared with the mature oocyte (Moreau et al., 1978). It is possible that in *Mediaster* oocytes, the Na^+-K^+ pump is not activated by increases in $[Na^+]_i$ of the magnitude of those in Fig. 5, or that the rate of its operation is such as to result in a decrease in $[Na^+]_i$ too slow to be detected in our recordings. The

maintenance of low $[\text{Na}^+]_i$ in the immature oocyte under normal conditions suggests that the passive leak of Na^+ into the cell must be quite slow. The relative Na^+ permeability of the oocyte is indeed very small, judging from the proximity of the resting potential to the K^+ equilibrium potential and the extremely high membrane resistance in cells in which the resting K^+ conductance has been blocked (Hagiwara et al., 1978; Hagiwara and Yoshii, 1979).

Effect of Low External pH

In several experiments, the effects of decreasing the external pH to values below 5.0, using biphthalate-buffered ASW, were examined. We found that at $\text{pH} = 4.0$, the inward rectifier currents were reduced to 12–40% of control. As described above, at $\text{pH} 5.0$, the currents were reduced to 88–100% of control. The block of inward rectification at $\text{pH} 4.0$ could not be accounted for by a change in intracellular pH. An example of the effect of $\text{pH} 4.0$ biphthalate ASW on intracellular pH is shown near the end of the record in Fig. 2. Exposure of this cell to $\text{pH} 4.0$ for 12 min slowed the alkalization that occurred after the removal of acetate 30 min earlier, but caused no net acidification. Nonetheless, the inward rectifier currents were reduced to 40% of their control value by this solution. We could detect no substantial voltage dependence to the block by external H^+ . The effects of low external pH were incompletely and only slowly reversible: superfusion of $\text{pH} 7.8$ ASW for 30–60 min produced a return of the currents to only 50–90% of the control value.

Thus there appears to be a binding site of external H^+ , distinct from that for intracellular H^+ , whose protonation also results in the block of this channel.

DISCUSSION

We have shown that decreasing the intracellular pH blocks inwardly rectifying potassium currents in immature oocytes of starfish. The results are consistent with the binding of three ions to a site with a $\text{p}K$ of 6.26 at or near the inner surface of the membrane. The block is not voltage dependent, and the activation kinetics of the current are not affected by changes in pH_i .

The fact that three protonatable groups act cooperatively to block the channel means that small changes in pH_i between 5.8 and 6.8 have fairly large effects on inward rectification. In addition, the increase in $[\text{Na}^+]_i$ that occurred in our experiments (Fig. 5) may have caused us to underestimate the effect of pH_i on the currents in the range of 7.09 (normal) to 6.8. In a few experiments, increasing the pH_i by ~ 0.4 unit caused a reduction in the current, which suggests that the normal pH_i is an optimum value so that inward rectifier currents decrease when the pH_i is changed by a sufficient amount in either direction. In this regard, the effect of $[\text{H}^+]_i$ on the inward rectifier is different from that of $[\text{Na}^+]_i$. The resting $[\text{Na}^+]_i$ of the oocyte is approximately at the midpoint of the Na^+ binding curve ($K_m = 35$ mM), so that small increases or decreases in $[\text{Na}^+]_i$ cause the current to increase or decrease, respectively (Hagiwara and Yoshii, 1979). The same distinction can be made with the effect of pH_i on delayed rectification in the squid giant axon (Wanke et al., 1979), in which increasing the pH_i from the normal value increases, and decreasing the pH_i decreases, the currents.

It has been reported that decreasing the external pH blocks the inward rectifier in starfish oocytes with an apparent pK substantially higher than indicated in our results with external H^+ (Hagiwara et al., 1978). These results were caused by the presence of the permeant buffer acetate in the Ringer's solution, and were due to changes in internal, not external pH. By the same argument, it is possible that decreased internal, not external pH (Hagiwara et al., 1975), might block the inward Ca^{2+} currents in starfish oocytes (see Sharp and Thomas, 1981, for a review of the problem of using permanent weak acids to change extracellular pH or to substitute for external Cl^-).

The experiments of Fig. 5 showed that $[Na^+]_i$ increased substantially when the pH_i was decreased. This should affect the shape of the titration curve shown in Fig. 4 A and perhaps alter the pK . For example, if we assume that the same changes in $[Na^+]_i$ recorded in Fig. 5 occurred during the experiment in Fig. 3, then when the pH_i was changed from 7.23 to 6.52 and the inward rectifier currents were decreased to 88% of their control value, $[Na^+]_i$ increased from 24 to 32 mM. This change in $[Na^+]_i$ by itself would have increased the inward rectifier currents by 17% (Hagiwara and Yoshii, 1979). If we further assume, for example, that H^+ and Na^+ act at independent sites on the channel, then at $pH_i = 6.52$, the currents have actually been reduced to 75% of the control value, not 88% (that is, had the pH_i alone been changed). Whatever the nature of the interaction between Na^+ and H^+ , the actual titration curve is likely to be shifted somewhat to the right and may be altered in steepness from that in Fig. 4. Thus, inward rectification is probably slightly more sensitive to changes in pH_i , especially near the physiological range, than the experiment of Fig. 3 indicates. It would be especially interesting to examine the relationship between pH_i and inward rectification in skeletal muscle fibers (see Blatz, 1980), where there is no effect of $[Na^+]_i$ on the currents (Hestrin, 1981).

The resting pH_i value obtained in these experiments is substantially more alkaline than the value that would be in equilibrium with the membrane potential of -70 mV. The same is true for a large variety of excitable cells (Roos and Boron, 1981). The mature sea urchin egg appears to be an exception, since the resting pH_i of 6.84 (Shen and Steinhardt, 1978) is almost exactly in equilibrium with the resting membrane potential of -70 to -75 mV (see Jaffe and Robinson, 1978). In a variety of cells, increases in $[Na^+]_i$ have been recorded as a result of decreasing pH_i (e.g., Thomas, 1977; Moody, 1981). There is substantial evidence that this results from the operation of a Na^+-H^+ exchange mechanism which serves to regulate pH_i (Roos and Boron, 1981). The nonequilibrium distribution of H^+ in the starfish oocyte and the increase in $[Na^+]_i$ when the pH_i is lowered suggest that Na^+-H^+ exchange may be a part of the pH_i regulating system in this cell. In mature sea urchin eggs, fertilization triggers a Na^+ -dependent H^+ efflux (Johnson et al., 1976), but whether this is a true Na^+-H^+ exchange is a matter of controversy (see Shen and Steinhardt, 1979). Clearly it would be of interest to investigate pH_i regulation in oocytes in a manner similar to previous studies on neurons (see, for example, Thomas, 1977; Moody, 1981).

The sensitivity of inward rectification to changes in the concentrations of

intracellular ions is of particular interest in oocytes. In *Mediaster*, as well as in other species of starfish, inward rectification decreases substantially during *in vitro* maturation of the oocytes by 1-methyladenine (Miyazaki et al., 1975; J. Lansman, personal communication). Inward calcium and outward potassium currents elicited by depolarization also change during the maturation process (Miyazaki et al., 1975; Miyazaki and Hirai, 1979; Miyazaki, 1979). The effect of these changes appears to be to increase the amplitude of the fertilization potential so that an effective electrical block to polyspermy (Jaffe, 1976) develops in the mature egg (Miyazaki and Hirai, 1979; Miyazaki, 1979). Both the active transport and the intracellular concentration of ions are affected by the maturation process in various oocytes (Moreau et al., 1978; Lee and Steinhardt, 1981). It is possible that changes in pH_i or $[Na^+]_i$ during maturation cause the emergence of electrical properties characteristic of the mature starfish oocyte.

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