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Ionic and charge-displacement currents evoked by temperature jumps in *Xenopus* oocytes

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Membrane currents were recorded in voltage-clamped oocytes of *Xenopus laevis*. Currents were produced in response to temperature jumps imposed by a heating lamp. Responses were larger when the animal (pigmented) hemisphere of the oocyte was illuminated as compared to the vegetal hemisphere; they arose because of a thermal effect as they were attenuated by removal of infrared wavelengths. The temperature jump responses comprised two distinct components: (i) a slow maintained current, which inverted direction at a membrane potential of about −25 mV and, (ii) a fast transient current, which at all potentials examined (−160 to +30 mV), was inward at the onset of a light flash and outward at the offset. The slow component probably arises through temperature-dependent changes in the 'leakage' current of the oocyte, and measurements of reversal potentials in solutions of different ionic composition indicated that currents carried by Na^+ and H^+ ions contribute to the response. In contrast, the fast component was not altered by changes in composition of the bathing solution. This observation, together with the finding that the charge movements associated with the on and off transients were of similar magnitude, suggest that the fast current may arise because of the displacement of charges across the plasma membrane.

Introduction

It was previously reported (Miledi *et al.* 1987) that rapid changes in temperature, imposed by a heating lamp, can provide a novel technique to study the phosphoinositide signalling pathway in *Xenopus* oocytes. Large oscillatory currents were evoked by cooling steps applied to oocytes in which the phosphoinositide system was activated, probably because the equilibrium of a feedback element in the pathway was disturbed. Although resting oocytes showed only small currents in response to the heating light, it now seemed worthwhile to investigate these responses further. One motivation arose from the introduction of photolabile 'caged' compounds (Gurney & Lester 1987; Kaplan & Somlyo 1989). Of these, caged inositol trisphosphate has already proved very useful to study intracellular messenger systems in the oocyte (Parker 1988; Miledi & Parker 1989; Parker & Miledi 1989). Because strong illumination of the oocyte is required to photolyse caged compounds loaded into the cell, it was necessary to discover what responses might be evoked by the light *per se*, as distinct from those produced by the liberation of free messenger from its 'cage'. Secondly, it seemed that modulation

by temperature of resting currents in the oocyte might provide a useful approach to identify and characterize these currents, which are otherwise rather intractable to experimental study. This paper describes briefly the resting ionic currents modulated by temperature, and also presents evidence that temperature steps evoke a novel response that appears to arise from a displacement of charges across the plasma membrane.

Methods

Experiments were done on fully grown immature oocytes of *Xenopus laevis*, from which the surrounding cell layers were removed by treatment with collagenase, followed by mechanical stripping (Miledi & Parker 1984). Oocytes were voltage clamped by using a two electrode system (Sumikawa *et al.* 1989) and, to decrease clamp noise, both the recording and current-passing electrodes were broken to a low (less than 1 MΩ) resistance. Records of membrane current were photographed from an analogue storage oscilloscope, or were stored on floppy discs, by using a digital oscilloscope, for subsequent computer analysis. During recording, the oocytes were continuously superfused with Ringer solution (composed in millimoles per litre): NaCl, 120; KCl, 2; $CaCl_2$, 1.8; HEPES, 5; at pH 7.0) at room temperature (22–24 °C). Rapid temperature changes were imposed by applying flashes of light from a continuous 75 W xenon arc lamp. The duration of the flash was set by an electrically actuated shutter, and the light was focused onto the oocyte through a Zeiss epifluorescence system and a 6 × Neofluor objective lens. Unless otherwise noted, no filters were imposed in the light path, and the usual dichroic mirror in the epifluorescence system was replaced by a right angle prism. Oocytes were usually positioned with their animal (pigmented) hemisphere facing the light. The diameter of the light spot was adjusted by a diaphragm so as to just cover the whole hemisphere. A photomultiplier was used to monitor the light incident on the oocyte. To estimate the temperature rise produced by the heating light, the oocyte was replaced by a blackened thermocouple bead, of similar size. With the solution flowing at the usual rate, the temperature of the thermocouple rose by about 10 °C during continuous illumination.

Results

Currents evoked by light flashes

Light flashes evoked a complex series of currents in voltage-clamped oocytes, as illustrated in figure 1*a*. An important point was first to establish whether these arose from a heating effect of the light on the oocyte, or from some other cause. In particular, the chlorided silver wires which were used to make contact with the microelectrodes, sometimes show photosensitivity, and might thus introduce artefactual responses. However, this possibility could be discounted, because the current responses were abolished when the light spot was displaced so as to be clear of the oocyte, but to still illuminate the shanks of either the recording or current passing electrodes. Similarly, no responses were seen when the light was arranged as an annulus, with a central dark spot covering the oocyte, surrounded by a ring of light falling on the shanks of both electrodes.

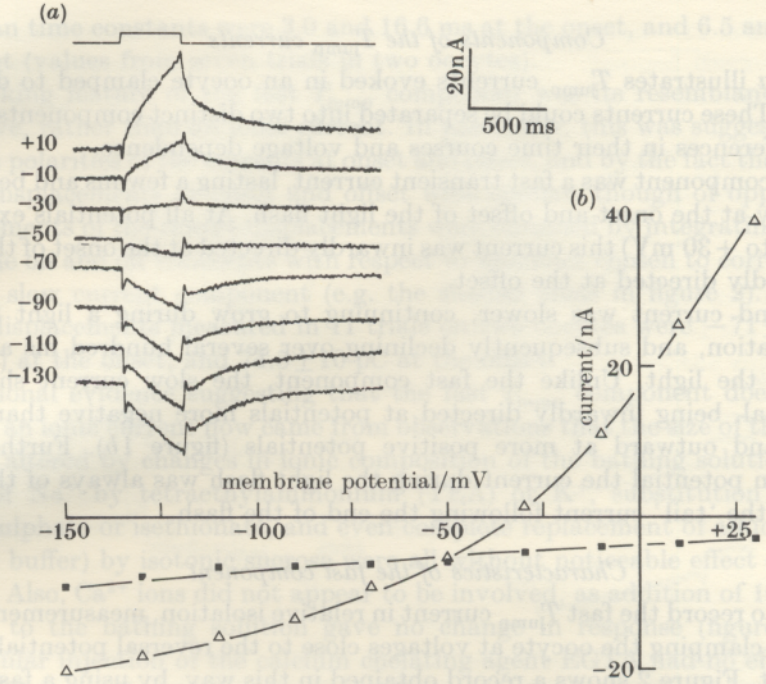

FIGURE 1. Membrane currents evoked by temperature jumps, and their voltage dependence; (*a*) traces show membrane currents evoked by a heating light flash in an oocyte clamped to different potentials (indicated in mV next to each trace). The upper trace shows the timing of the light flash. Current traces were low-pass filtered at 200 Hz; (*b*), Voltage-dependence of the different components of the T_{jump} current; (△), the size of the slow component, measured just before the end of the light flash; (■), the size of the initial transient current, measured at the onset of illumination. Data from the same oocyte as (*a*).

Further evidence implicating the oocyte itself in the generation of the responses was that appreciable responses were obtained only when the animal (pigmented) hemisphere was illuminated, and illumination of the vegetal hemisphere gave no, or very small, responses. For this reason, all experiments described here were done with the animal hemisphere facing the light.

To determine whether a temperature change, rather than light *per se*, was the important factor, a heat reflecting mirror (type 5740, Oriel Corp.) was placed in the light path. This reduced the sizes of the light flash responses by about one half, consistent with its expected attenuation of the heating effect of the light. The mirror is specified by the manufacturer to reduce the heating effect by about 60%, but to transmit visible and ultraviolet wavelengths with little attenuation. Furthermore, ultra-violet light was ineffective, as no currents were seen when a dichroic mirror was used to restrict illumination to wavelengths shorter than about 520 nm. Thus it seems that the current responses arise because of temperature changes in the oocyte, and we will refer to them as temperature jump (T_{jump}) currents.

Components of the T_{jump} currents

Figure 1*a* illustrates T_{jump} currents evoked in an oocyte clamped to different potentials. These currents could be separated into two distinct components, on the basis of differences in their time courses and voltage dependence.

The first component was a fast transient current, lasting a few ms and beginning immediately at the onset and offset of the light flash. At all potentials examined (−160 mV to +30 mV) this current was inwardly directed at the onset of the light, and outwardly directed at the offset.

The second current was slower, continuing to grow during a light flash of 400 ms duration, and subsequently declining over several hundred ms after extinguishing the light. Unlike the fast component, the slow current showed a clear reversal, being inwardly directed at potentials more negative than about −30 mV, and outward at more positive potentials (figure 1*b*). Furthermore, at any given potential the current during the light flash was always of the same polarity as the 'tail' current following the end of the flash.

Characteristics of the fast component

In order to record the fast T_{jump} current in relative isolation, measurements were made while clamping the oocyte at voltages close to the reversal potential for the slow current. Figure 2 shows a record obtained in this way, by using a fast sweep speed and minimal (500 Hz) filtering. Following the onset of the light flash the current rose to a peak within about 1 ms. This rise time is comparable to the opening time of the shutter, and may therefore be artefactually limited. Peak currents evoked at the maximal intensity of the illuminator ranged between about 5 and 15 nA in different oocytes. Following the peak, the current declined within 10–20 ms, and at the offset of the light flash a similar, though inverted, current response was seen. Usually, the peak current at the 'off' was smaller than at the 'on', and the decay of the current was slower. The decay of the current at both the onset and offset of the light could be fitted well by the sum of two exponentials.

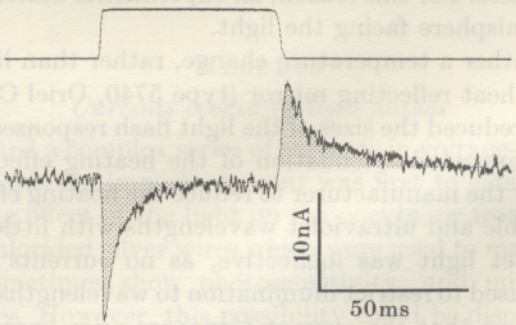

FIGURE 2. Fast components of the T_{jump} current, recorded in an oocyte clamped at a potential (−18 mV) close to the equilibrium of the slow component. The upper trace monitors the light flash, and the lower shows membrane current, low-pass filtered at 500 Hz. Shading indicates the areas integrated to estimate the charge displacements associated with the transients.

The mean time constants were 3.0 and 16.6 ms at the onset, and 6.5 and 59 ms at the offset (values from seven trials in two oocytes).

A striking feature of the fast T_{jump} component was its resemblance to a capacitative, rather than an ionic current. In particular, this was suggested by the opposite polarities of the currents at onset and offset, and by the fact that the total charge displacements at onset and offset were similar, though of opposite sign. Measurements of the charge displacements were obtained by integrating the areas under the on and off transients with respect to baselines chosen to correct for any residual slow current component (e.g. the shaded areas in figure 2). The mean charge displacements measured in 11 trials on two oocytes were -71 ± 7 pC (s.e. of mean) at the onset, and 74.5 ± 10 pC at the offset.

Additional evidence suggesting that the fast T_{jump} component does not arise through an ionic current flow came from observations that the size of the response was not altered by changes in ionic composition of the bathing solution. Substitution of Na^+ by tetraethylammonium (TEA) or K^+, substitution of Cl^- by methylsulphate or isethionate, and even complete replacement of all ions (except HEPES buffer) by isotonic sucrose were all without noticeable effect on the fast current. Also, Ca^{2+} ions did not appear to be involved, as addition of 10 mM Mn^{2+} or La^{3+} to the bathing solution gave no change in response (figure 3*a*), and intracellular injection of the calcium chelating agent EGTA had no effect (figure 3*b*). Finally, no effects were observed following bath application of tetrodotoxin (6 μM), lidocaine (1 mM) and dihydrouabain (1 mM).

As illustrated in figure 1*b*, the voltage dependence of the fast T_{jump} current was slight, but in this and three further oocytes examined, the current decreased as the membrane potential was made increasingly positive. It was not possible to obtain records at potentials more positive than +30 mV because of the activation of various voltage-dependent currents (Baud *et al.* 1982; R. M. Woodward, I. Parker & R. Miledi, unpublished data), but extrapolation indicated that the current would not reduce to zero until polarization to potentials of at least +80 to +100 mV.

Characteristics of the slow component

The results described here were obtained from oocytes with high (greater than 1 MΩ) input resistance, and these gave slow T_{jump} currents of 10–20 nA at −60 mV. Much larger currents (100 nA or more) could be obtained in oocytes with low input resistance. In particular, large responses were evoked shortly after impalement of the electrodes, but subsequently decreased as the oocyte membrane sealed around the electrodes.

The mean reversal potential for the slow current was -25 ± 1.7 mV (s.e. of mean, 19 oocytes). This voltage is close to the chloride equilibrium potential in the oocyte (Kusano *et al.* 1982), but several observations indicated that chloride ions are not primarily responsible for carrying the slow T_{jump} current. One indication came from two oocytes that showed spontaneous oscillations in chloride current (Kusano *et al.* 1982), because the reversal potential for the oscillations was 3–4 mV more negative than for the T_{jump} current. To further explore the ionic basis of the T_{jump} current, the reversal potential of the response was determined while bathing the oocyte in solutions of different ionic composition. For the oocyte illustrated in

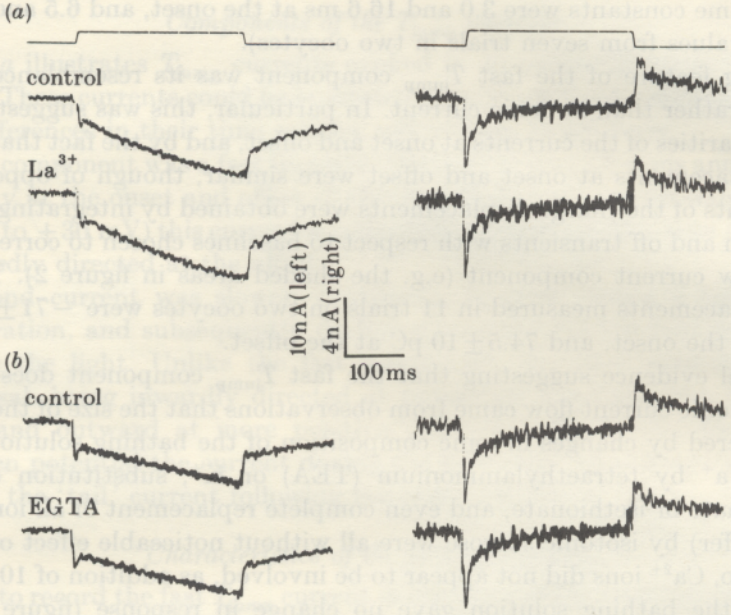

FIGURE 3. The T_{jump} currents do not depend upon calcium. Traces show membrane currents, recorded on the left at a potential of −60 mV, and on the right at potentials (−15 mV in (*a*) −23 mV in (*b*)) close to the equilibrium of the slow component. The upper traces in each frame monitor the light flash; (*a*) control records in normal Ringer solution (upper), and after addition of 10 mM La^{3+} to the bathing solution (lower); (*b*) records from a second oocyte, obtained before (upper) and after (lower) loading the oocyte with 20 pmol EGTA.

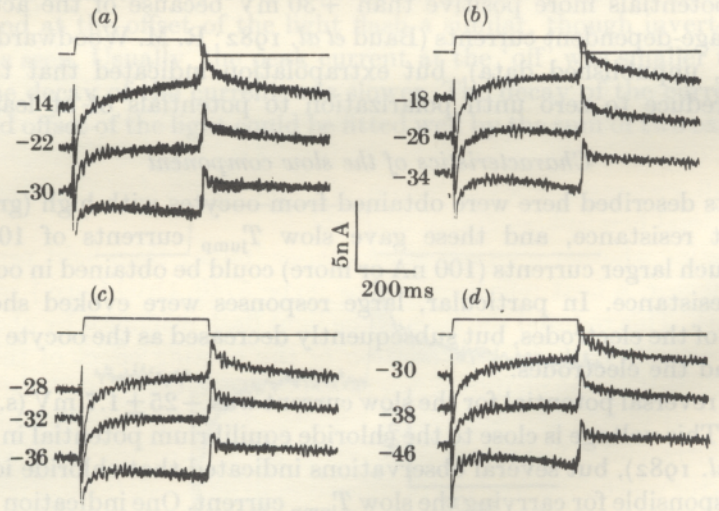

FIGURE 4. Reversal potentials of the slow T_{jump} component in solutions of different ionic composition. In each frame, the top trace monitors the light flash, and the lower traces show currents recorded at the clamp potentials indicated (in millivolts). All records are from a single oocyte; (*a*) the oocyte was bathed in normal Ringer solution (control); (*b*) solution in which NaCl was replaced by Na-isethionate; (*c*) one half of the NaCl in the Ringer solution was replaced by KCl; (*d*) NaCl was replaced by TEA-Cl.

figure 4 the reversal potential in normal Ringer solution was −22 mV (figure 4*a*), and complete replacement of Cl^- ions by isethionate changed the reversal only slightly to −26 mV. Similar results were obtained in a further two oocytes where Cl^- was substituted by methylsulphate. In contrast, complete substitution of Na^+ by tetraethylammonium (TEA), and partial substitution by K^+ gave larger shifts in reversal to potentials of, respectively, −38 and −32 mV (figure 4*c*, *d*). Thus it seems that although Cl^- contributes little to the slow T_{jump} current, its role in modulation of a Na^+ current may be important. Furthermore a role for H^+ ions was suggested by the finding that changes in extracellular pH altered the reversal potential. In two oocytes, decreasing pH from 7.0 to 6.5 shifted the reversal by 4–6 mV more positive, whereas increasing pH to 7.5 shifted the reversal 5 mV more negative.

Calcium ions did not appear to be involved in the generation of the slow T_{jump} current. Addition of La^{3+} to the bathing solution to block calcium entry (figure 3*a*), and intracellular injection of EGTA (figure 3*b*), caused little change in magnitude or reversal potential of the response.

Discussion

The results show that temperature steps, applied to oocytes by a heating lamp, evoke two types of membrane current response, with different properties and probably different origins. Both of these responses were quite small (tens of nA or less) with the optical system employed, and are unlikely to cause serious problems in experiments where powerful illumination is used to photolyse caged compounds loaded into the oocyte (Parker 1988; Miledi & Parker 1989; Parker & Miledi 1989). In particular, the currents were prominent only when the light included infrared wavelengths, and when the animal (pigmented) hemisphere of the oocyte was illuminated. As the optimal wavelengths for photolysis of caged compounds are in the ultraviolet (Gurney & Lester 1987; Kaplan & Somlyo 1989), the temperature effects can be greatly reduced by appropriate filtering. Furthermore, maximal responses to photolysis of caged inositol trisphosphate are obtained by illumination of the vegetal rather than the animal hemisphere, presumably because there is no pigment to screen the caged compound loaded into the oocyte from light (Parker & Miledi 1989).

The T_{jump} responses were obtained in oocytes that were treated with collagenase to remove follicular and other enveloping cells, so that they must arise across the oocyte membrane proper. The difference in sensitivity between the animal and vegetal hemispheres is likely to arise because the pigment in the animal hemisphere absorbs visible and infrared light, thus giving rise to a greater temperature increase. However, it remains possible that the pigment might play some more direct role in generating the responses, and differences in numbers of membrane channels between the two hemispheres (Miledi & Parker 1984) could also be a factor.

Because the slow component of the T_{jump} current showed a strong dependence upon voltage and ionic composition of the bathing solution, it almost certainly arises through temperature-dependent modulation of resting ionic fluxes. Furthermore, the lack of effect of dihydrouabain indicates that changes in activity of the

electrogenic Na^+-pump (Eisner *et al.* 1987) do not contribute appreciably to the response. Changes in reversal potential of the current were seen following alterations in Na^+ and H^+ concentrations, but were smaller with changes in Cl^- or Ca^{2+}. This suggests that currents carried by Na^+ and H^+ ions may influence the resting potential in defolliculated oocytes, but it is difficult from the present results to assess the relative importances of different ions, as the sensitivities of he respective conductances to temperature are not known.

The fast component of the T_{jump} response appeared as a transient inward current at the beginning of a light flash, and as a transient outward current after the flash. The areas under the on and off responses were similar, and the responses were impervious to changes in ionic composition of the bathing solution. They probably arise, therefore, from a displacement of charges across the plasma membrane, rather than from a flux of ions through membrane channels. The largest charge displacements observed were about 100 pC, corresponding to the movement across the membrane of roughly 6×10^8 electron charges. Because the animal hemisphere of the oocyte has a surface area of about 6×10^6 μm^2 (assuming a diameter of 1.2 mm and surface infolding that increase the area tenfold (Dascal 1987)), this gives a minimum estimate for the charge density of about 100 per μm^2. In fact, the available number of mobile charges is probably greater than this, because the maximum output of the illumination system was not sufficient to saturate the response.

The mechanism giving rise to the fast T_{jump} current is at present unclear, but several possibilities may be considered. One is that changes in temperature alter the capacitance of the cell membrane, giving rise to charging currents. However, this seems unlikely, as responses were still obtained when the potential across the membrane was reduced to zero. A different explanation is suggested by similarities between the fast T_{jump} current and the charge displacement currents previously observed in nerve and muscle cells following changes in membrane potential, which are thought to arise from the movement of charged particles in the membrane (Almers 1978). The fast T_{jump} current might originate in a similar way if the oocyte possesses electrical dipoles (e.g. on protein molecules) that are unequally distributed across the membrane. The distribution of charges will be given by the Boltzmann equation, which includes terms for both temperature and membrane potential. When the temperature is stepped to a higher value, the charges will approach closer toward an even distribution, thus giving rise to a displacement current. In this model, the fast T_{jump} current should reduce to zero as the membrane potential is altered so as to distribute charges equally across the membrane. Measurements such as those in figure 1 indicate that this would happen only at strongly positive potentials, suggesting that charge is very asymmetrically distributed across the resting membrane. We do not yet know what molecules in the membrane might carry the charges, but candidates include the various ion channels in the oocyte (Miledi 1982; Miledi & Parker 1984; Baud *et al.* 1984) that, because of their voltage sensitivity, are presumed to possess mobile charges.

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