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Transient potassium current in native *Xenopus* oocytes

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Depolarization of follicle-enclosed oocytes of *Xenopus laevis* obtained from some donors elicits, in addition to other responses, a fast transient outward current. After holding the membrane potential at −100 mV this response begins to be activated by depolarizations to around −30 mV, and increases progressively as the voltage is raised further. A striking characteristic is that the current recovers only slowly (several seconds) from inactivation following a depolarizing pulse. Because of its outward direction and insensitivity to removal of extracellular chloride or addition of tetrodotoxin, the current probably arises largely through a flux of potassium ions. The current was abolished after treatment of oocytes with collagenase to remove enveloping cells, and although it was blocked by barium and zinc ions, tetraethylammonium was relatively ineffective. In addition, the potassium current was unaffected by 5 mM manganese, suggesting that it does not arise as a consequence of an influx of calcium into the oocyte.

Introduction

Oocytes of *Xenopus laevis* possess a variety of ion channels, which give rise to membrane currents upon depolarization of the cell. The list of currents so far observed includes a calcium-dependent chloride current (Miledi 1982; Barish 1983), a slow sodium current induced by strong depolarization (Baud *et al.* 1982), a transient sodium current (Parker & Miledi 1987), and a maintained potassium current (Peres *et al.* 1985). These currents are of interest in their own right. Also, because of the increasing popularity of the oocyte as a translation system for the functional expression of exogenous membrane channels, it is important to learn more about those channels already present in the 'native' oocyte. Here, we extend the list of 'native' voltage-activated currents by describing a fast transient outward current, which appears to be carried by potassium ions and is independent of extracellular calcium.

Methods

Experiments were made on ovarian follicle-enclosed oocytes of *Xenopus laevis*, and on oocytes from which the enveloping cells were removed by collagenase treatment (Kusano *et al.* 1982; Miledi & Parker 1984). These were placed in a bath continuously perfused with Ringer solution at room temperature (22–24 °C), and were voltage-clamped using a two-electrode system (Miledi 1982; Gundersen *et al.*

1983). The membrane potential was usually held at −100 mV, from which it was stepped briefly (usually 120 ms) to various potentials. Current–difference records (see below) were obtained by the use of a digital storage oscilloscope (Nicolet 3091), with pulses given at various intervals.

Results

Fast outward current activated on depolarization

Depolarization of oocytes of *Xenopus laevis* to potentials around 0 mV elicits passive capacitative and leakage currents, often accompanied by a slowly rising and falling outward current due to chloride ions moving through channels opened by a preceding influx of calcium into the oocyte (Miledi 1982; Barish 1983). More rarely, a transient inward current is also apparent due to the activation of voltage-dependent sodium channels (Parker & Miledi 1987). The present report concerns a further response to depolarization, which is seen as a rapidly rising and decaying outward current.

Figure 1*a* shows records from a follicle-enclosed oocyte which displayed this fast outward current, but in which the sodium current was absent and the outward chloride current was small. Depolarization from −90 to −20 mV elicited a tran-

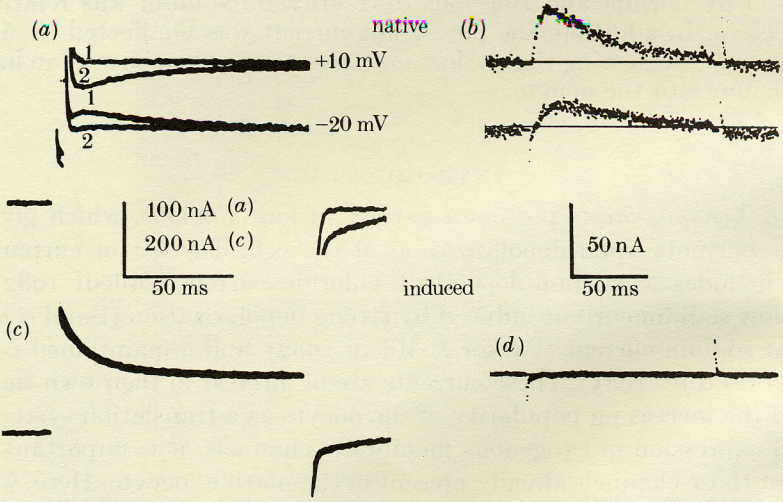

Figure 1. (*a*, *b*) Fast outward currents evoked by depolarization in a 'native' follicle-enclosed oocyte from *Xenopus*. (*a*) Superimposed traces showing membrane currents evoked by two pairs of depolarizing pulses from −90 mV to −20 mV and +10 mV. The oocyte was stepped to each potential for 120 ms, and the interval between the pulses in each pair was 1 s. Numbers next to the traces identify the responses to the first and second pulses in each pair. In this, and other figures, upward deflections correspond to outward membrane currents. (*b*) Traces show current differences obtained by subtraction of the responses to paired depolarizations to −20 mV and +10 mV shown in (*a*). (*c*) Transient outward potassium current evoked in a collagenase-treated oocyte which was injected with messenger RNA from rat cerebral cortex. Superimposed traces show almost identical currents evoked by a pair of depolarizing pulses from −100 to −20 mV, at an interval of 1 s. (*d*) Subtraction of the responses to paired depolarizations in (*c*) showing almost no current difference.

sient outward current, which decayed within about 50 ms. A characteristic of this current (described in more detail later) was that it recovered only slowly from inactivation. Thus a second identical depolarizing pulse applied after an interval of 1 s elicited mainly passive currents, and the difference between the two current records gave a measure of the fast outward current in isolation. It was therefore convenient to study this current by using a digital storage oscilloscope to subtract the current responses evoked by pairs of identical depolarizing pulses applied at an interval of 1 s (figure 1*b*). This procedure was especially useful at potentials close to 0 mV, where the fast outward current was otherwise obscured by the rising phase of the calcium-dependent chloride current.

The results presented here are based on recordings from oocytes obtained from a single donor; but, over the years, we have occasionally seen similar currents in oocytes from other donors in London and Irvine. The mean size of the fast outward current, in ten follicle-enclosed oocytes was 36 ± 3.5 nA (s.e. of mean), measured by subtraction of currents in response to paired depolarizing pulses to 0 mV. Three oocytes from the same donor, which were treated with collagenase to remove enveloping cells, all failed to show any detectable fast outward current.

As described later, the fast outward current probably arises through activation of potassium channels. Transient voltage-activated potassium currents can also be induced in the oocyte by injection of messenger RNA from brain and other sources (Gundersen *et al.* 1983), but these heterogenic currents show properties different to the endogenous current in native oocytes. Specifically, the induced potassium currents were seen in collagenase-treated oocytes, and they recovered rapidly from inactivation, so that paired depolarizations at an interval of 1 s evoked virtually identical current responses (figure 1*c*, *d*).

Voltage dependence of activation

The voltage dependence of the fast outward current was determined by stepping the clamp potential to various levels during two identical depolarizing pulses, and recording the difference in currents elicited by the two pulses in each pair (see figure 1). Reponses were first detected when the membrane potential was raised to -30 mV, and further increases gave currents of progressively greater size (figure 2*a*). At all potentials (-30 to $+50$ mV) the current was in an outward direction, and it increased almost linearly with voltage above -25 mV (figure 2*b*).

The rising phase of the current was relatively slow during depolarization to levels just above threshold and, for example, at -20 mV the peak current was recorded 10–20 ms after the onset of depolarization. At more positive potentials the current increased more rapidly, and the rising phase was often not well resolved in the present recordings. Different from this, the time-course of decline of the current during maintained depolarization did not show any obvious dependence upon membrane potential, and two oocytes examined over the voltage range -20 to $+40$ mV showed no systematic changes in half-time of decay. During depolarization to 0 mV, the mean half-time of decay of the current was 30.6 ± 3.5 ms (s.e. of mean, eight oocytes).

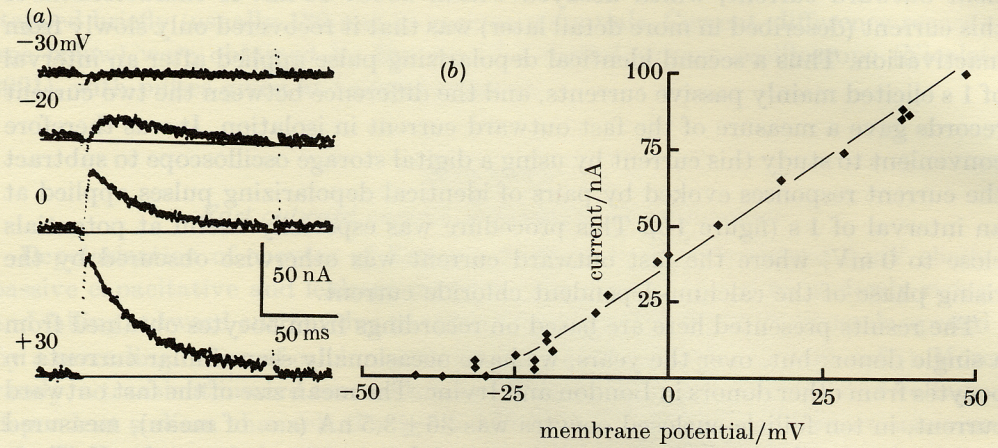

FIGURE 2. Voltage dependence of activation of the fast outward current. (*a*) Traces show current derived by paired-pulse subtraction, evoked by depolarizations from −100 mV to the potentials indicated (in millivolts). (*b*) Variation in peak size of the current with potential. Data are from the same oocyte as in (*a*). Similar results were obtained in a further three oocytes.

Ionic basis of the fast outward current

The voltage dependence of the fast outward current indicates that it cannot be due to a flux of sodium ions because the current was outward over the voltage range −30 to +50 mV, whereas both native and induced sodium currents in the oocyte are inward at these potentials (Gundersen *et al.* 1983; Parker & Miledi 1987; Sigel 1987). Also, the response was unaffected by addition of tetrodotoxin (300 nM) to the bathing solution. Calcium ions may also be ruled out as the charge carriers directly responsible for the current because calcium influx into the oocyte would similarly give rise to inward membrane currents. It was also unlikely that chloride ions could have been responsible for the response, as it was seen as an outward current with depolarization to −30 mV, even though the chloride equilibrium potential in the oocyte is more positive than this (*ca.* −20 to −25 mV (Kusano *et al.* 1982; Barish 1983)). To strengthen this conclusion, we reduced the chloride concentration in the bathing solution to 10% of the normal level, by replacement with methylsulphate ions. This procedure shifts the chloride equilibrium potential to positive voltages but, nevertheless, the fast outward current was almost unchanged in this solution, and remained in an outward direction even at potentials of −20 and −10 mV. Therefore, we conclude that the fast outward current is carried largely by an efflux of potassium ions.

Action of blocking agents

The fast potassium current might arise because the oocyte membrane contained potassium channels which are directly gated by membrane potential. Alternatively, calcium-activated potassium channels might be opened subsequent to an influx of calcium through the voltage-gated calcium channels known to be present in the oocyte (Miledi 1982; Barish 1983). To distinguish between these possibilities we added manganese to the bathing solution because this agent blocks calcium

entry in the oocyte (Miledi 1982; Parker *et al.* 1985). At a concentration of 5 mM, manganese did not appreciably alter the fast potassium current (figure 3*a*), even though the calcium-dependent chloride current in the same oocyte was suppressed.

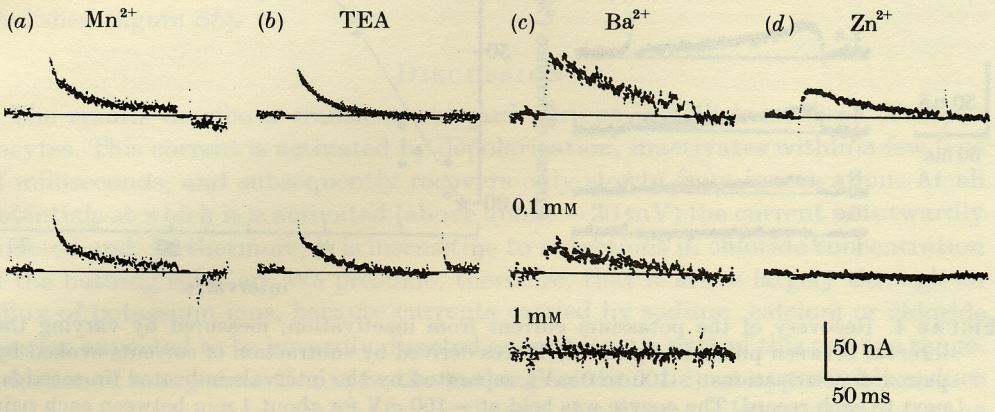

FIGURE 3. Pharmacology of the fast potassium current. Traces show responses to depolarization from −100 to 0 mV, derived by paired-pulse subtraction. In each frame the upper trace is a control record obtained in normal Ringer solution, whereas the lower trace was obtained after adding various agents to the bathing solution. (*a*) Effect of 5 mM $MnCl_2$. (*b*) Effect of 20 mM TEA bromide. (*c*) Ringer solution contained $BaCl_2$ at concentrations of 0.1 mM (middle trace) and 1 mM (lower trace). (*d*) Action of 0.1 mM $ZnCl_2$. Records in (*a*) and (*b*) are from a single oocyte, those in (*c*) and (*d*) are from two further oocytes. The rising phase of the potassium current was not resolved in (*a*) and (*b*).

Voltage-gated potassium channels in many tissues are blocked by tetraethylammonium (TEA) ions, although the sensitivity of different types of potassium channels varies widely (Stanfield 1983). The fast potassium current in the oocyte was relatively resistant to TEA, showing little block even at a concentration of 20 mM (figure 3*b*). In contrast, the current was reduced to about one half by barium at a concentration of 0.1 mM, and was almost completely abolished by 1 mM barium (figure 3*c*). Zinc ions were also effective in blocking the potassium current, which was abolished at a concentration of 1 mM (figure 3*d*).

Inactivation and recovery from inactivation

As mentioned above, the potassium current recovered only slowly from inactivation when the oocyte was repolarized to −100 mV following a depolarizing pulse of few hundred milliseconds duration. To monitor the rate of recovery, we applied two depolarizing pulses separated by a variable interval during which the oocyte was clamped to −100 mV. At short intervals, the difference between the two current traces was large, because there was little recovery of the potassium current at the time of the second pulse. However, as the pulse interval was made longer, the current difference became smaller (figure 4*a*) as the potassium current evoked by the second pulse recovered in size towards the control value. Figure 4*b* shows measurements of the rate of recovery from inactivation, derived in this way in two oocytes. Complete recovery of the response occurred only after an interval of about 15 s at −100 mV, and for intervals between about 1 and 10 s the response

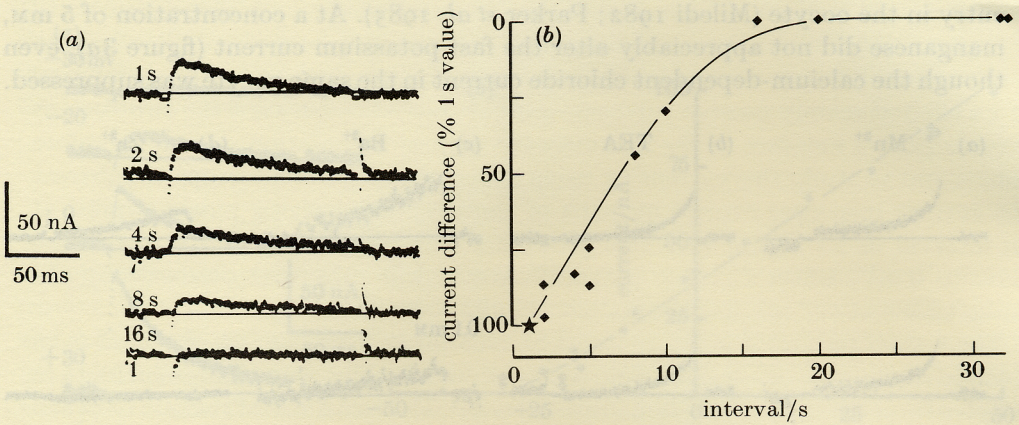

FIGURE 4. Recovery of the potassium current from inactivation, measured by varying the interval between pulses in a pair. (*a*) Traces derived by subtraction of currents evoked by paired depolarizations (−100 to 0 mV), separated by the intervals indicated (in seconds) next to each record. The oocyte was held at −100 mV for about 1 min between each pair of stimuli. (*b*) Difference in currents evoked by the first and second pulses of a pair, plotted against pulse interval. Points are single measurements from two oocytes, and are expressed as a percentage of the mean value in each oocyte at a pulse interval of 1 s.

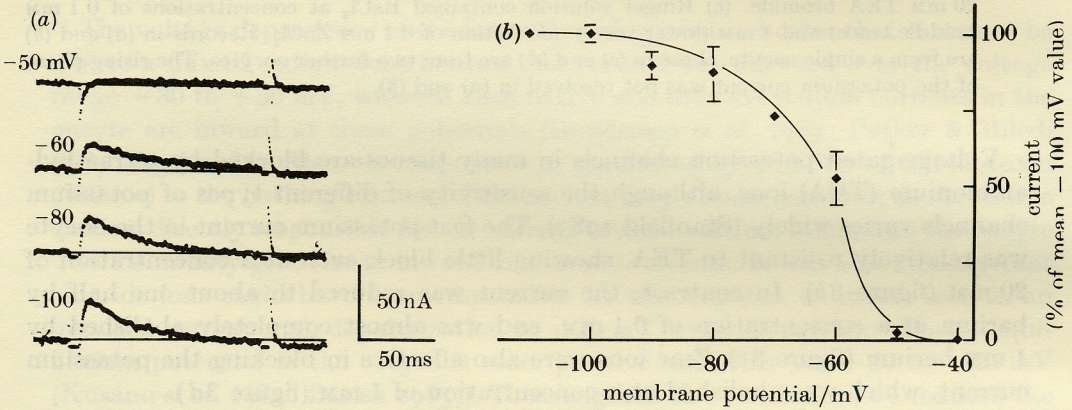

FIGURE 5. Steady-state inactivation of the potassium current. (*a*) Currents derived by paired-pulse subtraction with depolarization to 0 mV, after holding the oocyte at different potentials (indicated in millivolts) for about 1 min. (*b*) Dependence of size of the current with holding potential. Data are from three oocytes, and are expressed as a percentage of the mean current in each oocyte evoked by depolarization from −100 to 0 mV. Bars indicate ±1 s.e. of mean. Points without bars are mean values from two or three observations.

to the second pulse appeared to grow about linearly with time. Our usual recording procedure was to measure current-differences with a pulse interval of 1 s. Extrapolation of the relation in figure 4 indicates that these measurements would slightly underestimate (by about 10%) the true size of the outward potassium currents.

The steady-state inactivation of the fast potassium current was determined by clamping the oocyte to different potentials for about one minute, and then

recording the potassium current in the usual way by subtraction of responses to paired depolarizations to 0 mV (figure 5*a*). Raising the holding potential from −100 to −60 mV gave a reduction in the potassium current to about one half, whereas at a holding potential of −50 mV the current was almost completely abolished (figure 5*b*).

Discussion

The results descibe a transient outward current in follicle-enclosed *Xenopus* oocytes. This current is activated by depolarization, inactivates within a few tens of milliseconds, and subsequently recovers only slowly from inactivation. At all potentials at which it is activated (above about −30 mV) the current is outwardly directed and, furthermore, it is insensitive to reductions in chloride concentration of the bathing solution. We presume, therefore, that it arises largely through an efflux of potassium ions, because currents carried by sodium, calcium or chloride ions are expected to be inwardly directed over at least a part of this voltage range. Addition of manganese (5 mM) to the bathing solution did not appreciably reduce the transient potassium current, even though this blocks calcium entry into the oocyte and abolished the calcium-dependent chloride current. Thus the response appears not to arise through calcium-activated potassium channels (Schwarz & Passow 1983) opened as a result of calcium influx through voltage-gated channels but, instead, the potassium channels may be directly operated by the membrane potential. The cellular localization of these potassium channels is at present uncertain. Removal of follicular and other enveloping cells with collagenase abolished the transient potassium current in the oocyte, suggesting that it may arise in the follicular cells, which are electrically coupled to the oocyte proper (Browne *et al.* 1979; Woodward & Miledi 1987). However, we cannot as yet exclude the alternative possibility that collagenase impaired the functioning of channels located in the oocyte membrane.

A voltage-activated potassium current has previously been observed in frog (*Rana esculenta*) oocytes (Peres *et al.* 1985), as well as in *Xenopus* oocytes (R. Miledi, unpublished observations). However, that current showed important differences from the one described here. In particular, it activates slowly (several hundred milliseconds), but is well maintained throughout depolarizations lasting several seconds. Also, the current in *Rana* oocytes became apparent at more negative potentials (*ca.* −50 mV) than the transient potassium current in *Xenopus* oocytes, and was still present after manual removal of the follicular cell layer.

Depolarization-activated potassium currents display a wide range of properties in different species and tissues, but they may be broadly divided into two classes (Hille 1984): slowly activating, maintained currents ('K' type), and rapidly inactivating currents ('A' type). The transient potassium current in the oocyte thus appears to correspond more closely to the A-type current. Potassium currents with a similar, transient, time-course can also be induced in the oocyte after injection of messenger RNA from brain (Gundersen *et al.* 1983, 1984). However, these exogenous responses differ in some respects from the current in native oocytes. Most notably, they recover rapidly from inactivation, so that two depolarizing pulses applied at an interval of 1 s elicit almost identical responses.

Together with the fast sodium current we have previously described (Parker & Miledi 1987), the transient potassium current could provide the basis for oocytes to generate action potentials of the type found in nerve and muscle cells. However, although action potentials could be elicited in oocytes induced to express large numbers of exogenous sodium and potassium channels after injection with foreign messenger RNA (Gundersen *et al.* 1984), it is unlikely that action potentials would occur in native oocytes. This is because even the largest sodium and potassium currents that we have recorded are too small, and brief, to charge the large membrane capacitance of the oocyte. In addition, the transient potassium current is present to an appreciable extent in oocytes from only a small proportion of donors, so that it is unclear whether it serves any functional role. Several other membrane responses, including sensitivity to acetylcholine (Kusano *et al.* 1982) and GABA (R. Miledi, unpublished observations), a fast sodium current (Parker & Miledi 1987) and a chloride current activated on hyperpolarization (Parker & Miledi 1988), are also found in oocytes from only some donors. In all these cases it may be that the respective channels and receptors arise because of an apparently random expression of genes which are of importance at later stages in the development of the frog.

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