Proc. R. Soc. Lond. B **238**, 369–381 (1990)
Printed in Great Britain

A slowly inactivating potassium current in native oocytes of *Xenopus laevis*

By I. Parker and I. Ivorra

Laboratory of Cellular and Molecular Neurobiology, Department of Psychobiology, University of California Irvine, California 92717, *U.S.A.*

(*Communicated by R. Miledi, F.R.S. – Received* 30 *August* 1989)

Membrane currents were recorded in voltage-clamped oocytes of *Xenopus laevis* in response to voltage steps. We describe results obtained in oocytes obtained from one donor frog, which showed an unusually large outward current upon depolarization. Measurements of reversal potentials of tail currents in solutions of different K^+ concentration indicated that this current is carried largely by K^+ ions. It was strongly reduced by extracellular application of tetraethylammonium, though not by Ba^{2+} or 4-aminopyridine. Removal of surrounding follicular cells did not reduce the K^+ current, indicating that it arises across the oocyte membrane proper. Activation of the K^+ conductance was first detected with depolarization to about −12 mV, increased with a limiting voltage sensitivity of 3 mV for an e-fold change in current, and was half-maximally activated at about +10 mV. The current rose following a single exponential time-course after depolarization, with a time constant that shortened from about 400 ms at −10 mV to about 15 ms at +80 mV. During prolonged depolarization the current inactivated with a time constant of about 4 s, which did not alter greatly with potential. The K^+ current was independent of Ca^{2+}, as it was not altered by addition of 10 mM Mn^{2+} to the bathing medium, or by intracellular injection of EGTA. Noise analysis of K^+ current fluctuations indicated that the current is carried by channels with a unitary conductance of about 20 ps and a mean open lifetime of about 300 ms (at room temperature and potential of +10 to +20 mV).

Introduction

Immature oocytes of *Xenopus laevis* have been found to possess a surprising diversity of ion channels that give rise to membrane currents on depolarization; the list of these is still growing. Those so far described include Ca^{2+} channels (Miledi 1982; Barish 1983; Dascal *et al.* 1986), tetrodotoxin sensitive (Parker & Miledi 1987*b*) and insensitive (Baud *et al.* 1982) Na^+ currents, and transient K^+ currents (Parker & Miledi 1988*a*). As we have discussed before (Parker & Miledi 1987*b*, 1988*a*), some of these currents are prominent only in oocytes obtained from a small proportion of donor frogs. Even then, they may be so small or brief that they would not be expected to influence the electrical potential of the oocyte. Although we cannot yet attribute any function to such currents it is, nevertheless, important to characterize them when afforded the opportunity by a frog yielding oocytes with a prominent response. One reason is that because of the increasing popularity

of the oocyte as an expression system for exogenous channels (Dascal 1987; Miledi *et al.* 1989), it is important to be aware of those channels that may already be present in the native oocyte. Secondly, even if the channels do not serve a function in the oocyte, they may be expressed by genes that become important at later stages of development. By studying the occurrence of various types of channel in the oocyte we may gain clues about the factors that regulate their expression. Furthermore, it may actually be easier to study the channels in the oocyte, rather than when they are expressed later in development in smaller and less accessible cells.

In this paper we describe a novel K^+ current, activated by depolarization, which has properties markedly different to those of K^+ currents previously described in anuran oocytes (Peres *et al.* 1985; Parker & Miledi 1988*a*).

Methods

The experiments described here were done on oocytes of *Xenopus laevis*, obtained from a single donor frog supplied by Xenopus 1 (Ann Arbor, Michigan). After killing the frog by decerebration and pithing, oocytes at stages V and VI (Dumont 1972) were dissected from the ovary, and prepared in three ways: (i) epithelium removed (e.r.) oocytes; the oocyte dissected free of inner ovarian epithelium, but still surrounded by follicular cells (Miledi & Woodward 1989); (ii) manually de-folliculated oocytes; e.r. oocytes rolled across a poly-L-lysine-coated slide to remove follicular cells (Miledi & Woodward 1989); (iii) oocytes treated with collagenase (c.t. oocytes) to remove all surrounding cell layers (Sumikawa *et al.* 1989).

Electrophysiological recordings were made by using a two-electrode voltage clamp (Miledi 1982; Sumikawa *et al.* 1989). The membrane potential was usually held at -100 mV, from where it was stepped briefly to various potentials. Records of membrane current were photographed from an analogue storage oscilloscope and were also stored on floppy discs by a digital oscilloscope (Nicolet 3091). For noise analysis of current fluctuations, the clamp current was band-pass filtered at 0.03 or 0.1 Hz to 50 Hz, and directly sampled at 10 ms intervals by computer for analysis by using the SPAN package supplied by J. Dempster (University of Strathclyde, U.K.). Power spectra were computed for 512 point segments of records, and control spectra at -20 mV were subtracted from spectra obtained during activation of the K^+ current. The mean channel lifetime (τ) and current (i) were estimated by calculating $\tau = 1/(2\pi f_c)$ and $i = S(0)/4I\tau$: where $S(0)$ is the zero-frequency asymptote of the power spectrum, f_c is the half-power frequency of the spectrum and I is the mean K^+ current.

During recording, oocytes were continuously superfused with normal Ringer solution at room temperature (20–23 °C). This solution had the composition (in millimoles per litre): NaCl, 120; KCl, 2; $CaCl_2$, 1.8; HEPES, 5; at pH 7.0. Low Cl^- and low Na^+ solutions were made by substituting, respectively, sodium methylsulphate and choline chloride for NaCl. High K^+ solutions were made by substituting KCl for NaCl. Intracellular injections of EGTA were made as described previously (Miledi & Parker 1984; Parker & Miledi 1987*a*).

Results

Outward current activated by depolarization

This paper is based on recordings in oocytes obtained from a single donor frog, which showed a prominent outward current on depolarization. We have subsequently seen similar currents in oocytes from a second donor. The main features of this current are illustrated in figure 1. It was activated only by depolarization potential was stepped further up to at least +80 mV. The current rose over a few tens or hundreds of milliseconds after the onset of depolarization, becoming faster at more positive potentials, and declined over several seconds during maintained depolarization. Evidence presented later indicates that this current is carried by K^+ ions, so we will refer to it as the slow K^+ current.

All oocytes examined from both donors showed a slow K^+ current. The mean size in oocytes from the first donor, measured at +50 mV after subtraction of passive leakage currents, was 705±86 nA (standard error (s.e.) of mean,

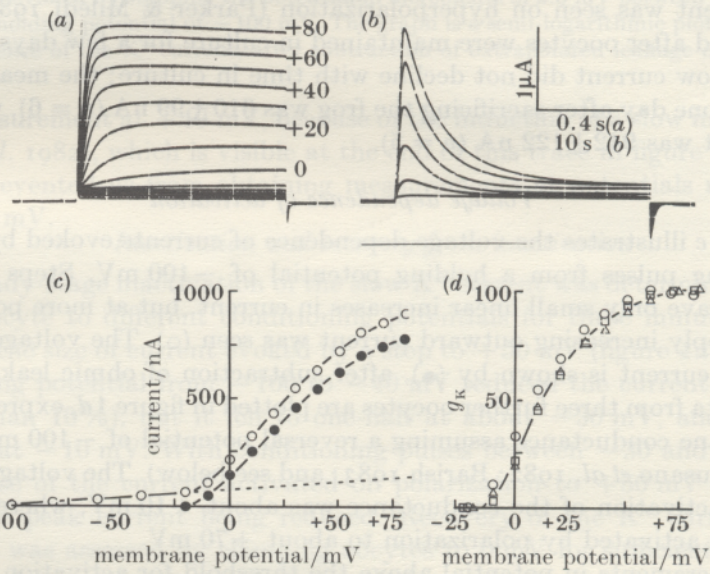

FIGURE 1. Outward current activated by depolarization of native oocytes. (*a*) Superimposed traces show membrane currents evoked by stepping the membrane potential to potentials between −80 and +80 mV, from a holding potential of −100 mV. The potential was stepped in 20 mV increments between −80 and −20 mV, and then increased in 10 mV increments. Potentials during the steps are indicated (mV) next to some of the traces. In this, and other figures, upward deflections correspond to outward membrane currents. (*b*) Records from a second oocyte, obtained at slower sweep speed to show inactivation of the current. Potential steps were from a holding potential of −100 mV to −10, 0, +10, +20, +30 and +40 mV. (*c*) Current–voltage relation measured in a third oocyte, from records like those in (*a*). (○), The peak outward current during a 1 s depolarizing step, measured with respect to the holding current at −100 mV; (●), the relation derived after subtraction of extrapolated ohmic leakage currents (----). (*d*) Voltage dependence of the K^+ conductance (g_K), measured in three oocytes (△, ○, □). Conductances were calculated assuming a reversal potential of −100 mV, and are scaled as a percentage of the maximum for each oocyte.

28 oocytes; range 120–2140 nA). In contrast, oocytes obtained from hundreds of other donors have shown either no detectable current, or small currents that were often obscured by other currents activated on depolarization, but could sometimes be seen in isolation in oocytes where the transient outward current (Miledi 1982) was absent.

The slow K^+ current was still present in oocytes from which the follicular cells were removed, indicating that it arises across the oocyte membrane proper. The mean current in 17 e.r. oocytes (i.e. oocytes still surrounded by follicular cells) was 525 ± 83 nA, whereas in 11 oocytes defolliculated manually or by collagenase treatment the current was 898 ± 156 nA. There was no apparent correlation between the presence of the slow K^+ current in oocytes from this donor and the presence of other membrane currents. Depolarization did not elicit a detectable calcium-dependent chloride current (Miledi 1982; Barish 1983), fast Na^+ current (Parker & Miledi 1987*b*), or fast K^+ current (Parker & Miledi 1988*a*), and prolonged polarization to very positive potentials induced only a slight maintained Na^+ current (Baud *et al.* 1982). Shortly after removing the oocytes from the ovary a Cl^- current was seen on hyperpolarization (Parker & Miledi 1988*b*), but this disappeared after oocytes were maintained in culture for a few days. Different to this, the slow current did not decline with time in culture; the mean current (at +50 mV) one day after sacrificing the frog was 610 ± 99 nA ($n = 6$), whereas after five days it was 942 ± 122 nA ($n = 5$).

Voltage dependence of activation

Figure 1*c* illustrates the voltage dependence of currents evoked by progressive depolarizing pulses from a holding potential of −100 mV. Steps up to about −20 mV gave only small linear increases in current, but at more positive potentials a steeply increasing outward current was seen (○). The voltage dependence of the K^+ current is shown by (●), after subtraction of ohmic leakage currents. Similar data from three further oocytes are plotted in figure 1*d*, expressed in terms of membrane conductance assuming a reversal potential of −100 mV for the K^+ current (Kusano *et al.* 1982; Barish 1983; and see below). The voltage giving half-maximal activation of the conductance was about +10 mV, whereas it became maximally activated by polarization to about +70 mV.

Small increments in potential above the threshold for activation gave steeply increasing currents (figure 2). For the oocyte illustrated, the current first became detectable at about −12 mV, and the limiting voltage sensitivity corresponded to an e-fold change in current for a change in potential by about 3 mV.

The rising phase of the slow K^+ current could be fitted well by a single exponential function at all potentials, with the exception of the first few milliseconds following the potential step, when the clamp current was not well resolved. The time constant of the rise became progressively faster at more positive potentials (figure 3), reducing from about 400 ms at −10 mV to about 15 ms at +80 mV. The decay of the current during prolonged polarization also followed a single exponential timecourse, but showed a much smaller voltage dependence than did the rising phase. For the oocyte illustrated in figure 3, the decay time constant was about 10 s at −10 mV, shortened to 3 s at +10 mV, but then became slightly longer with further polarization to +40 mV. Some error may have been introduced

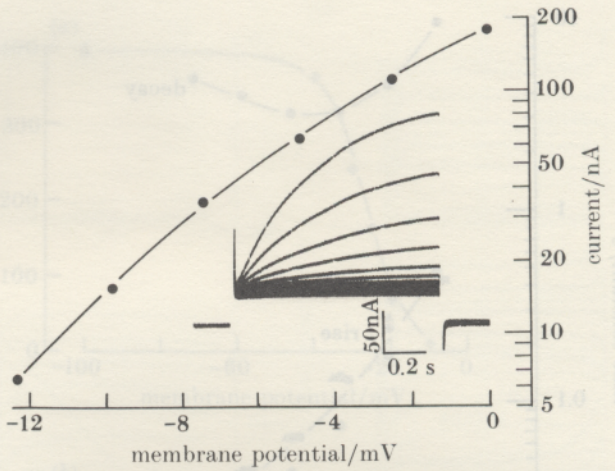

FIGURE 2. Activation of K^+ current by depolarizations close to threshold. The inset shows currents evoked by stepping the potential in increments of 2.5 mV between −30 and 0 mV, from a holding potential of −100 mV. The graph is a semi-logarithmic plot of the voltage-dependence of the K^+ current, after subtraction of extrapolated leakage currents.

in the measurement at +40 mV, because of the induction of a slow inward current (Baud *et al.* 1982), which is visible at the end of this trace in figure 1*b*. Also, this current prevented us from obtaining measurements at potentials more positive than +40 mV.

Inactivation and recovery from inactivation

The steady-stage inactivation of the slow K^+ current was determined by clamping the oocyte to different conditioning potentials for three minutes, and then recording the size of current evoked by a step to +50 mV (figure 4*a*). Raising the conditioning potential from −100 to −40 mV reduced the current only slightly (by less than 10%), but it fell to one-half at about −30 mV, and was almost abolished at −10 mV. With conditioning pulses between −30 and −10 mV the rising phase of the current activated on polarization to +50 mV was speeded, despite the peak current being reduced. Recovery of the K^+ current from inactivation was assessed by clamping oocytes to 0 mV for sufficient time for the current to delince to a steady value, and then recording the currents evoked on returning to this potential following hyperpolarizing steps of various durations and sizes. Figure 4*b* illustrates the effect of hyperpolarizing steps of fixed duration (1 s) to varying potentials. Almost no recovery was seen following a step to −50 mV, but increasingly large currents were evoked as the steps were made progressively more negative. In similar experiments (not shown), the timecourse of recovery from inactivation was followed by using steps of different duration. With a conditioning pulse to −100 mV, the current recovered to one half of the maximal value after about 300 ms. However, when the conditioning pulse was reduced to −50 mV recovery was much slower and incomplete. Following a step to −50 mV for 8 s the current approached a maximal value that was only one-quarter of that following a step to −100 mV, and the half-time of recovery to this maximal level was about 3 s.

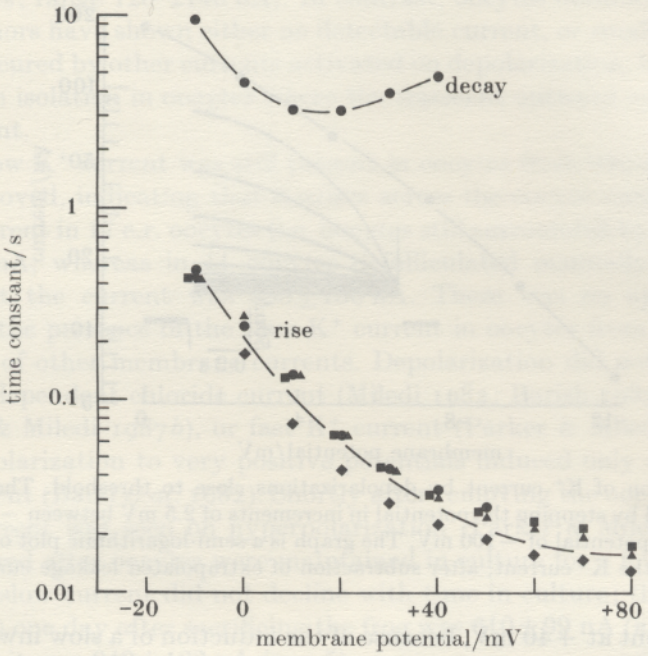

FIGURE 3. Voltage-dependence of the time constants for activation and inactivation of the K^+ current. Data are shown from four oocytes for activation (▲, ●, ◆) and from a further oocyte (same as figure 1*b*) for inactivation.

Ionic basis of the slow outward current

The ion fluxes underlying the slow outward current were investigated by measuring the reversal potentials of tail currents evoked on stepping the clamp potential back to different levels following a brief depolarization (100 ms to +40 mV) to activate the current (figure 5*a–d*). When oocytes were bathed in normal Ringer, outwardly directed tail currents were seen on returning the potential to values more positive than about −60 mV, but we were unable to obtain a clear reversal of the tail currents (figure 5*a*). The apparent inward tails visible in the records at −70 and −80 mV persisted when the conditioning depolarization was reduced or shortened so as to evoke little or no outward current and thus do not reflect a reversal of this current.

Nevertheless, the finding of an outward tail at −50 mV already suggested that the current must be carried largely by K^+ ions, as fluxes of Na^+, Ca^{2+}, and Cl^- ions are all expected to give inward currents at this potential (Kusano *et al.* 1982; Barish 1983). To confirm this, we raised the K^+ concentration in the bathing fluid to 20 mM or more. It was then possible to observe a clear reversal of the tail current; the reversal potential shifted progressively to more positive voltages as the K^+ concentration was raised (figure 5*b–d*). Figure 5*e* shows a semi-logarithmic plot of the dependence of reversal potential for K^+ concentrations between 20 and 120 mM. The measurements lie well on a straight line, which has a slope of 56 mV per decade change in K^+ concentration, and extrapolates to a reversal of about

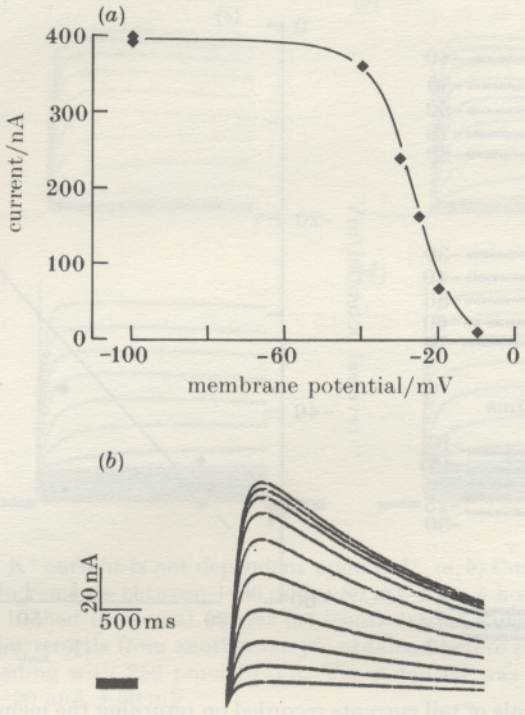

FIGURE 4. (*a*) Steady-state inactivation curve for the K^+ current. Each point shows the current evoked by a step (1 s duration) to +50 mV after holding the oocyte for 3 min at the indicated potential. Following each test, the oocyte was clamped at −100 mV for several seconds, to allow complete recovery from inactivation caused by the test pulse. (*b*) Recovery from inactivation measured by clamping the oocyte to different potentials during a 1 s step. The oocyte was held at 0 mV and stepped in 10 mV increments between −50 and −140 mV. Progressively larger currents were evoked on returning to 0 mV as the steps were made increasingly negative.

−100 mV in normal (2 mM K^+) Ringer. This slope value is very close to that expected if the current were passing through channels selective only to K^+ ions (58 mV per decade at 20 °C).

To further examine any contribution of Na^+ or Cl^- fluxes to the current, we substituted these ions whilst measuring the reversal potential of tail currents evoked in the presence of 20 mM K^+. Replacement of most (100 mM out of 120 mM) of the Cl^- in the bathing solution by methylsulphate shifted the reversal only slightly, from −48 to −52 mV, and replacement of all but 5 mM Na^+ by choline gave an even smaller shift, to −50 mV.

The slow K^+ *current does not depend upon* Ca^{2+}

The surface membrane of *Xenopus* oocytes often contains voltage-gated Ca^{2+} channels (Miledi 1982; Barish 1983), so that the slow K^+ current might arise if the oocytes also possessed many K^+ channels activated by the rise in intracellular free Ca^{2+} resulting from influx of Ca^{2+} ions (compare this with results in Boton *et al.* (1989)). However, this possibility could be discounted, as addition of 10 mM Mn^{2+}

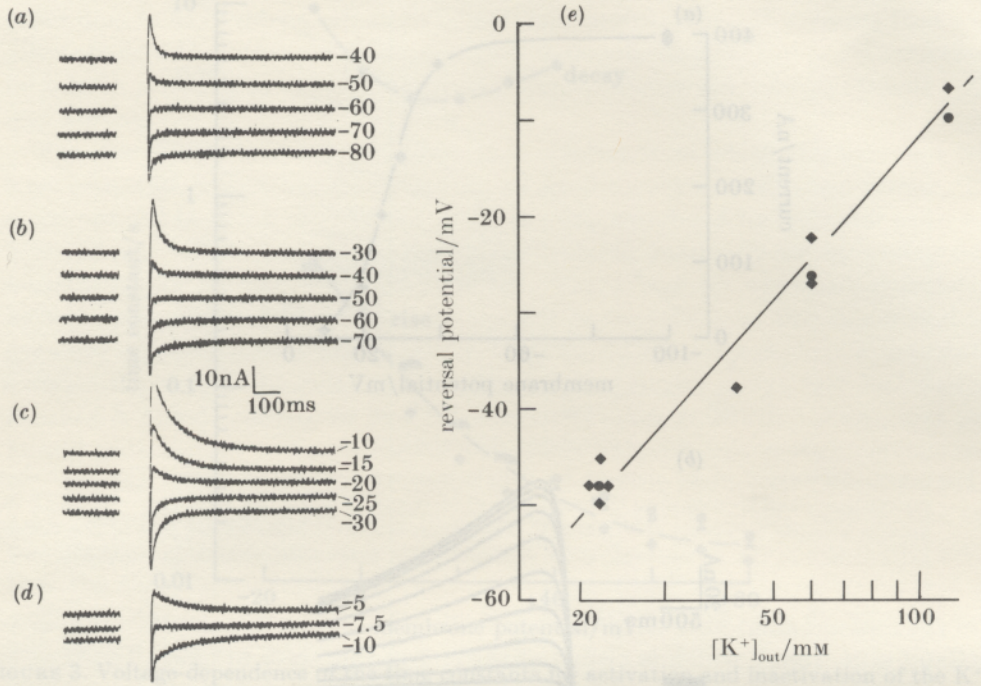

FIGURE 5. Reversal potentials of tail currents recorded on returning the membrane potential to various levels following a 100 ms step to +40 mV. (*a–d*) Tail currents in a single oocyte bathed in solutions of different K^+ concentration; (*a*), 2 mM; (*b*), 20 mM; (*c*) 60 mM; (*d*) 120 mM. Each frame shows superimposed traces of currents at the holding potentials indicated (in mV). (*e*) Dependence of reversal potential on extracellular K^+ concentration. Data are from two oocytes (●, ◆). Line is drawn by eye.

to the bathing solution so as to block influx of Ca^{2+} through membrane channels (Miledi 1982; Miledi & Parker 1984) did not appreciably reduce the slow K^+ current (figure 6*a*, *b*). Furthermore, the channels mediating the K^+ current appear to be independent of intracellular free Ca^{2+}, as injection of the calcium chelating agent EGTA had little effect. Figure 6*c*, *d* shows results from one oocyte loaded with 250 pmol EGTA; an amount sufficient to block the Ca^{2+} activation of Cl^- channels in the oocyte membrane (Parker & Miledi 1987*a*; Boton *et al.* 1989). After EGTA loading, the input resistance of the oocyte declined but, in this and a further two oocytes, the slow K^+ current was virtually unchanged.

Additional evidence against the slow K^+ current arising through Ca^{2+}-activated K^+ channels came from the use of toxins that specifically block these classes of channels (Hugues *et al.* 1982; Castle *et al.* 1989). Thus the current was unaffected following addition to the bathing solution of apamin (50 μM) or venom from *Leiurus quinquestriatus* (0.1 mg ml^{-1}).

Pharmacology

Because we had shown the slow outward current to be carried by K^+ ions, the effects of agents known to block K^+ currents in other cells were examined on the oocyte (Stanfield 1983; Hille 1984). Tetraethylammonium (TEA) ions at a

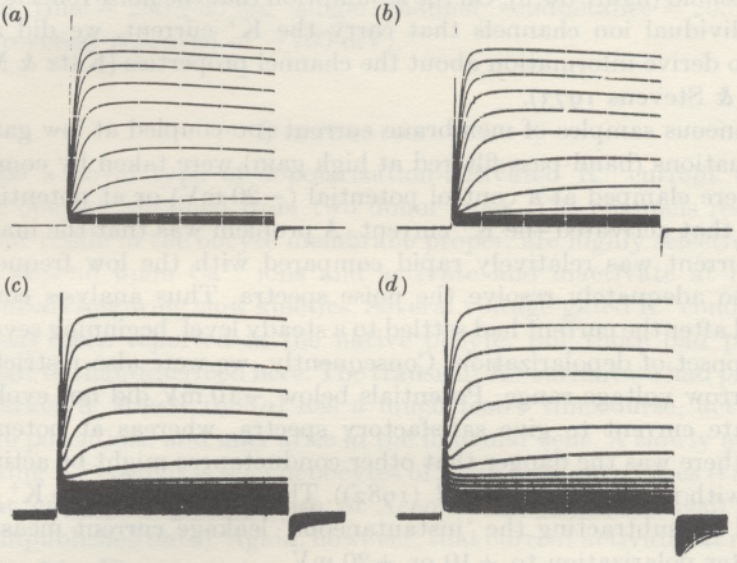

FIGURE 6. The K^+ current is not dependent upon Ca^{2+}. (*a*, *b*) Currents evoked by voltage steps in 10 mV increments between −90 and +80 mV from a holding potential of −100 mV in an oocyte bathed in normal Ringer (*a*) (control) and Ringer including 10 mM $MnCl_2$ (*b*). (*c*, *d*) Similar records from another oocyte obtained before (*c*) (control) and after (*d*) intracellular loading with 250 pmol EGTA. The potential was stepped in 10 mV increments between −90 and +60 mV.

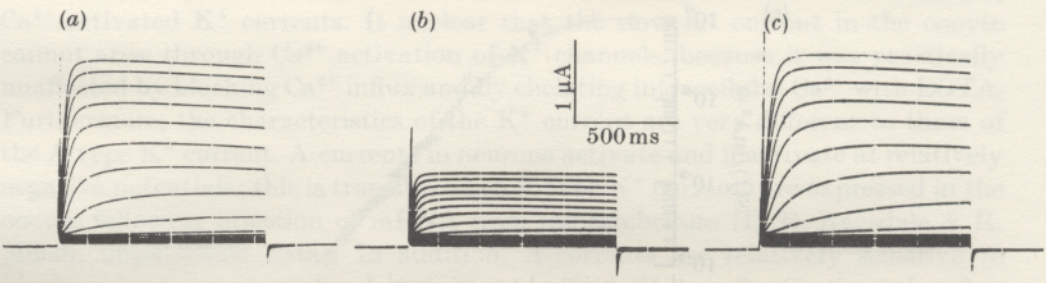

FIGURE 7. Action of (*b*) TEA and (*c*) Ba^{2+} ions on the slow K^+ current. Each frame shows superimposed current traces elicited by steps in 10 mV increments from −90 to +80 mV. Results from a single oocyte, clamped at a holding potential of −100 mV; (*a*), control.

concentration of 20 mM reduced the slow K^+ current to about one-third at all voltages examined (figure 7*a*, *b*), but addition of 10 mM Ba^{2+} was without noticeable effect (figure 7*c*). The following K^+-channel blocking agents also failed to alter the slow K^+ current: extracellularly applied Cs^{2+} (10 mM), Zn^{2+} (1 mM) and 4-aminopyridine (1 mM). Finally, we observed that acidification of the bathing solution to pH 5.6 did not affect the current, and that it was unchanged following intracellular injection of *ca.* 50 pmol cyclic AMP.

Characteristics of single-channel properties derived by noise analysis

During depolarizations that activated the slow K^+ current there was a concomitant increase in membrane current noise as compared with that at potentials just

below threshold (figure 8*a*, *b*). On the assumption that the noise reflects the activity of the individual ion channels that carry the K^+ current, we did a statistical analysis to derive information about the channel properties (Katz & Miledi 1972; Andersen & Stevens 1973).

Simultaneous samples of membrane current (DC-coupled at low gain) and current fluctuations (band-pass filtered at high gain) were taken by computer while oocytes were clamped at a control potential (−20 mV) or at potentials (+10 or +20 mV) that activated the K^+ current. A problem was that the inactivation of the K^+ current was relatively rapid compared with the low frequency cut-off required to adequately resolve the noise spectra. Thus analysis could only be performed after the current had settled to a steady level, beginning several seconds after the onset of depolarization. Consequently, we were also restricted to work over a narrow voltage range. Potentials below −10 mV did not evoke sufficient steady-state current to give satisfactory spectra, whereas at potentials above +20 mV there was the danger that other conductances might be activated (compare this with results in Baud *et al.* (1982)). The magnitude of the K^+ current was calculated by subtracting the 'instantaneous' leakage current measured immediately after polarization to +10 or +20 mV.

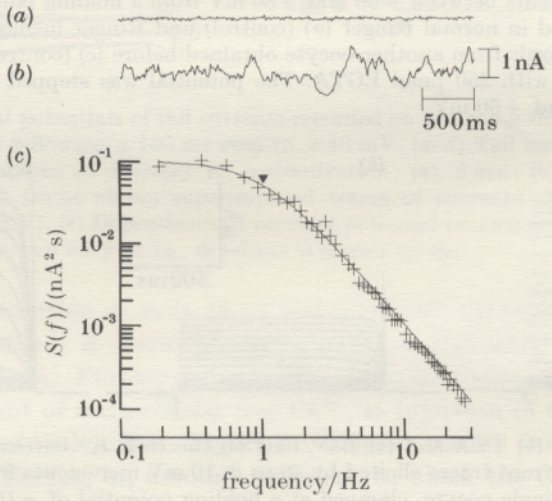

FIGURE 8. Membrane current noise during activation of the K^+ current. (*a*, *b*) Sample traces of computer-digitized records of noise fluctuations from an oocyte clampled at (*a*) −20 mV and (*b*) +20 mV. Currents were band-pass filtered at 0.03–50 Hz, and were sampled at 10 ms intervals. (*c*) Power spectrum of K^+ channel noise at a clamp potential of +20 mV. The spectrum is an average of 48 data blocks of 512 samples, after subtraction of the control noise spectrum at −20 mV. A Lorenzian curve was fitted by computer to the data, and the arrow marks the half-power frequency (1.03 Hz). The mean K^+ current, after subtraction of leakage current, was 53 nA.

Figure 8*c* shows the power spectrum derived in one oocyte clamped at +20 mV, after subtraction of the background noise spectrum at −20 mV. The data are well fitted by a single Lorentzian component and, for the oocyte illustrated, the half-power frequency of the spectrum corresponds to a mean channel open time of 150 ms. Mean values, obtained from nine recordings in three oocytes were; mean

channel open time = 280±30 ms, single channel conductance = 20.5±1.8 pS (assuming a reversal potential of −100 mV).

Discussion

We describe a novel type of depolarization-activated K^+ current that was prominent in oocytes obtained from two donor frogs. The channels responsible for this current reside in the oocyte membrane proper, are highly selective for K^+ ions, do not depend upon Ca^{2+} ions and activate and inactivate at relatively positive potentials following slow kinetics. Several voltage-gated K^+ conductances have previously been reported in the native oocyte, but those had properties clearly different to that described here. The transient K^+ current we had previously described (Parker & Miledi 1988*a*) has a much faster timecourse, activates at more negative potentials, and may arise in the follicular cells. A slowly activating and inactivating K^+ current is seen in oocytes of *Rana esculenta* (Peres *et al.* 1985), and a similar current is present also in *Xenopus* oocytes (R. Miledi & R. M. Woodward, unpublished data). Again, however, that current activates at relatively negative (*ca.* −50 mV) potentials.

Many different types of depolarization-activated K^+ currents have been described in other cells (for reviews see; Hille (1984); Kaczmarek & Levitan (1987); Moczydlowski *et al.* (1988); Castle *et al.* (1989)). These are generally classified into three groups, according to their voltage dependence, kinetics and gating mechanisms: (i) delayed rectifiers, (ii) transient outward rectifiers or A-currents and (iii) Ca^{2+}-activated K^+ currents. It is clear that the slow K^+ current in the oocyte cannot arise through Ca^{2+} activation of K^+ channels, because it was practically unaffected by blocking Ca^{2+} influx and by chelating intracellular Ca^{2+} with EGTA. Furthermore, the characteristics of the K^+ current are very different to those of the A-type K^+ current. A-currents in neurons activate and inactivate at relatively negative potentials; this is true also when A-type K^+ currents are expressed in the oocyte following injection of mRNA from rat cerebellum (D. R. Ragsdale & R. Miledi, unpublished data). In addition, A-currents are relatively sensitive to blockage by 4-aminopyridine but resistant to TEA (Hille 1984; Castle *et al.* 1989). In contrast, the K^+ current described here activated only with strong depolarizations to beyond about −15 mV and although it was partially reduced by high (20 mM) concentrations of TEA, it was insensitive to 4-aminopyridine. By elimination, therefore, the slow K^+ current in the oocyte seems best classified as a delayed rectifier, and several characteristics are consistent with this. For example, the kinetics of activation and inactivation fall within the wide range reported for delayed rectifier channels, as does the single-channel conductance (Hille 1984; Moczydlowski *et al.* 1988). On the other hand, some characteristics are unusual. Most notably, the potential giving half-maximal activation of the current is about +10 mV, whereas the corresponding value for delayed rectifier channels is consistently around −25 mV in various amphibian and mammalian tissues (Adrian *et al.* 1970; Pappone 1980; Cahalan *et al.* 1985). This difference in voltage dependence of activation is unlikely to result from some peculiarity of the oocyte membrane, as exogenous Na^+ and K^+ channels expressed in the oocyte show a voltage-dependence like that in their native cells (Gundersen *et al.* 1983; Sigel

1987). Instead, it presumably arises from differences in the channel molecules themselves. Another unusual feature is the insensitivity of the oocyte K^+ current to Ba^{2+}, Cs^{2+} and 4-aminopyridine, as these agents generally block delayed rectifier currents (Hille 1984; Castle *et al.* 1989).

The finding of a large K^+ current in native oocytes emphasizes that caution is needed when interpreting data from experiments by using the oocyte to study channels expressed from exogenous mRNA. For example, it has recently been shown that the properties of A-type K^+ channels expressed from brain mRNA are modulated by a relatively low molecular mass fraction of mRNA that, by itself, does not cause expression of K^+ currents (Rudy *et al.* 1988). As some native oocytes express K^+ channels, it is possible that other oocytes that do not show K^+ currents might still possess modulatory proteins like that encoded by brain mRNA. We had previously considered a similar possibility in regard to the expression of exogenous Na^+ channels, and this is clearly a potential complication to bear in mind when studying many other types of exogenous channels and receptors expressed in the oocyte.

It is not yet clear whether the slow K^+ current serves any function in the oocyte. The currents we describe are large enough to strongly influence the cell potential, but responses of this size were apparent only in oocytes from two donors out of many hundreds examined. Furthermore, because of inactivation, the K^+ current would be effective for only a short time following abrupt depolarization, and would be expected to have little effect on the steady resting potential. It may be more appropriate, therefore, to ask whether the chance occurrence of the large K^+ current in oocytes from one frog might have deleterious effects. For example, if the K^+ channels persist after oocyte maturation, they might reduce or slow the depolarization that accompanies fertilization and that is thought to act as a fast block to polyspermy (Cross & Ellinson 1980).

We are grateful to John Dempster for providing the spectral analysis program, and to Rico Miledi for the program to analyse data stored by the Nicolet oscilloscope. This work was supported by grants GM39831 and NS23284 from the U.S. Public Health Services.

References

Adrian, R. H., Chandler, W. K. & Hodgkin, A. L. 1970 Voltage clamp experiments in striated muscle fibres. *J. Physiol., Lond.* **208**, 607–644.

Andersen, C. R. & Stevens, C. F. 1973 Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *J. Physiol., Lond.* **235**, 655–691.

Barish, M. E. 1983 A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol., Lond.* **342**, 309–325.

Baud, C., Kado, R. T. & Marcher, K. 1982 Sodium channels induced by depolarization of the *Xenopus laevis* oocyte. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3188–3192.

Bolton, R., Dascal, N., Gillo, B. & Lass, Y. 1989 Two calcium-activated chloride conductances in *Xenopus* oocytes permeabilized with the ionophore A23187. *J. Physiol., Lond.* **408**, 511–534.

Cahalan, M. D., Chandy, K. G., DeCoursey, T. E. & Gupta, S. 1985 A voltage-dependent potassium channel in human T lymphocytes. *J. Physiol., Lond.* **358**, 197–237.

Castle, N. A., Haylett, D. G. & Jenkinson, D. H. 1989 Toxins in the characterization of potassium channels. *Trends Neurosci.* **12**, 59–65.

Cross, N. L. & Ellinson, R. P. 1980 A fast block to polyspermy in frog mediated by changes in membrane potential. *Devl Biol.* **75**, 187–198.

Dascal, N. 1987 The use of *Xenopus* oocytes for the study of ion channels. *CRC crit. Rev. Biochem.* **22**, 317–387.

Dascal, N., Snutch, T. P., Lubbert, H., Davidson, N. R. & Lester, H. A. 1986 Expression and modulation of voltage-dependent calcium channels after RNA injection in *Xenopus* oocytes. *Science, Wash.* **231**, 1147–1150.

Dumont, J. N. 1972 Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morph.* **136**, 153–180.

Gundersen, C. B., Miledi, R. & Parker, I. 1983 Voltage-operated channels induced by foreign messenger RNA in *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **220**, 131–140.

Hille, B. 1984 *Ionic channels of excitable membranes.* Sunderland, Massachusetts: Sinauer Associates.

Hughes, M., Romey, G., Duval, D., Vincent, J. P. & Lazdunski, M. 1982 Apamin as a selective blocker of calcium dependent potassium channels in neuroblastoma cells. Voltage clamp and biochemical characterization of the toxin receptor. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1308–1312.

Kaczmarek, L. K. & Levitan, I. B. 1987 *Neuromodulation. The biochemical control of neuronal* excitability. New York: Oxford University Press.

Katz, B. & Miledi, R. 1972 The statistical nature of the acetylcholine potential and its molecular components. *J. Physiol., Lond.* **224**, 665–699.

Kusano, K., Miledi, R. & Stinnakre, J. 1982 Cholinergic and catecholaminergic receptors in the *Xenopus* oocyte membrane. *J. Physiol., Lond.* **328**, 143–170.

Miledi, R. 1982 A calcium-dependent transient outward current in *Xenopus laevis* oocytes. *Proc. R. Soc. Lond.* B **215**, 491–497.

Miledi, R. & Parker, I. 1984 Chloride current induced by injection of calcium into *Xenopus* oocytes. *J. Physiol., Lond.* **357**, 173–183.

Miledi, R. & Woodward, R. M. 1989 The effect of defolliculation on the membrane current responses of *Xenopus* oocytes. *J. Physiol., Lond.* **416**, 601–621.

Miledi, R., Parker, I. & Sumikawa, K. 1989 Transplanting receptors from brains into oocytes. In *Fidia award lecture series* (ed. J. Smith), vol. 3, pp. 57–90. New York: Raven Press.

Moczydlowski, E., Lucceri, K. & Ravidran, A. 1988 An emerging pharmacology of peptide toxins targeted against potassium channels. *J. Membr. Biol.* **105**, 95–111.

Pappone, P. A. 1980 Voltage-clamp experiments in normal and denervated mammalian skeletal muscle fibres. *J. Physiol., Lond.* **306**, 377–410.

Parker, I. & Miledi, R. 1987*a* Injection of inositol 1,3,4,5-tetrakisphosphate into *Xenopus* oocytes generates a chloride current dependent upon intracellular calcium. *Proc. R. Soc. Lond.* B **232**, 59–70.

Parker, I. & Miledi, R. 1987*b* Tetrodotoxin-sensitive sodium current in native *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **232**, 289–296.

Parker, I. & Miledi, R. 1988*a* Transient potassium current in native *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **234**, 45–53.

Parker, I. & Miledi, R. 1988*b* A calcium-independent chloride current activated by hyperpolarization in *Xenopus* oocytes. *Proc. R. Soc. Lond.* B **233**, 191–199.

Peres, A., Bernardini, G., Mancinelli, E. & Ferroni, A. 1985 A voltage-dependent K^+ channel controlling the membrane potential in frog oocytes. *Pflügers Arch. Eur. J. Physiol.* **403**, 41–46.

Rudy, B., Hoges, J. H., Lester, H. A. & Davidson, N. 1988 At least two mRNA species contribute to the properties of rat brain A-type potassium channel expressed in *Xenopus* oocytes. *Neuron* **1**, 649–658.

Sigel, E. 1987 Properties of single sodium channels translated by *Xenopus* oocytes after injection with messenger ribonucleic acid. *J. Physiol., Lond.* **386**, 73–90.

Stanfield, P. R. 1983 Tetraethylammonium ions and the K^+ permeability of excitable cells. *Rev. Physiol. biochem. Pharmacol.* **97**, 1–67.

Sumikawa, K., Parker, I. & Miledi, R. 1989 Expression of neurotransmitter receptors and voltage-activated membrane channels from brain mRNA in *Xenopus* oocytes. In *Methods in neurosciences*, vol. 1 (ed. P. M. Conn), pp. 30–45. Florida: Academic Press.