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SLOWLY INACTIVATING POTASSIUM CHANNELS INDUCED IN *XENOPUS* OOCYTES BY MESSENGER RIBONUCLEIC ACID FROM *TORPEDO* BRAIN

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SUMMARY

1. Poly(A^+) messenger RNA was extracted from the electric lobe and medulla of *Torpedo* and injected into oocytes of *Xenopus laevis*. The synthesis and processing of proteins coded by the injected messenger RNA led to the incorporation of voltage-activated channels in the oocyte membrane.

2. A large, well maintained outward current was recorded from injected oocytes in response to depolarization. Non-injected oocytes did not show this current. The reversal potential of the current varied according to the Nernst equation with external potassium concentration, indicating that it was largely carried by potassium ions.

3. The maintained potassium current was not reduced by manganese (5 mM) or lanthanum ions (0·1 mM).

4. Tetraethylammonium and aminopyridines blocked the potassium current. The block produced by 3,4-diaminopyridine was enhanced by previous activation, but diminished by strong depolarization.

5. The amplitude of the potassium current increased over the approximate voltage range −30 to +50 mV, but reduced at more positive potentials. The decline of the current during maintained depolarization became slower as the membrane potential was made more positive, and the rate of onset of the current became faster.

6. Estimates from noise analysis indicated that the slow potassium current passes through channels with a mean lifetime of about 14 ms and conductance of 14 pS (at −10 mV and room temperature).

7. Injection of the messenger RNA also induced the formation of fast sodium and potassium channels activated by voltage, and channels activated by kainate.

INTRODUCTION

Native oocytes of *Xenopus laevis* show a number of voltage-activated membrane currents, including a calcium-dependent chloride conductance (Miledi, 1982; Barish, 1983), a sodium conductance which develops during prolonged depolarization (Baud, Kado & Marcher, 1982), a transient inward current activated by hyperpolarization (Peres & Bernadini, 1983; C. B. Gundersen, R. Miledi & I. Parker, unpublished

observations) and slow potassium and chloride currents not yet characterized (R. Miledi, unpublished observations). In addition to these 'native' membrane channels, we have recently found that exogenous poly(A^+) messenger RNA (mRNA), when injected into oocytes, is translated and forms functional voltage-activated channels in the oocyte membrane. For example, transient sodium and potassium currents could be recorded from oocytes which had been injected with mRNA derived from rat brain or cat muscle (Gundersen, Miledi & Parker, 1983*b*), as well as human brain (Gundersen, Miledi & Parker, 1984*b*) and chick brain (K. Sumikawa, R. Miledi & I. Parker, unpublished observations). Here, we describe the properties of a slowly inactivating potassium current, which was expressed following injection of mRNA from the brain of *Torpedo*.

METHODS

Experiments were made on oocytes of *Xenopus laevis* (diameter 0·8–1·3 mm), which had been injected with poly(A^+) mRNA obtained from the brains of *Torpedo marmorata* and *T. ocellata*, caught in the Bay of Naples. The animals were anaesthetized with tricaine and after exposing the brain, it was divided into two portions. One consisted of the forebrain plus cerebellum. The other consisted of the electric lobe and the underlying medulla. The electric lobe contains, almost exclusively, the neurones that directly innervate the electric organ (electromotor neurones), while the medulla contains many other neurones, including those in the oval nucleus, which controls the electromotor neurones (Szabo, 1954). Messenger RNA prepared from this portion of the brain was injected into oocytes, using methods previously described (Miledi & Sumikawa, 1982; Gundersen, Miledi & Parker 1983*a*, *b*). For simplicity we refer to this messenger preparation as *Torpedo* electric lobe mRNA, although it should be remembered that it was derived from both electric lobe and medulla.

Some oocytes were treated with collagenase (Sigma type 1, 500 u. ml^{-1} for about 1 h at room temperature) to remove follicular cells (Kusano, Miledi & Stinnakre, 1982). Electrophysiological measurements were made as described previously (Kusano *et al.* 1982; Miledi, 1982). In most experiments a potassium chloride-filled micro-electrode was used for voltage recording, and a potassium acetate electrode was used for current injection. The oocyte was usually voltage clamped at a potential of −100 mV, from which it was stepped momentarily to different potentials and the ensuing currents were recorded. Usually, oocytes were continuously perfused with Ringer solution (composition (mM): NaCl, 120; KCl, 2; $CaCl_2$, 1·8; HEPES, 5; pH 7·2) at a temperature of 11–23 °C whilst recording was carried out. High-potassium solutions were made by addition of potassium chloride to normal Ringer solution. The results presented are based on experiments on thirty-one *Torpedo* electric lobe mRNA-injected oocytes, from five donors.

For noise analysis of the slow potassium current, records of current fluctuations were band-pass filtered at 0·1–50 Hz (24 dB per octave roll off) and digitized at a 10 ms sampling interval. Power spectra were computed for 512 point segments of records, using a fast Fourier transform algorithm. Control spectra at a holding potential of −100 or −60 mV were subtracted from spectra obtained during activation of the maintained outward current. The mean channel lifetime (τ) and current (i) were estimated after subtraction of control spectra, by calculating $\tau = 1/(2\pi f_c)$ and $i = S(0)/2I\tau$: 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 potassium current.

RESULTS

Maintained outward current after injection of mRNA from electromotor lobe

Depolarization of *Xenopus* oocytes which had been injected with mRNA from the *Torpedo* electric lobe elicited membrane currents which were strikingly different from those in control (non-injected) oocytes (Fig. 1). The most obvious current in *Torpedo*

electric lobe mRNA-injected oocytes was a large, well maintained outward current, which was activated by polarization to potentials more positive than about −30 mV (Fig. 1*A*). Evidence presented later indicates that this current is carried largely by potassium ions, and it will be referred to as the slow potassium current. Nearly all

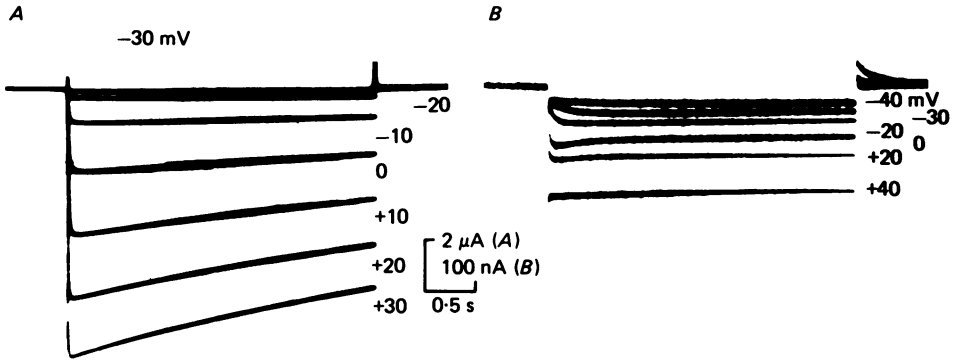

Fig. 1. Membrane currents recorded from an oocyte injected with *Torpedo* electric lobe mRNA (*A*), and a non-injected oocyte from the same donor (*B*). In each frame, the oocyte was voltage clamped at −100 mV and currents were recorded in response to steps to the potentials indicated. In this, and other Figures, outward currents correspond to downward deflexions. Note the difference in recording gain between *A* and *B*. The oocyte in *A* had been enucleated before injection of mRNA. Temperature, 23 °C.

injected oocytes gave an outward current of several microamperes at a potential of +40 mV, and some oocytes gave currents as large as 20–40 μA. In contrast, non-injected oocytes from the same donors showed no appreciable slow potassium current (Fig. 1*B*; see also Miledi, 1982; Barish, 1983).

The slow potassium current was clearly generated by the oocyte membrane proper, because it was present in oocytes which had been treated with collagenase to remove follicular and other enveloping cells. Furthermore, the current was still present in oocytes which had been enucleated before injection of mRNA, or which had been maintained continuously in actinomycin D to inhibit synthesis of mRNA (Adamson & Woodland, 1977; Gundersen, Miledi & Parker, 1984*a*). It is therefore unlikely that the proteins forming the channels responsible for the slow potassium current were incorporated because the injection of foreign mRNA triggered the transcription of the appropriate messengers from the oocyte's own genome. Instead, the relevant protein almost certainly arose as a direct result of the translation of the injected mRNA.

Other membrane currents in oocytes injected with Torpedo electric lobe mRNA

In addition to the slow potassium current, a number of other currents were elicited by depolarization of injected oocytes. These comprised principally the following currents.

(i) A fast transient inward current, which was blocked by tetrodotoxin (TTX). This current was similar to the sodium current induced in oocytes following injection of mRNA from cat muscle, or from chick, rat and human brains (Gundersen *et al.* 1983*b*,

1984*a*, *b*). The properties of the inward current will be described in a future paper. Most records shown here were obtained at sweep speeds too slow to allow this current to be clearly seen. Also, some experiments were made in the presence of TTX (330 nM), to block the inward sodium current.

(ii) A fast transient outward current, decaying within a few tens of milliseconds, which resembled the rapidly inactivating potassium current seen in oocytes injected with mRNA from rat brain, cat muscle (Gundersen *et al.* 1983*b*) and human brain (Gundersen *et al.* 1984*b*). This current was seen in only a few oocytes, and was small in comparison with the slow outward potassium current. Fig. 6 shows records where a small, fast outward current was superimposed on the slow potassium current.

(iii) A transient outward current, due to an influx of chloride ions. This current resembled the calcium-activated transient outward (T_{out}) current in native oocytes (Miledi, 1982; Barish, 1983). Both injected and non-injected oocytes showed this current, and its time course and amplitude varied considerably between different oocytes. Some experiments were made using manganese or lanthanum ions to block selectively the T_{out} current (see following section), and allow the slow potassium current to be studied in relative isolation. However, this was often not necessary, since in many oocytes the size of the T_{out} current was very small compared to the slow potassium current.

The slow potassium current is not calcium dependent

Fig. 2*A* shows records from an oocyte injected with *Torpedo* electric lobe mRNA, which gave a large transient outward current in addition to the slow potassium current. Depolarization to potentials of +10 and +20 mV elicited an outward current which became obvious after a latency of a few hundred milliseconds, but which was not present at 0 mV or +30 mV. This component of the current resembled the T_{out} current seen in native oocytes (Miledi, 1982; Barish, 1983), although in the case illustrated, its time course was slower. The T_{out} current decayed after a few seconds, and similar to the native T_{in} current, it was suppressed by depolarizations to potentials of about +40 mV, and was reversibly abolished by addition of manganese to the bathing solution. Fig. 2*B* illustrates the blocking of this current by manganese (5 mM); note also that the tail current, seen on repolarization from potentials which elicited a T_{out} current, was also abolished. In contrast, the maximal amplitude of the maintained outward current elicited at a potential of +50 mV was virtually unchanged.

The voltage dependence of activation of the slow potassium current was, however, shifted to more positive potentials in the presence of manganese. This is shown in Fig. 2*C*, where the current–voltage relationship of the maintained current was measured 300 ms after the beginning of the pulse, at a time before the onset of the T_{out} current. The curve obtained in the presence of manganese was shifted along the voltage axis by about 15 mV to more positive potentials, but followed the same sigmoid relationship as in normal Ringer solution. Most probably this effect arose due to changes in surface potential of the oocyte membrane. A shift of this magnitude in surface potential would be expected from addition of 5 mM-manganese (Hille, Woodhull & Shapiro, 1975).

The calcium-dependent T_{out} current in native oocytes is blocked, almost

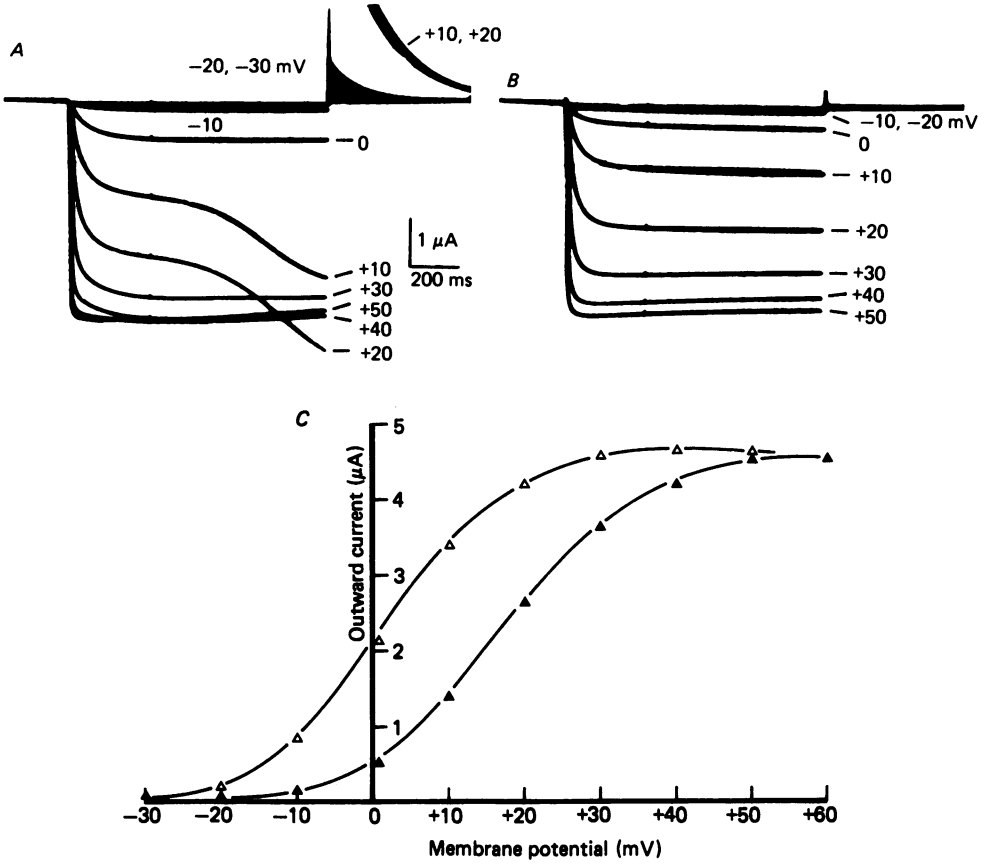

Fig. 2. Slow outward currents in an oocyte injected with *Torpedo* electric lobe mRNA. Temperature in all records was 12 °C. The oocyte was collagenase treated. *A*, membrane currents in normal Ringer solution. The oocyte was voltage clamped at −100 mV and depolarized during pulses of 1 s duration to the potentials indicated. Polarization to potentials more positive than −20 mV elicited a maintained outward current. Pulses to +10 and +20 mV elicited in addition a slowly developing outward current, which was followed by a large inward tail current at the end of the pulse. *B*, records obtained from the same oocyte as *A*, but with 5 mM-manganese added to the bathing solution. *C*, current–voltage relationships for the maintained outward current, obtained from the same oocyte as *A* and *B*. Open symbols are measurements made in normal Ringer solution, whilst filled symbols are data obtained in the presence of 5 mM-manganese. Measurements were made as the difference between the base-line current at −100 mV, and the current elicited 300 ms after the beginning of the depolarizing pulse.

irreversibly, by low concentrations of lanthanum ions (R. Miledi & I. Parker, unpublished observations). In oocytes injected with *Torpedo* electric lobe mRNA, 100 μM-lanthanum blocked the T_{out} current elicited by depolarization to 0 mV, whilst leaving the component due to the slow potassium currrent. At +40 mV the amplitude of the slow potassium current was almost unchanged after the addition of lanthanum.

All this indicates that the slow potassium current does not involve an influx of calcium ions into the oocyte.

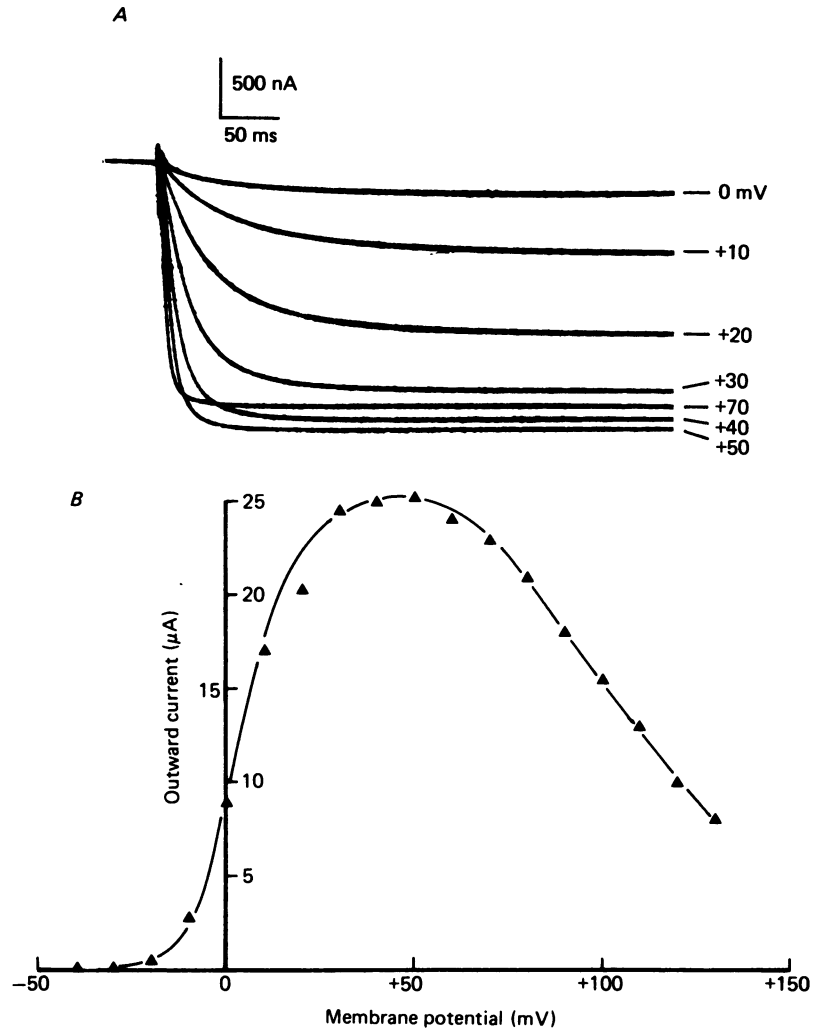

Fig. 3. Current–voltage relationship of the maintained outward current recorded from oocytes injected with *Torpedo* electric lobe mRNA. *A*, membrane currents elicited by polarization to the potentials indicated, from a holding potential of −100 mV. Note that the current at +70 mV rises faster, but is smaller than at +40 and +50 mV. Bathing solution included 5 mM-manganese and 330 nM-TTX. Temperature, 13 °C. *B*, current–voltage relationship plotted from a different oocyte. The passive resistance of the oocyte (measured between −140 and −60 mV) was about 2 MΩ. Thus, even at a potential of +100 mV the extrapolated 'leakage' current would have been only 100 nA, and negligible in comparison with the slow potassium current. Curve was drawn by eye. Temperature, 22 °C.

Voltage dependence of the slow potassium current

Fig. 3*A* shows currents recorded from an oocyte injected with *Torpedo* electric lobe mRNA in response to depolarizations to different potentials, from a holding potential of −100 mV. Manganese (5 mM) was added to the bathing solutions to block the calcium-dependent T_{out} current, and TTX (330 nM) to block the sodium current. The

rate of onset of the maintained outward current increased markedly with increasing depolarization, and for the oocyte illustrated the half-rise time of the current was about 50 ms at 0 mV, 25 ms at +30 mV and 7 ms at +60 mV (temperature 12 °C).

In contrast to the monotonic increase in rate of rise with increasing positivity, the plateau level of the current reached a maximum at +40 to +60 mV, and then

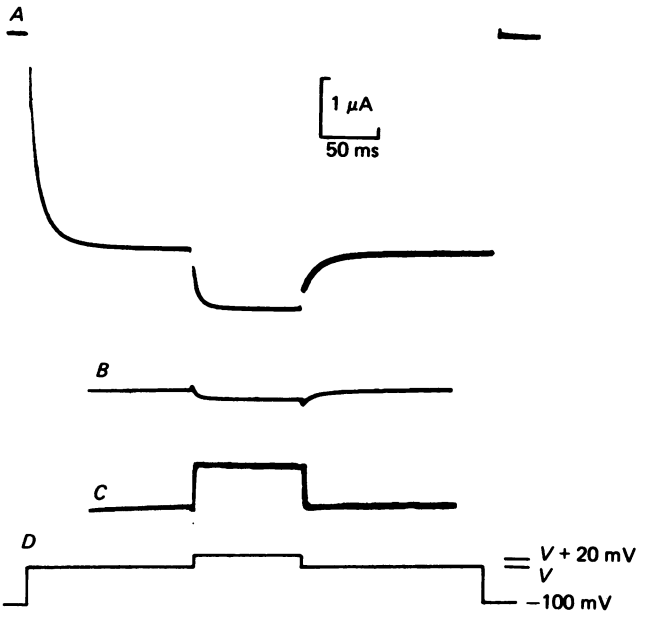

Fig. 4. Relaxations of the maintained outward current following 20 mV polarizing steps applied during polarization to various potentials. The pulse protocol is illustrated in *D*. The membrane was polarized to potentials *V* of +20 mV in *A*, +30 mV in *B* and +50 mV in *C*. Temperature was 21 °C. The oocyte was injected with *Torpedo* electric lobe mRNA and was collagenase treated.

declined (Fig. 3*A* and *B*). At a potential of +130 mV, the slow potassium current elicited in Fig. 3*B* had reduced to about one-quarter of the maximal value at +50 mV.

Fig. 4 shows currents recorded in response to 20 mV polarizing pulses applied during longer polarizations to various potentials. Stepping the potential from +20 to +40 mV gave a rapid increase in current, followed by a relatively slow increase to a new steady level (Fig. 4*A*). On returning to +20 mV there was again a rapid fall and this was followed by a relaxation which was slower than the corresponding phase of the rise. In contrast to this, a step from +30 to +50 mV gave little change in current (Fig. 4 *B*), whilst a step from +50 to +70 mV produced a rapid decrease in current (Fig. 4 *C*).

The slow potassium current is blocked by tetraethylammonium

The slow potassium current recorded from oocytes injected with *Torpedo* electric lobe mRNA was rapidly and almost completely abolished by tetraethylammonium (TEA) bromide or chloride, at a concentration of 10 mM (Fig. 5). In the presence of

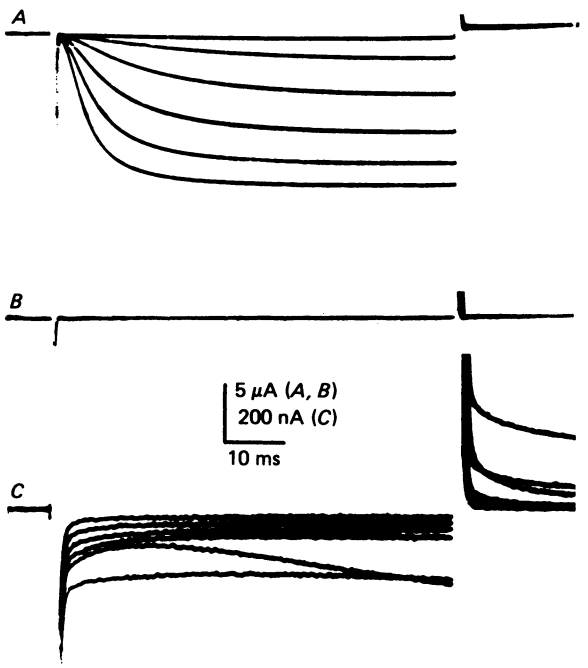

Fig. 5. Blocking of the maintained outward current by tetraethylammonium ions (TEA). Records were obtained from a collagenase-treated oocyte which had been injected with *Torpedo* electric lobe mRNA. TTX (330 nM) was present in all bathing solutions. Temperature was 14 °C. *A*, membrane currents elicited in normal Ringer solution. The oocyte was held at a potential of −100 mV, and stepped to potentials of (from top to bottom) −20, −10, 0, +10, +20 and +30 mV. *B*, block of the outward current in the presence of 10 mM-TEA. The oocyte was depoolarized to +30 mV. *C*, currents remaining in the presence of 10 mM-TEA, recorded at a higher gain. The oocyte was depolarized to potentials of (from top to bottom) −80, −60, −40, −20, 0 and +30 mV. A small transient outward current was present at 0 mV, whilst the steady outward current was very small, even at a potential of +30 mV.

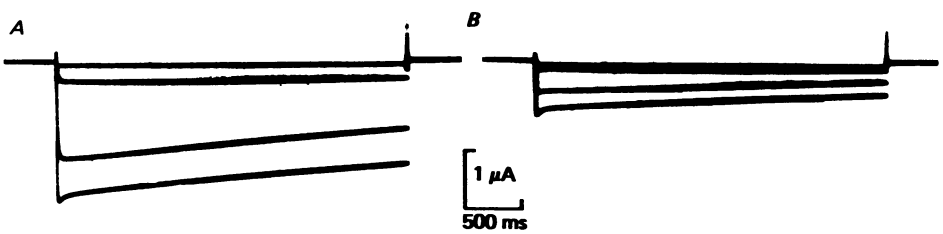

Fig. 6. Reduction of the maintained outward current by 100 μM-TEA. Each frame shows currents elicited by depolarizations to potentials of (from top to bottom) −30, 0, +20 and +40 mV. All solutions included lanthanum (100 μM), to block the calcium-dependent T_{out} current. Records were obtained before (*A*), and a few minutes after (*B*) adding 100 μM-TEA bromide to the perfusion solution. Temperature was 21 °C. The oocyte was injected with *Torpedo* electric lobe mRNA and was collagenase treated.

TEA, any remaining maintained outward current in the oocyte illustrated was less than 40 nA at a potential of +30 mV, whilst the current in normal Ringer solution was about 13 μA. After addition of TEA, a comparatively small transient outward current remained, and also a small maintained outward current (Fig. 5*C*) which may have been partly due to a maintained chloride current seen in native oocytes (R. Miledi, unpublished observations). Thus, 10 mM-TEA reduced the maintained potassium current by a factor of at least several-hundredfold.

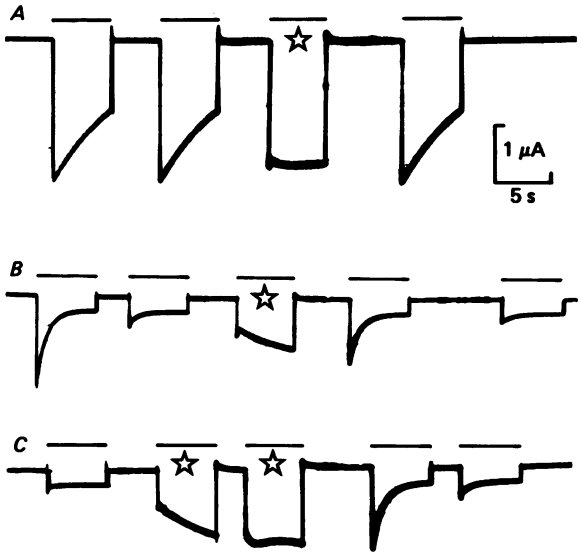

Fig. 7. Blocking action of DAP on the maintained outward current. The oocyte was clamped at −100 mV and depolarized during the times indicated by the bars. All depolarizations were to +20 mV, except for those marked by a star, which were to +100 mV. Temperature was 22 °C. Oocyte was injected with *Torpedo* electric lobe mRNA and collagenase treated. *A*, control records obtained in normal Ringer solution. *B*, records obtained after the oocyte had been exposed to DAP for 5 min and then washed in normal Ringer solution for 6 min. The initial voltage step in this trace was the first one applied since the oocyte was exposed to DAP. *C*, trace obtained a few minutes after that in *B*, with the oocyte remaining in normal Ringer solution.

Lower concentrations of TEA were also effective in reducing the size of the slow potassium current. Fig. 6 shows the effect of 100 μM-TEA on the currents elicited from an oocyte injected with *Torpedo* electric lobe mRNA. The amplitude of the slow outward current was reduced by more than one-half, but the voltage dependence of the current, and its decline during a long depolarization, were not appreciably altered. After washing out the TEA for a few minutes, the amplitude of the current recovered to close to the control value.

Effects of aminopyridines on the slow potassium current

The slow potassium current was blocked by 3,4-diaminopyridine (DAP), but this action showed several interesting features, which were not seen with TEA (Fig. 7). In particular, the block was enhanced by moderate depolarization, but could be

partially reversed by strong depolarization. Furthermore, the current remained reduced even 30 min after the DAP had been washed out.

In control solution, successive steps to +20 mV elicited outward currents which repeated almost identically, and were not altered by an intervening step to +100 mV (Fig. 7*A*). In contrast, when DAP (1 mM) had been applied and then washed out for several minutes, a step to +20 mV elicited a smaller and rapidly declining current (Fig. 7*B*). A second pulse to +20 mV evoked an outward current which was only slightly greater than the passive leakage current. This 'activity-dependent' block of the slow potassium current could be partially reversed by pulses to +100 mV. During the strong depolarization the outward current increased, and the current elicited by a subsequent pulse to +20 mV showed partial recovery (Fig. 7*B* and *C*). However, the current during this pulse declined rapidly, and again showed an activity-dependent block, even though DAP was not present in the bathing solution.

Records similar to those in Fig. 7*B* and *C* were obtained from other oocytes where 1 mM-DAP was still present in the bathing solution. The action of DAP appeared to be little diminished even after washing in normal Ringer solution for 30 min.

The maintained outward current was also blocked by 1 mM-4-aminopyridine. With this drug there was little 'activity dependence', and the current elicited by the first step to +20 mV was almost completely blocked. Depolarization to +100 mV did not appreciably reduce the block, and the current did not recover after washing for several minutes.

Reversal potential of the slow potassium current

To measure the reversal potential of the slow potassium current, we recorded the tail currents elicited when the membrane potential was repolarized to different levels, following voltage steps which activated outward membrane currents. A complication in these experiments was that large tail currents often resulted because of current flow through the calcium-activated chloride channels responsible for the T_{out} current (Miledi, 1982; Barish, 1983). This current decayed quite slowly (Fig. 2*A*), and tended to obscure the faster tail currents arising from current flow through the potassium channels. However, the calcium-dependent chloride tail currents could be blocked by manganese (Fig. 2*B*), leaving a more rapidly decaying current (Fig. 8*A* and *B*).

The tail currents recorded in the presence of manganese were abolished by addition of 10 mM-TEA, and were not seen when the initial depolarization was reduced below the threshold for eliciting a maintained outward current. Thus, the current relaxations recorded upon repolarization most probably arose because the membrane channels responsible for generating the slow potassium current remained open for a short time after repolarization.

In the presence of manganese, repolarization to a potential of −100 mV gave a fast inward tail current. When the potential following the depolarizing pulse was made more positive, the tail current inverted direction at about −90 mV and became outward (Fig. 8*A*). Also, the decline of the current became slower as the membrane was repolarized to less negative potentials, suggesting that the closing rate of the channel is voltage dependent.

The inversion of the tail current at a potential of −90 to −100 mV indicates that potassium must be the main ion responsible for carrying the slow outward current,

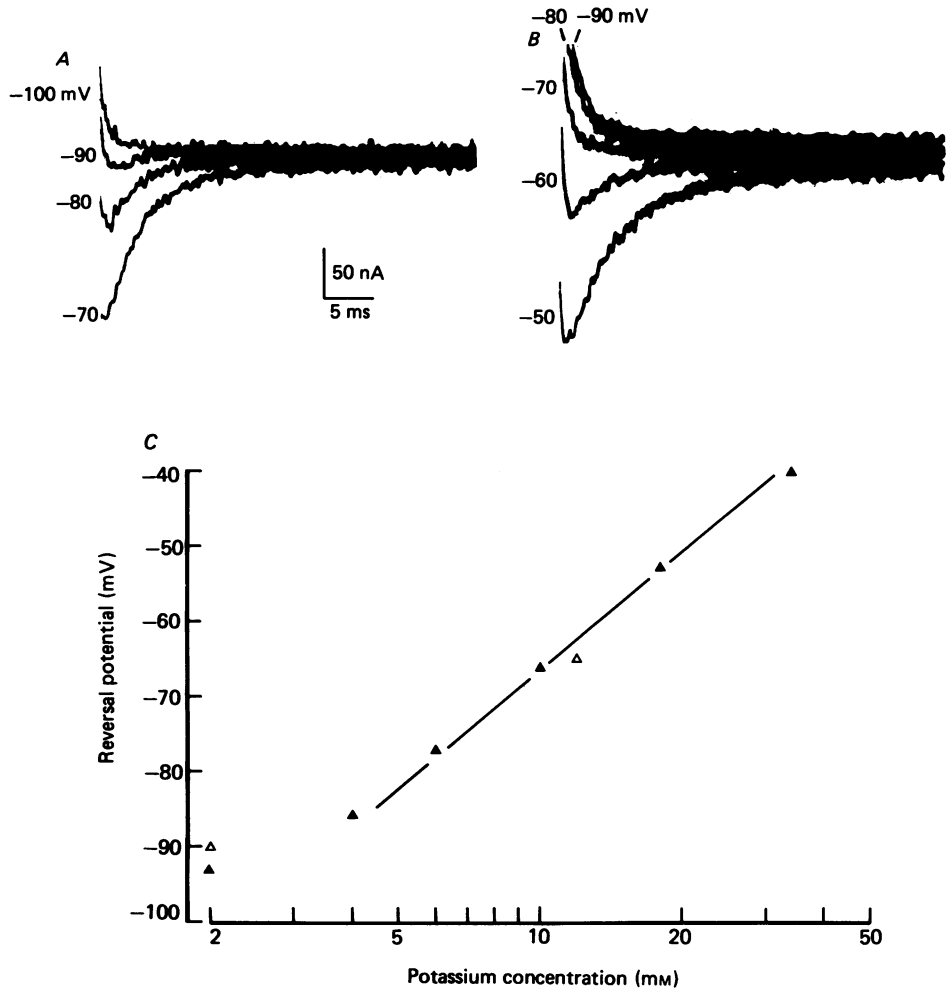

Fig. 8. Reversal potential of tail currents after activation of the maintained potassium current. *A* and *B*, tail currents elicited by repolarizing the oocyte to different potentials following a depolarizing pulse of 3 s duration to +30 mV which elicited a maintained outward current of about 1·5 μA. Figures next to the traces indicate (in mV) the potentials to which the membrane was repolarized. The oocyte was injected with *Torpedo* electric lobe mRNA, and was collagenase treated. Ringer solutions included 5 mM-manganese. Temperature was 12 °C. *A*, records obtained in normal Ringer solution containing 2 mM-potassium. The tail current inverted direction at a potential between −100 and −90 mV. *B*, records obtained in Ringer solution containing 10 mM-potassium. The reversal potential lay between −70 and −60 mV. *C*, dependence of the tail current reversal potential on the potassium concentration in the bathing solution. Reversal potentials were estimated from records similar to those in Fig. 4, but using voltage steps of 2 mV for more accurate measurements. Data from two oocytes (different symbols), both of which had been injected with *Torpedo* electric lobe mRNA and were collagenase treated. Temperature was 12–13 °C. The potassium concentration is plotted on a logarithmic scale. The line drawn through the data has a slope of 54 mV per decade change in potassium concentration.

since this is close to the potassium equilibrium potential in *Xenopus* oocytes, whereas the chloride equilibrium potential is around −24 mV (Kusano *et al.* 1982; Barish, 1983). Further evidence for the involvement of potassium was obtained by measuring the reversal potential of the tail currents in solutions containing elevated potassium concentrations.

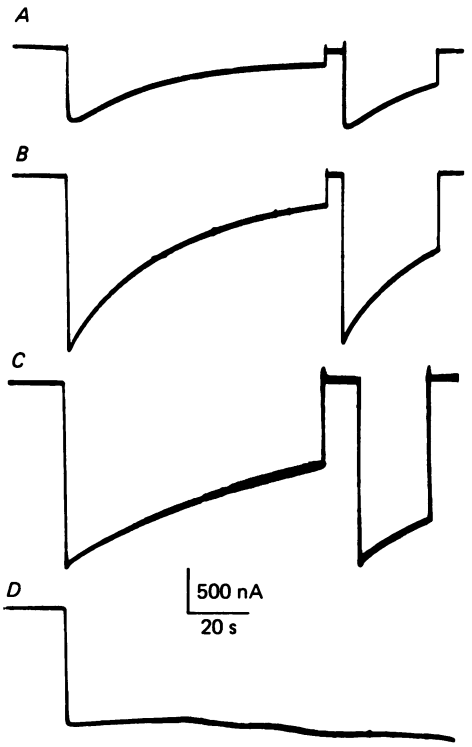

Fig. 9. Decline of the outward potassium current during long depolarizations. The oocyte was clamped at −100 mV and stepped to potentials of +10 mV (*A*), +30 mV (*B*), +70 mV (*C*) and +100 mV (*D*). In *A–C* two steps were applied, with the potential returned to −100 mV in between. In *D* the pulse was maintained until after the end of the trace. Temperature was 12 °C.

Fig. 8*B* illustrates tail currents recorded from an oocyte bathed in Ringer solution containing 10 mM-potassium. In this solution, the current inverted direction between −60 and −70 mV. Reversal potentials measured from two oocytes in solutions containing various potassium ion concentrations are plotted in Fig. 8*C*. At concentrations above about 5 mM, the reversal potential varied as a logarithmic function of potassium concentration, showing a change of about 54 mV per decade concentration change. This is very close to the value expected for a purely potassium-selective channel on the basis of the Nernst equation (56 mV per decade at 12 °C).

The voltage dependence of activation of the maintained outward current during positive-going pulses appeared little changed in solutions containing between 2 and 34 mM-potassium. This contrasts with the activation of the 'anomalous' inward potassium current seen in a number of preparations, where the voltage dependence

shifts with changes in external potassium concentration (Hagiwara & Takahashi, 1974; Leech & Stanfield, 1981).

Slow inactivation of the potassium current

Over a time scale of hundreds of milliseconds, the outward potassium current appeared very well maintained (Fig. 2*B*), but during depolarizations lasting many seconds a slow decline was evident (Figs. 6, 7 and 9). The decline in current during the pulse was voltage dependent, and became slower at more positive potentials. For example, the oocyte illustrated in Fig. 9 showed a half-decay time of about 30 s at a potential of +10 mV (temperature 12 °C). This increased to 75 s at +70 mV, and at +100 the current did not show any clear decline, even during a depolarization lasting several minutes.

In the case of Fig. 9*D*, the outward current during the 2 min depolarization would have transported about $1{\cdot}5 \times 10^{-9}$ mol potassium; even so, there was no sign of a fall in current due to depletion of potassium in the oocyte. This is not surprising, because the large volume of the cell (about 1 μl) would have caused the over-all intracellular potassium concentration to fall by only about 1·5 mM.

Recovery of the potassium current from 'inactivation' was found to occur rapidly when the membrane was repolarized. When a second pulse was applied a few seconds after a long pulse, the amplitude of the current was the same as that at the start of the first pulse. This occurred in spite of an appreciable decline in current during the initial pulse (Fig. 9*A–C*).

Characteristics of single potassium channels as derived from noise analysis

During depolarizations which activated the slow potassium current, there was a concomitant increase in membrane current noise (Fig. 10*A*). This noise was still present in oocytes treated with manganese to block the T_{out} current, but was almost completely abolished when the slow potassium current was blocked by TEA. Thus, the noise probably reflects the activity of the individual ion channels which carry the slow potassium current.

To analyse the noise fluctuations, oocytes were clamped during alternate periods of about 1 min duration to control potentials (−100 or −60 mV) and test potentials (−10 or −5 mV). High gain, a.c.-coupled recordings of membrane current were made throughout the control periods, and from test periods, beginning about 10 s after the onset of the pulse. Some experiments were made without blocking the T_{out} current; this would not have contributed to the noise fluctuations, since the T_{out} current decayed almost completely within 10 s of the onset of depolarization. In agreement, similar results were obtained in the presence of 5 mM-manganese. Test potentials to −10 or −5 mV were chosen to ensure that only a small fraction of the slow potassium current was activated (cf. Fig. 3*B*). Estimates of the mean slow potassium current were made by subtracting the extrapolated passive 'leakage' current from the mean total current elicited during the depolarizing pulses. In two experiments, TEA (10 mM) was added after recording the noise runs; here, the leakage current was directly measured as the current remaining when the oocyte was depolarized in the presence of TEA. Statistical analysis of the noise fluctuations was made using techniques described previously (Katz & Miledi, 1972; Anderson & Stevens, 1973;

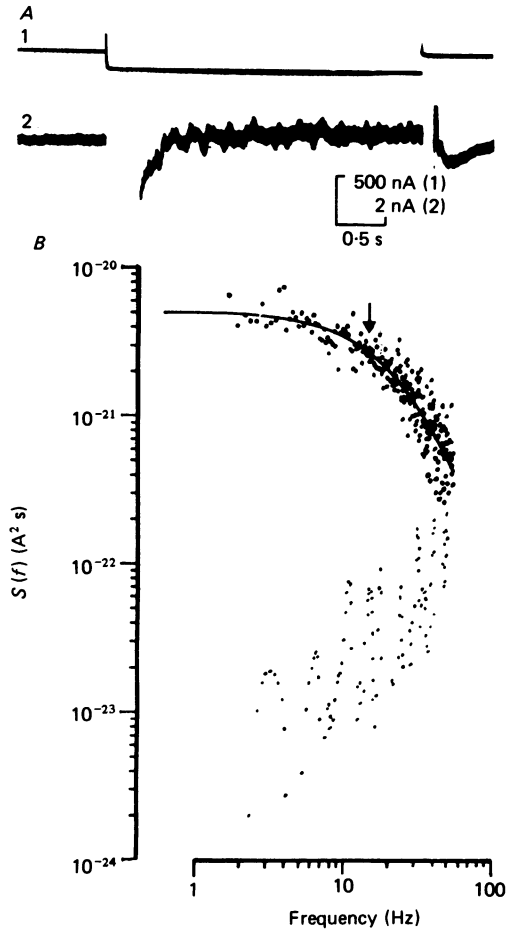

Fig. 10. Membrane current noise during activation of the maintained outward current. *A*, currents recorded at low gain (1) and high gain (2) in response to steps from −100 mV to −10 mV. The high-gain recording was band-pass filtered at 1–20 Hz. Temperature was 21 °C. *B*, upper curve shows power spectrum of potassium channel noise, obtained from a *Torpedo* electric lobe mRNA-injected oocyte in Ringer solution including 5 mM-manganese. The spectrum is an average of twenty-six data blocks of 512 samples, recorded at a potential of −5 mV. Samples were taken during three 60 s periods of depolarization, beginning about 10 s after onset of the pulse. Control noise spectra at −60 mV have been subtracted. The half-power frequency of the spectrum is marked by the arrow, and was 12 Hz. The lower curve (small dots) shows the spectrum obtained at the same voltages after the slow potassium current had been blocked by 10 mM-TEA. The mean current at −5 mV in normal Ringer solution (plus manganese) was 165 nA, and this was reduced to 50 nA after adding TEA. Temperature was 21 °C. Different oocyte from *A*.

and see Methods) to derive estimates of the properties of the elementary channels responsible for the maintained outward current.

Fig. 10*B* illustrates the average power spectrum of slow potassium current noise at −5 mV, obtained from a *Torpedo* electric lobe mRNA-injected oocyte in the presence of 5 mM-manganese. The power spectrum of noise obtained during the same voltage step after blocking the slow potassium current with 10 mM-TEA is also shown

(lower curve, Fig. 10*B*). Over most of the frequency range explored, this residual noise was negligibly small compared to the noise due to the slow potassium current. For the oocyte illustrated in Fig. 10*B*, the mean lifetime and conductance of the potassium channels, as derived from the noise analysis, were 13 ms and 20 pS respectively. Mean values obtained from a total of five oocytes were: mean lifetime = 13·8 ± 1·4 ms (± 1 S.E. of mean), conductance = 14·0 ± 1·1 pS (temperature = 21–23 °C, clamp potential = −10 or −5 mV).

Induction of kainate receptors

In addition to the voltage-activated channels involved in the generation of the slow and fast potassium currents and the sodium current, the mRNA from the electromotor lobe also induced the incorporation of drug receptors activated by kainate. However, compared to other brain mRNA preparations which we have used previously (Gundersen *et al.* 1983*a*, *b*, 1984*a*, *b*; Miledi, Parker & Sumikawa, 1982), the *Torpedo* electric lobe mRNA was poor at inducing drug-activated channels, even though substantial numbers of voltage-activated channels were formed.

DISCUSSION

Induction of membrane channels coded by Torpedo brain mRNA

Xenopus oocytes injected with mRNA from the electric lobe and medulla of *Torpedo* developed a potassium conductance which was activated by depolarization, and could give currents as large as 40 μA. Control (non-injected) oocytes did not show this conductance, whilst the potassium channels were still incorporated following injection of *Torpedo* electric lobe mRNA into oocytes where the transcription of the oocyte's own genome was blocked by enucleation or by actinomycin. Thus, the proteins comprising the membrane channel were almost certainly translated by the injected exogenous mRNA. Since the conductance of the single potassium channel is about 14 pS, it appears that more than 10^7 channels were incorporated into the membrane of some oocytes.

At present, we do not know the cellular source of the mRNA responsible for the channels induced in the oocyte. However, it seems likely that the potassium and sodium channels may have derived from the electromotor neurones, since these large cells (60–80 μm diameter) are present in considerable numbers (100000) in the electric lobes (Albé-Fessard & Buser, 1954; Szabo 1954). Albé-Fessard & Buser (1954) showed that the electromotor neurones give action potentials, but it was not determined whether these were due to sodium or calcium channels. We hope to explore the origin of the mRNA more thoroughly, by taking only the neuronal cell bodies of the electric lobe to prepare mRNA (cf. Schmid, Stadler & Whittaker, 1982).

The present preparation of *Torpedo* electric lobe mRNA was not very effective at inducing receptors to neurotransmitters, even though such receptors are present in the native cells. Electromotor neurones in some fish species have electrical synapses (Bennett, 1970; Pappas, Waxman & Bennett, 1975), but chemical synapses have been described in *T. marmorata* (Roberts & Ryan, 1975), and Albé-Fessard & Buser (1954) show records of synaptic potentials. Moreover, our mRNA preparation included the medulla, which must have many neurones with transmitter receptors. In particular,

we have not so far detected responses to taurine or β-alanine, which are the most prevelant amino acids in *Torpedo* brain (Davies & Dowe, 1979).

It would be interesting to find out if the mRNA from the electric lobe of fishes which have electrical contacts is able to translate and incorporate these membrane channels into the oocyte membrane. Quite possibly, some cells in the brains that we have used (*Torpedo*, rat, chick and human: Gundersen *et al.* 1983*a*, *b*, 1984*a*, *b*; Miledi *et al.* 1982) have electrical contacts. These may have given some 'gap junction channels' in the oocyte, but their detection could have eluded us. The electric lobe of fish, possessing many cells with electrical contacts, would be a favourable source of mRNA to study the synthesis and processing of gap junctions.

Characteristics of the slow potassium channels

The chief characteristics of the maintained potassium current are: (i) it inactivates very slowly during maintained depolarization; (ii) it is blocked almost completely by 10 mM-TEA; (iii) over the approximate voltage range -30 to $+50$ mV, the current grows, but begins to decline with depolarization beyond about $+50$ mV; and (iv) the current is not blocked by manganese (5 mM) or lanthanum (100 μM). Some of these properties contrast markedly with those of the transient potassium current which is induced in oocytes injected with mRNA from rat brain (Gundersen *et al.* 1983*b*). This declines within about 100 ms of the onset of depolarization, and is only partially reduced in size by TEA.

Several different types of voltage-activated potassium currents have been described in a variety of excitable cells. These include: (i) a very slowly inactivating current found in squid axon (Hodgkin & Huxley, 1952; Ehrenstein & Gilbert, 1966) and skeletal muscle (Adrian, Chandler & Hodgkin, 1970); (ii) a transient current which has been recorded from many cell types, including egg cells, and molluscan and vertebrate neurones (see, for example, Barrett, Barrett & Crill, 1980; Hagiwara, Yoshida & Yoshii, 1981); and (iii) a calcium-dependent potassium current (Schwartz & Passow, 1983). The slow, potassium-selective membrane channels induced in the oocyte membrane following injection of mRNA from *Torpedo* electric lobe seem to resemble most closely the maintained potassium channels in nerve and muscle, whilst poly(A^+) mRNA from adult rat brain induces largely the transient type of potassium channel.

Potassium channels in squid axons and nerve terminals are blocked by TEA, but only if the TEA is injected intracellularly (Hagiwara & Tasaki, 1957; Katz & Miledi, 1969; Armstrong, 1971). In this respect, the maintained potassium current in the oocyte resembles more the potassium current in vertebrate nerve fibres and terminals (Hille, 1970; Katz & Miledi, 1977), which can be blocked by external TEA. The blocking action of 3,4-diaminopyridine on the maintained potassium channels in the oocyte shows several interesting features, which were not apparent with 4-aminopyridine or TEA; in particular, the block is enhanced by previous activation, but is partly reversed by strong depolarization. Furthermore, all these actions persist for many minutes after removing the drug from the bathing solution. 4-Aminopyridine has been used clinically for treatment of Eaton Lambert syndrome and myasthenia gravis (Lundh, Nilsson & Rosen, 1977, 1979), but because of the diversity of their actions, other aminopyridines might be better for this purpose. The use of human

potassium channels 'transplanted' into the oocyte (Gundersen *et al.* 1984*b*) may facilitate the study of these drugs.

A curious feature of the potassium current in oocytes injected with *Torpedo* electric lobe mRNA is the decline of current with increasing polarization to potentials above about +50 mV. The driving force for potassium efflux is expected to continue to increase at these potentials, so that the decline in current must presumably arise either from a voltage-dependent decrease in single-channel conductance, or from a reduction in the number of open channels. At present we cannot clearly distinguish between these possibilities. Several drug-activated membrane channels also show rectification (Gundersen *et al.* 1983*a*, 1984*a*; Miledi, Nakajima & Parker, 1980) and it will be interesting to see if any common mechanisms are responsible for this phenomenon.

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