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Voltage-operated channels induced by foreign messenger RNA in *Xenopus* oocytes

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Poly(A)$^+$ messenger RNA (mRNA) extracted from rat brains or from cat muscles was injected into *Xenopus laevis* oocytes. This led to the incorporation of voltage-operated Na^+ and K^+ channels into the oocyte membrane. These channels are not normally present in the oocyte and presumably result from the synthesis and processing of proteins coded by the injected mRNA. Tetrodotoxin blocked the Na^+ channels induced by mRNA derived from either innervated or denervated muscle.

INTRODUCTION

About 10 years ago Miyazaki *et al.* (1974) found that tunicate oocytes were able to generate action potentials, and since then action potentials have been observed in oocytes of other species (for reviews see Hagiwara & Jaffe (1979) and Takahashi (1979)). These potentials are generally caused by an increase in membrane permeability to calcium ions, although tunicate oocytes (Miyazaki *et al.* 1974), as well as oocytes from the starfish (Miyazaki *et al.* 1975) and the mouse (Okamoto *et al.* 1977), have action potentials with Ca^{2+} and Na^+ components. Neither component is blocked by tetrodotoxin (TTX), and in the mouse the Na^+-dependent component appears to be due to Na^+ passing through the calcium channels (Yoshida 1983). In contrast, amphibian oocytes are in general inexcitable, although maturing oocytes of *Rana pipiens* have action potentials, caused by Na^+ moving through TTX-resistant channels (Schlichter 1983*a*, *b*).

Oocytes from the South African frog *Xenopus laevis* are normally unable to generate action potentials (Kusano *et al.* 1982; Baud *et al.* 1982). However, Cl^- action potentials can occasionally be seen (Miledi, unpublished), and Na^+ channels appear after maintaining a strong depolarization. These Na^+ channels inactivate very slowly, if at all, and are blocked by TTX but only at high concentrations (Baud *et al.* 1982). Thus *Xenopus* oocytes are normally unable to generate Na^+ or Ca^{2+} action potentials of the type common in nerve and muscle cells. The question arises whether the *Xenopus* oocyte membrane is unable to incorporate the appropriate channels, or whether the channels are absent because the required proteins are not produced by the oocyte. We have examined this question by injecting into *Xenopus* oocytes poly(A)$^+$-mRNA derived from sources rich in cells capable of generating action potentials.

Methods

Oocytes were dissected from the ovaries of adult frogs (*Xenopus laevis*). They were injected with poly(A)$^+$-RNA obtained from brains of Wistar rats, or cat limb muscles, by the procedure of Miledi & Sumikawa (1982) or by a modification of this protocol in which the initial tissue (10 g) homogenization was performed in a blender with 100 ml each of homogenization buffer and phenol. After centrifugation the aqueous phase was extracted once with an equal volume of chloroform, then with chloroform–phenol (0.5 volume of each) and finally with chloroform (0.5 volume). Also, the final step of the oligo(dT) chromatography employed buffer that had been warmed to 45 °C to elute the poly(A)$^+$-RNA.

For electrophysiological study the oocytes were in a bath perfused with frog Ringer solution (NaCl 115 mM, KCl 2 mM, $CaCl_2$ 1.8 mM, HEPES 5 mM, pH about 7) and the membrane was voltage clamped (Kusano *et al.* 1982; Miledi 1982). The membrane potential was usually held at -100 mV, from which it was stepped momentarily to different potentials and the ensuing membrane currents were recorded directly on film, or averaged by using a Datalab recorder.

Results

In normal, non-injected oocytes the main membrane currents obtained with depolarizing pulses were essentially of two kinds. In some oocytes the current–voltage relation was nearly linear, corresponding to a passive membrane (Kusano *et al.* 1982; Baud *et al.* 1982). In other oocytes a transient outward current was generated when the membrane was depolarized beyond about -30 mV. This current is caused by chloride ions moving through channels that are opened by a preceding influx of calcium into the oocyte (Miledi 1982). There are also maintained currents, but in this paper we are concerned only with transient currents that inactivate within seconds.

Transient currents after injection of rat brain mRNA

A few days after injecting the oocytes with poly(A)$^+$-mRNA extracted from rat brains, the currents elicited by depolarizing pulses were importantly different from those occurring in control oocytes. Three main currents were observed: two outward currents, both transient but one fast and the other slow, and a transient inward current (figure 1). These currents were clearly generated by the oocyte membrane proper, because they were still present after removing the follicular and other cells with collagenase (Kusano *et al.* 1982). Furthermore, the fast outward and the inward currents were induced by the injection of rat brain mRNA because they were not seen in oocytes (from the same donor) that were either non-injected or were injected with mRNA extracted from *Torpedo* electroplaques, which are electrically inexcitable.

Outward currents

The two transient outward currents are illustrated in figure 1*a*, the slow one at 0 mV and the fast one at $+40$ mV. The slow current begins to activate at about

−30 mV, reaches a maximum amplitude at about +10 mV, becomes smaller as the potential is made more positive and vanishes at about +40 mV. This current is blocked by Mn^{2+} (5 mM) or La^{3+} (50–100 μM). In these and other respects the slow, transient outward current of oocytes injected with mRNA is the same as that seen frequently in normal oocytes and is also carried by Cl^- ions (Miledi 1982). Although this current is also seen in control oocytes, it appears to be more prominent in injected oocytes.

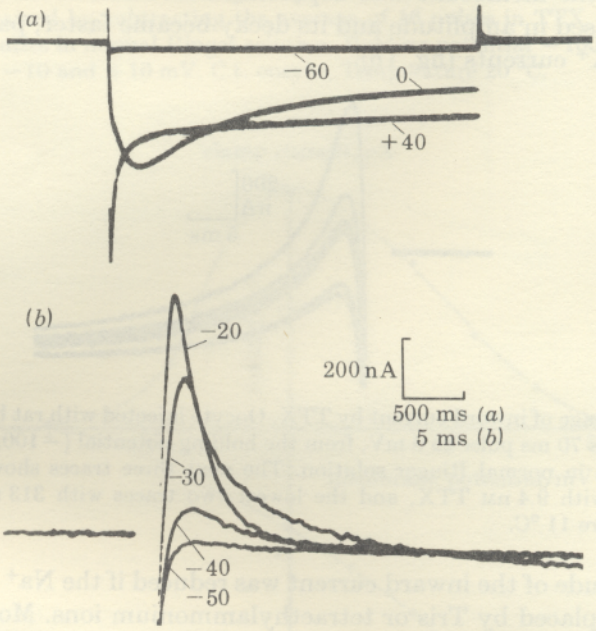

FIGURE 1. Membrane currents in an oocyte previously injected with rat brain mRNA. In this and subsequent figures inward currents are denoted by upward deflexions. Figures on the traces indicate the membrane potential (millivolts) during depolarizing pulses, from the holding potential (−100 mV). In (*a*) the trace labelled O shows a transient outward current that disappears when the potential is stepped to +40 mV. This leaves a faster transient outward current. (*b*) Inward currents elicited by depolarizing pulses (70 ms). Collagenase-treated (c.t.) oocyte. Temperature 18 °C.

The fast outward current begins to activate at about −50 mV and its amplitude increases continuously as the membrane is depolarized and taken even beyond +40 mV (cf. figure 4). Depending on relative amplitudes, it was sometimes obscured by the onset of the slower chloride current. But when the latter disappeared, at +40 mV, the fast current could be seen clearly (figure 1*a*).

Experiments in which the membrane potential was stepped to various levels, immediately after the peak of the fast outward current, showed that the tail current disappeared at about −100 mV, which corresponds to the equilibrium potential for K^+ in these oocytes (Kusano *et al.* 1982). Moreover, the fast current was reduced by tetraethylammonium ions, and further decreased by La^{3+} and Mn^{2+}.

These and other experiments indicate that K^+ is the main ion carrying the fast

outward current, and the effects of La^{3+} and Mn^{2+} suggest that part of this current may be consequent to an entry of calcium ions.

Inward current

In contrast to control oocytes, those injected with rat brain mRNA generated inward currents when their membrane was depolarized. An inward current was already detected when the membrane potential was stepped from −100 to −50 mV. As the membrane was depolarized further towards 0 mV, the inward current increased in amplitude and its decay became faster, partly because of the onset of the K^+ currents (fig. 1*b*).

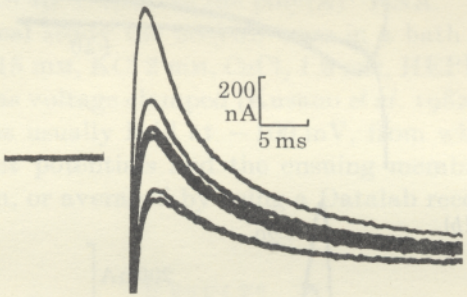

FIGURE 2. Blockage of inward current by TTX. Oocyte injected with rat brain mRNA. Currents elicited by a 70 ms pulse to 0 mV, from the holding potential (−100 mV). Top trace shows the current in normal Ringer solution. The next three traces show the currents during perfusion with 9.4 nM TTX, and the lowest two traces with 313 nM TTX. C.t. oocyte. Temperature 11 °C.

The amplitude of the inward current was reduced if the Na^+ in the bathing fluid was partly replaced by Tris or tetraethylammonium ions. Moreover, the current became nil at about +50 mV, which is close to the equilibrium potential for Na^+ in *Xenopus* oocytes (Kusano *et al.* 1982). These results indicate that Na^+ is the main ion responsible for the inward current, although a small Ca^{2+} component might be present. Mn^{2+} and La^{3+} reduced the inward current, but preliminary experiments suggest that this might result from a change in the characteristics of the Na^+ channels, rather than from a blockage of Ca^{2+} channels.

Effect of TTX on the induced currents

Tetrodotoxin (313 nM) had little or no effect on the outward currents but readily abolished the inward current induced by injection of rat brain mRNA. The block is illustrated in figure 2, which shows the currents elicited by pulses to 0 mV in an oocyte exposed to TTX. The top record shows the current obtained before applying the toxin, while the next three records show the decrease in current caused by exposing the oocyte to 9.4 nM TTX. At equilibrium this toxin concentration reduced the inward current to slightly less than half its initial amplitude. Increasing the TTX concentration to 313 nM almost completely blocked the inward current (lower two traces); and further increases in the concentration had little or no additional effect.

The time course of the Na^+ current was obtained by subtracting traces obtained

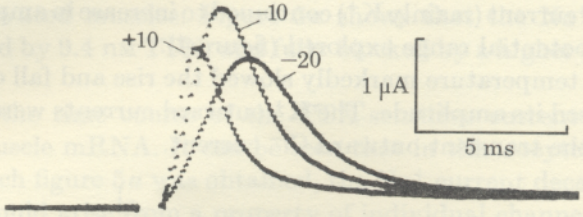

FIGURE 3. TTX-sensitive current in an oocyte injected with rat brain mRNA. Each trace shows the current obtained by subtracting the average of 16 pulses in TTX (313 nM) from the average of 16 pulses in normal Ringer solution. Holding potential −100 mV, depolarizing pulses to −20, −10 and +10 mV. C.t. oocyte, Temperature 20 °C.

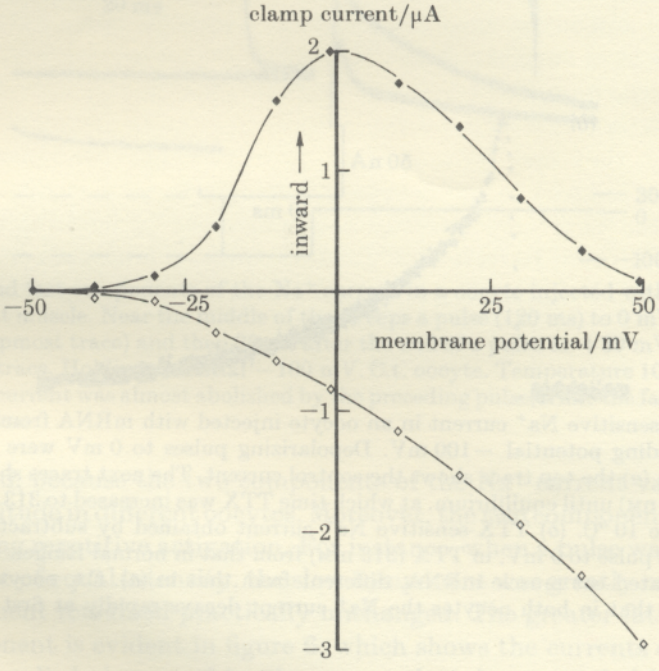

FIGURE 4. Peak amplitude of inward (Na^+) and outward (K^+) currents plotted against membrane potential in an oocyte injected with rat brain mRNA. Same oocyte as in figure 3. The upper part shows the TTX(313 nM)-sensitive inward current. The lower part shows the peak outward current (in TTX) after subtracting passive currents.

in TTX from those recorded in normal Ringer solution. Results with three pulses are shown in figure 3. Both rise and fall of the current became faster as the intensity of the depolarizing pulse was increased. Sometimes the decay occurred in two phases, suggesting that two different Na^+ channels may be involved.

Figure 4 illustrates the relation between amplitude of the TTX-sensitive current and the membrane potential. The maximum, at about 0 mV, was quite variable, ranging between more than 1 μA in some oocytes to very small currents in others. Sometimes no Na^{2+} current could be detected. With pulses beyond 0 mV the amplitude decreased and the current became nil at about +50 mV. In contrast,

the fast outward current (mainly K^+) continued to increase in amplitude throughout the membrane potential range explored (figure 4).

Lowering the temperature markedly slowed the rise and fall of the Na^+ current and also decreased its amplitude. The K^+ outward currents were also reduced and slowed, as was the transient outward Cl^- current.

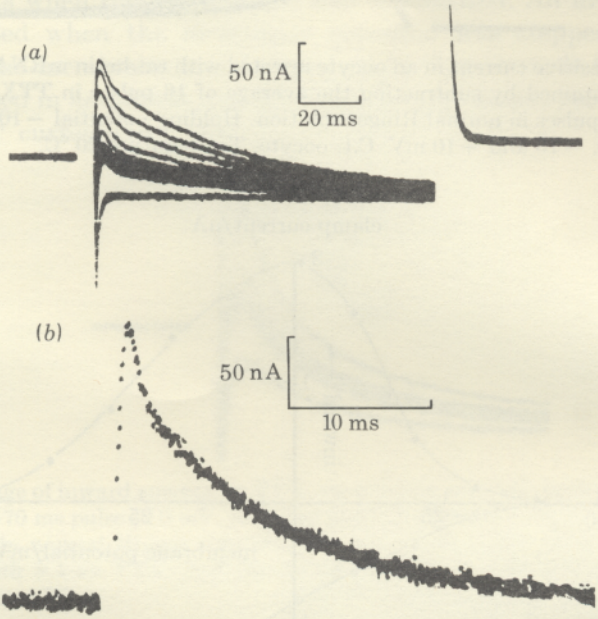

FIGURE 5. TTX-sensitive Na^+ current in an oocyte injected with mRNA from innervated cat muscle. Holding potential -100 mV. Depolarizing pulses to 0 mV were applied at 10 s intervals. In (*a*) the top trace shows the control current. The next traces show the currents in TTX (9.4 nM) until equilibrium, at which time TTX was increased to 313 nM. C.t. oocyte. Temperature 10 °C. (*b*) TTX-sensitive Na^+ current obtained by subtracting the current evoked by a pulse to 0 mV, in TTX (313 mM) from that in normal Ringer. Oocyte injected with innervated cat muscle mRNA, different from that in (*a*). C.t. oocyte. Temperature 13 °C. Note that in both oocytes the Na^+ current decays rapidly at first and then more slowly.

Current after injection of cat muscle mRNA

Outward currents

Oocytes were injected with mRNA isolated from either normal or denervated cat muscles, with qualitatively similar results. In general, the outward currents in these oocytes were smaller than those of 'rat brain oocytes'. A transient outward current, presumably due to K^+, was induced by cat muscle mRNA, but so far we have not examined whether this current includes more than one component.

Inward current

Cat muscle mRNA induced an inward current that resembled in many respects the inward current induced by rat brain mRNA, and which was similarly due to movement of Na^+ through channels in the oocyte membrane. These channels were blocked by TTX, regardless of whether they had been induced by mRNA from

innervated or denervated muscles. Figure 5*a* shows that the Na^+ current was substantially blocked by 9.4 nM TTX, and fully blocked by a higher concentration (313 nM) of TTX.

Figure 5*b* shows the time course of the TTX-sensitive current in an oocyte injected with cat muscle mRNA. It can be seen that in this oocyte, as well as in the oocyte from which figure 5*a* was obtained, the Na^+ current decayed with two time courses. This could arise from a property of individual channels or from the presence of different populations of channels. It appears that two Na^+ channels

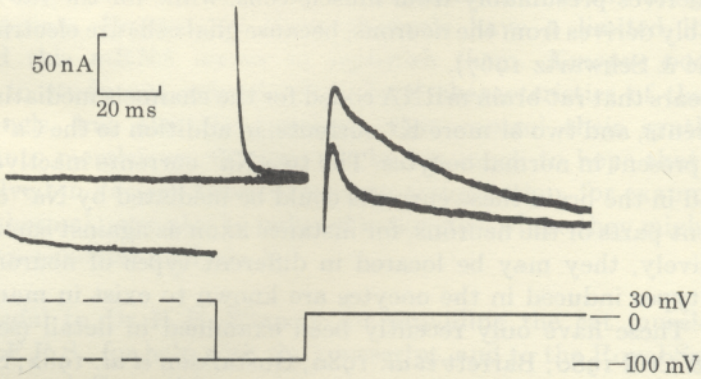

FIGURE 6. Slow and fast components of the Na^+ current in a oocyte injected with mRNA from innervated cat muscle. Near the middle of the sweeps a pulse (120 ms) to 0 mV was applied, first alone (topmost trace) and then 30 ms after the end of a pulse to +30 mV, as indicated in the lowest trace. Holding potential −100 mV. C.t. oocyte. Temperature 10 °C. Note that the slow Na^+ current was almost abolished by the preceding pulse, while the fast Na^+ current was reduced less.

may be involved, because the two components of the Na^+ current varied greatly in relative amplitude in different oocytes. Moreover, the two components 'fatigued' differently during repetitive activation. For instance, when a pulse was given 0.5 s after a first pulse of equal intensity, the slow component was greatly reduced while the fast component remained practically unchanged. The greater fatiguability of the slow component is evident in figure 6, which shows the currents elicited by a pulse to 0 mV applied alone, and by the same pulse when preceded by a pulse to +30 mV. The slow component was almost abolished by the conditioning pulse, whereas the fast component was reduced at most to about one-half.

DISCUSSION

The surface membrane of *Xenopus* oocytes contains receptors to substances that act as transmitters in the nervous system (Kusano *et al.* 1982). The presence of these native receptors makes the oocytes potentially very useful for the study of transmitter action. Recently, this possibility was greatly extended by injecting the appropriate mRNA, which enabled the oocyte to acquire 'foreign' receptors. In this way we have induced nicotinic acetylcholine receptors (Barnard *et al.* 1982; Miledi & Sumikawa 1982; Miledi *et al.* 1982*b*); receptors to GABA (Miledi *et al.* 1982*a*, 1983*a*), serotonin (Gundersen *et al.* 1983) and more recently glutamate and

kainate (Gundersen *et al.* 1984). These receptors were incorporated into the membrane, where they formed receptor–channel complexes that could be activated by the combination of a specific drug with its receptor. The present experiments show that, by similar procedures, the oocyte can be made to acquire channels that are opened by an electric potential change across the membrane. Our main finding is that the oocyte membrane can be induced to acquire voltage-operated channels similar to those present in neurons and muscles. The mechanism of the induction is, very probably, the translation of the injected mRNA. In muscle the responsible mRNA derives presumably from muscle cells, while for the rat brain most of it presumably derives from the neurons, because glial cells are electrically inexcitable (Krnjević & Schwartz 1967).

It appears that rat brain mRNA coded for the channels mediating two transient Na^+ currents, and two or more K^+ currents, in addition to the Ca^{2+}-dependent Cl^- channel present in normal oocytes. The two Na^+ currents inactivated at different rates, and in the brain these currents could be mediated by Na^+ channels located in different parts of the neurons, for instance axon as against soma and dendrites. Alternatively, they may be located in different types of neurons. Some of the channel types induced in the oocytes are known to exist in mammalian central neurons. These have only recently been examined in detail (see, for example, Barrett & Crill 1980; Barrett *et al.* 1980; Gustafsson *et al.* 1982; Schwindt & Crill 1980), and it will be interesting to compare their detailed properties in oocytes and neurons.

The Na^+ current of oocytes injected with cat muscle mRNA also had two components, whose relative amplitudes varied greatly in different oocytes. This is probably due to the existence of two different Na^+ channels, translated in different proportions. The two Na^+ channels differ in their rates of inactivation, and the channel that inactivates faster also recovers more quickly. Because of this, the slow component of the Na^+ current fatigues readily when pulses are repeated at a comparatively low rate, and this may help to explain the decrease in the amplitude of intracellular Ca^{2+} transients seen during repetitive activation of muscle fibres (Miledi *et al.* 1983*b*). It seems possible that in the muscle fibres the fast Na^+ channel is located in the surface membrane, and the slower Na^+ channel in the T-tubular membrane. In this context it is interesting that different properties of surface and T-tubule Na^+ channels have been observed in frog muscle, where a scorpion toxin appears to block only the surface channels (Jaimovich *et al.* 1982).

The two Na^+ currents induced by cat muscle mRNA, like those induced by rat brain mRNA, were blocked by TTX. Although there may be a small difference in the sensitivity of the two components to TTX, this is clearly not comparable with the TTX-resistance of Na^+ channels found in denervated rat muscles (Redfern & Thesleff 1971; Pappone 1980). In fact, 9.4 nM TTX reduced the Na^+ currents to about half in oocytes injected with mRNA derived from either innervated or denervated muscles. Perhaps the change that renders the Na^+ channels less sensitive to TTX after denervation does not reside in the structure of its constituent proteins, or it may be a consequence of a post-translational modification that does not occur in the oocyte.

Pending a more detailed analysis, the Na^+ currents induced by foreign mRNA

in the oocytes appear to be similar to those in neurons and muscles. The maximum rat brain or cat muscle Na^+ currents in the oocytes reached a few microamps, which is similar to the currents in cat motoneurons (Barrett & Crill 1980) and rat muscles (Adrian & Marshall 1977; Duval & Léoty 1980*a*, *b*; Pappone 1980). If the size of the single Na^+ channel current in the oocyte is approximately the same as in other cells (cf. Sigworth & Neher 1980), this would imply that the oocyte membrane incorporates more than a million Na^+ channels.

Finally, our experiments indicate that adult muscle and nerve cells contain mRNA species that code for the Na^+ and K^+ channels involved in the generation of action potentials. Presumably these channels have a limited life in the membrane and this mRNA serves to replenish them. *Xenopus* oocytes will probably help to study not only the functional characteristics of the voltage-operated channels, but also the processes that control their synthesis and incorporation into membranes. It may not be too much to hope that even the molecules involved in various types of sensory transduction, for example visual, mechanical or thermal, may also be induced in oocytes, where they might be more amenable to experimental analysis.

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