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Tetrodotoxin-sensitive sodium current in native *Xenopus* oocytes

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Depolarization of oocytes of *Xenopus laevis* usually elicits mainly passive currents, and a calcium-dependent chloride current. However, oocytes obtained from some donors show, in addition, a transient inward current on depolarization to potentials beyond *ca.* −40 mV. This current is abolished by tetrodotoxin at submicromolar concentrations, and is prolonged by veratrine; thus, it probably arises through sodium channels of a type similar to those found in nerve and muscle cells. However, the kinetics of the sodium currents varied between oocytes from different donors; this result suggests that genes encoding different sodium channels may be expressed in oocytes from different donors. The presence of these native channels may complicate experiments to study the expression of exogenous sodium channels encoded by foreign messenger RNAs injected into the ooctye.

Introduction

Oocytes from many species have been found to be electrically excitable, as a result of the presence in their cell membranes of channels activated by depolarization (for reviews see Hagiwara & Jaffe (1979); Takahashi (1979); Hagiwara (1983)). Voltage-dependent calcium channels are most commonly observed but, in addition, sodium-dependent currents have been described in oocytes from tunicates (Miyazaki *et al.* 1974), starfish (Miyazaki *et al.* 1975), mice (Okamoto *et al.* 1977; Yoshida 1983) and amphibia (Baud *et al.* 1982; Schlichter 1983). However, in all these cases, the properties of the sodium currents differed from those of 'classical' sodium currents like those in nerve and muscle cells (Hille 1984). In particular, the oocyte sodium currents showed little or no inactivation and were resistant to block by tetrodotoxin (TTX). In contrast to those responses, we describe here that depolarization of oocytes of *Xenopus laevis* sometimes generates a transient inward sodium current, which inactivates rapidly, is blocked by submicromolar concentrations of TTX, and is prolonged by veratrine.

Methods

Experiments were made on oocytes of *Xenopus laevis*, which were sometimes treated with collagenase to remove enveloping cells (Miledi & Parker 1984). For electrophysiological study, the ooctes were in a bath continuously perfused with

Ringer's solution at room temperature (20–25 °C), and were voltage-clamped with a two-electrode system (Miledi 1982; Gundersen *et al.* 1983). The membrane potential was usually held at −100 mV, from which it was stepped briefly (usually for 50 ms) to different potentials, and the ensuing membrane currents were photographed from analogue and digital storage oscilloscopes. Low-resistance-current electrodes were used to improve the ability of the clamp to charge the large membrane capacitance of the ooctye; despite this, the large capacitative transient prevented accurate recording during the first few milliseconds of a pulse.

Results

Transient inward current activated by depolarization

Oocytes of *Xenopus laevis* often show only passive currents on depolarization: a depolarizing step elicits an initial capacitative surge of current, followed by a maintained 'leakage' current, which varies roughly linearly with potential (Kusano *et al.* 1982). In addition, many oocytes show a slowly rising and falling outward current, when depolarized to around 0 mV, which is due to chloride ions moving through channels opened by a preceding influx of calcium into the oocyte (Miledi 1982; Barish 1983). More rarely, in experiments over several years, we have also detected sizeable brief inward currents; these are the subject of the present paper.

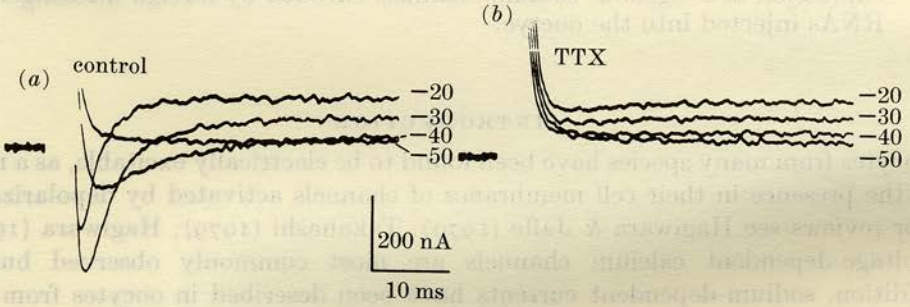

Figure 1. Transient inward sodium currents evoked by depolarization of a native (non mRNA-injected) oocyte to various potentials. Each frame shows superimposed records of membrane currents in response to a depolarizing step from a holding potential of −100 mV to the potentials indicated (in mV) next to each trace. Records in (*a*) were obtained in normal Ringer's solution; those in (*b*) show responses to the corresponding voltage steps applied after addition of TTX (600 nM) to the bathing medium. Downward deflections in this and other figures correspond to inward membrane currents. The oocyte was from donor F (see text), and was not collagenase-treated. Temperature 23 °C.

Figure 1(*a*) shows records of membrane currents in an oocyte which displayed this inward current. Depolarizations from the holding potential of −100 to −50 mV gave only passive currents. However, when the oocyte was stepped to −40 mV a transient inward current appeared, and this became larger when the potential was further decreased, reaching a maximum between about −20 and −30 mV. Addition of 300 or 600 nM tetrodotoxin (TTX) to the bathing medium abolished the transient inward current (figure 1*b*), indicating that it arises from

activation of voltage-dependent sodium channels. In many oocytes this sodium current was small, and difficult to discern in the presence of larger passive and calcium-activated chloride currents. Measurements were therefore made by recording currents on depolarization from −100 to −20 mV before and after adding TTX, and then subtracting these traces to give the TTX-sensitive sodium current (see, for example, figure 2). In this way, sodium currents could be detected in oocytes from most of the donors examined, although they were generally small (5 nA or less). However, some donors gave oocytes which showed consistently larger responses, that were occasionally as great as several hundred nanoamperes.

Sodium currents were observed both in intact follicles (i.e. oocytes surrounded by follicular and epithelial layers) and in oocytes that had been defolliculated by treatment with collagenase, but they were smaller in the collagenase-treated oocytes. For example, the mean response on depolarization to −20 mV was 358±96 nA (s.e.m.) in five non-treated oocytes, compared with 67±63 nA in five collagenase-treated oocytes, which were examined at the same time and obtained from the same donor. The time course of the sodium current was not obviously changed after collagenase treatment. Another factor that appeared to affect the size of the sodium current was the time at which oocytes were examined after removal from the ovary. Oocytes from the donor described above gave consistently large currents when tested one day after removal, but responses were almost completely absent in oocytes and follicles tested four days later. However, oocytes from another donor showed sodium currents of similar size when tested at various times up to eight days after removal (mean current = 66±5 nA; 14 oocytes, all collagenase-treated). The data presented in this paper are based on recordings in oocytes from these two donors, which we refer to as, respectively, donors F and S (for fast and slow; see below).

Properties of native sodium currents

Figure 2(*a*,*c*) shows the time course of the TTX-sensitive sodium current elicited by depolarization from −100 to −20 mV in oocytes from donors F and S. The rising phases of the currents were not well resolved in these records, but it is clear that the time course of decline of the currents during maintained depolarization was quite different between the two oocytes. The sodium current in figure 2*a* declined with a half-time of about 9 ms, whereas the corresponding value for the oocyte in figure 2*c* was about 1.5 ms. All oocytes from donor S showed a relatively slow sodium current, like that in figure 2*a*; the currents in oocytes from donor F were consistently fast (see figures 1*a* and 2*c*). This difference in time course of the currents in oocytes from different donors might have arisen because different types of sodium channel were expressed, or because the properties of the channel were altered by differences in lipid composition of the oocyte membrane. To examine the latter possibility, we recorded sodium currents in oocytes from donor S which had been injected with messenger RNA from rat brain, to induce the appearance of exogenous sodium channels (Gundersen *et al.* 1983). The induced sodium current (figure 2*b*) was much greater (3 μA) than the native response but was more transient, declining with a half-time of about 1.4 ms, similar to that of the native sodium current in oocytes from donor F.

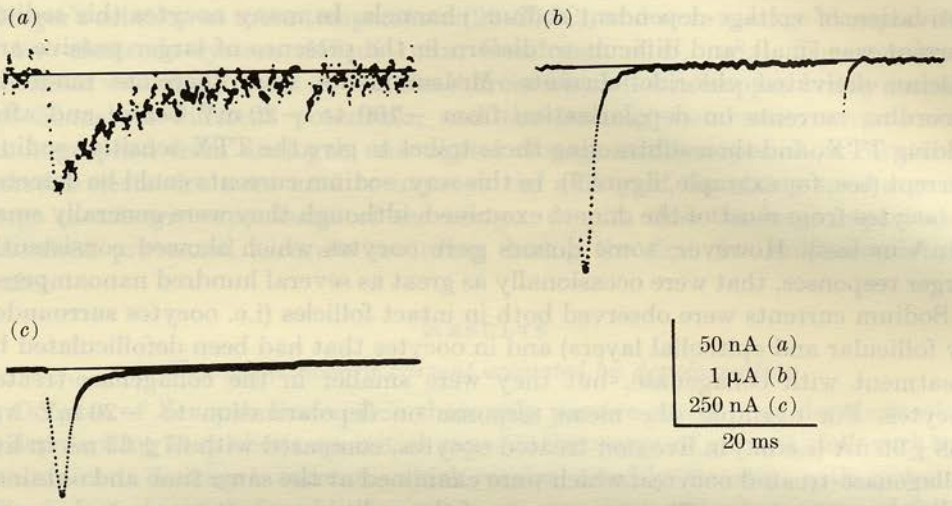

FIGURE 2. Native (*a*, *c*) and induced (*b*) sodium currents. Each frame shows sodium currents elicited by depolarization from −100 to −20 mV, and was derived by subtracting the currents elicited by identical potential steps in the absence and presence of TTX (300 or 600 nM). (*a*) Native sodium current in an oocyte from donor S; (*b*) induced sodium current in an oocyte from the same donor, which had been injected with mRNA from rat brain six days before recording; (*c*) native sodium current in an oocyte from donor F. Oocytes in (*a*) and (*b*) were collagenase-treated; the oocyte in (*c*) was untreated. Temperature 23 °C.

Differences in characteristics of the sodium currents in oocytes from the two donors were also apparent in their rates of recovery from inactivation. Oocytes from donor F showed rapid recovery, as illustrated in figure 3*a*, where two depolarizing pulses given at an interval of 100 ms elicited almost identical responses. In contrast, oocytes from donor S failed to give any appreciable response to a second stimulus, even after a longer interval of 200 ms (figure 3*b*).

The addition of veratrine (0.25 mg ml^{-1}) to the bathing solution dramatically slowed the inactivation of the sodium currents (figure 4). In the presence of

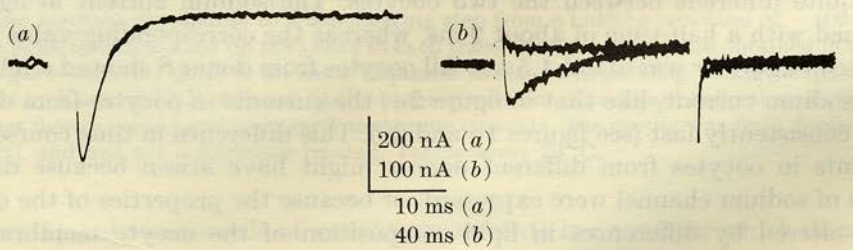

FIGURE 3. Recovery of sodium current from inactivation, monitored with paired pulses. Each frame shows two superimposed sweeps of membrane currents elicited by successive depolarizations from −100 to −20 mV (*a*) or −10 mV (*b*). The pulse duration was 100 ms, and the oocytes were repolarized to −100 mV for 100 ms (*a*) or 200 ms (*b*) between the pulses. Records in (*a*) were obtained from an oocyte (non-collagenase-treated) from donor F, and those in (*b*) were from an oocyte (collagenase-treated) from donor S. Temperature 24 °C.

veratrine a maintained inward current persisted throughout the depolarizing pulse, while the initial inward current was slightly smaller. The most striking difference was, however, seen when the oocyte was repolarized. Normally, no sodium tail currents were apparent when the membrane was repolarized (figure 2), but a few minutes after adding veratrine large and slowly decaying current relaxations were recorded (figure 4). Both the inward tail current and the maintained inward current during depolarization were blocked by TTX. Prolonged sodium currents could be detected after treatment with veratrine even in oocytes that showed very little transient current in the absence of this toxin.

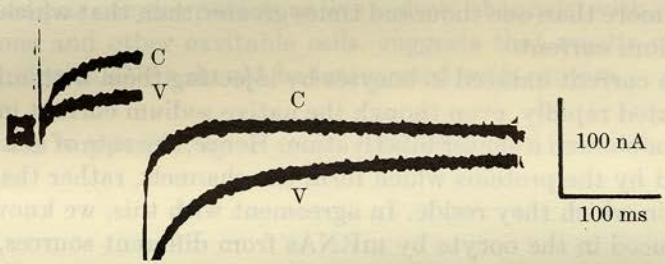

FIGURE 4. Induction of a prolonged sodium current following bath application of veratrine (0.25 mg ml^{-1}). The oocyte was depolarized from −100 to −20 mV for 100 ms. Superimposed traces show currents recorded before (C) and after (V) addition of veratrine. The oocyte had been treated with collagenase, and was obtained from a donor different from F or S. Temperature 22 °C.

DISCUSSION

We, and others, have previously reported that *Xenopus* oocytes do not normally posses functional sodium channels of the type which generate the action potential in nerve and muscle cells (Kusano *et al.* 1982; Baud *et al.* 1982; Gundersen *et al.* 1983; Sigel 1987). However, the present results indicate that a small proportion of donors yield oocytes which express an appreciable (tens or hundreds of nanoamperes) sodium current, and that small currents (*ca.* 5 nA) are common in oocytes from most donors. The membrane channels responsible for at least some of this current are located in the oocyte proper, because responses are seen in oocytes from which the follicular and other enveloping cells were stripped by treatment with collagenase. However, the response sizes were reduced by collagenase treatment. This may indicate that some sodium channels are present in the follicular cells, which are electrically coupled to the oocyte proper (Browne *et al.* 1979; Woodward & Miledi 1987). Alternatively, it could be that all the sodium channels reside in the oocyte membrane, but that some of these were damaged by the collagenase.

In those oocytes that gave responses sufficiently large for convenient study, after depolarization of potentials of −40 mV and above, the sodium current was seen as an inward current, which was transient and declined within a few tens of milliseconds or less. The current was abolished by tetrodotoxin, and prolonged by veratrine. Thus, the properties of the native sodium channels in the oocyte are

qualitatively similar to those of sodium channels in nerve and muscle (Hille 1970, 1984; Narahashi 1974; Catterall 1980), and to those of sodium channels from nerve and muscle transplanted to the oocyte by injection of exogenous mRNA (Gundersen *et al.* 1983, 1984; Sigel 1987).

A maintained sodium current has previously been described in *Xenopus* oocytes (Baud *et al.* 1982; Baud & Kado 1984), but that is clearly different from the transient current which we describe. In particular, the maintained current was seen only after induction by prolonged depolarization to positive membrane potentials, whereas the transient current inactivates rapidly during depolarization. Furthermore, the concentration of TTX required to block the maintained current (0.5 mM) was more than one thousand times greater than that which abolished the transient sodium current.

The sodium current induced in oocytes by injecting them with mRNA from rat brain inactivated rapidly, even though the native sodium current in oocytes from the same donor showed a slower inactivation. Hence, the rate of inactivation must be determined by the proteins which form the channels, rather than by the lipid environment in which they reside. In agreement with this, we know that sodium channels, induced in the oocyte by mRNAs from different sources, vary in their rates of inactivation and other characteristics (Gunderesen *et al.* 1983, 1984). Similarly, the native sodium currents in oocytes from different donors vary in their rates of inactivation and other characteristics; this observation suggests that these differences might also arise because of expression of different channel proteins. It will be interesting to see if the native oocyte channels correspond to any of those expressed in excitable cells in the adult frog. The sodium currents in frog node and muscle inactivate at a similar rate to that of the native sodium current in oocytes from donor F (Hille 1984), whereas the native current in donor S was slower and more like that of sodium channels in the heart.

At present it is unclear whether the transient sodium current has any function in the oocyte. In view of the small proportion of oocytes which show appreciable responses, this seems doubtful. Furthermore, even the largest sodium currents that we recorded (600 nA) would probably be insufficient to produce a regenerative action potential, because the duration of the current is too brief to discharge the large membrane capacitance of the oocyte (cf. Gundersen *et al.* 1984).

The use of *Xenopus* oocytes is becoming increasingly popular for the functional expression of sodium channels encoded by mRNAs extracted from various tissues (Gundersen *et al.* 1983, 1984; Sumikawa *et al.* 1984, 1986; Methfessel *et al.* 1986; Siegel 1987) or transcribed from cloned genes (Noda *et al.* 1986; Stühmer *et al.* 1987). For this purpose it would be convenient if the oocyte provided a 'blank slate', in the sense that it was totally devoid of native sodium channels and of translationally active mRNA encoding these channels. Our results indicate that this is not so. From the point of electrical recording of sodium currents there is little problem, because few donors give oocytes with appreciable sodium currents, and the native currents of a few nanoamperes which are more commonly found are insignificant as compared to the currents of several microamperes induced by exogenous mRNA. However, a more subtle complication arises from experiments

to determine whether the large subunit of brain sodium channels is sufficient, by itself, for functional activity. The sodium channel complex isolated from rat brain and muscle consist of a large subunit and one or two small subunits (Messner & Catterall 1985; Barchi *et al.* 1984). Injection of mRNA coding for only the large subunit was found to induce sodium currents in the oocyte (Sumikawa *et al.* 1984; Goldin *et al.* 1986; Noda *et al.* 1986; this result suggests that the presence of the small subunits is not required for the functioning of the channel. However, as noted by Noda *et al.* (1986), it is difficult to exclude the possibility that the *Xenopus* oocytes may themselves contain equivalents of the small polypeptides which associate with the exogenous large subunit to form functional channels. The finding that *Xenopus* oocytes possess native sodium channels, with properties like those in neurons and other excitable cells, suggests that results obtained after injection of foreign mRNAs should be interpreted with caution.

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