Effect of Lidocaine on the Slow Na$^+$ Channels of *Xenopus* Oocytes

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Abstract. The membrane of immature *Xenopus* oocytes is known to possess a peculiar type of sodium channels, which are not activatable unless the membrane has been depolarized for some time. Once induced by a long-lasting depolarization, the channels behave like voltage-dependent channels, but they slowly activate and apparently do not inactivate. In addition, these channels were shown to be insensitive to the toxins classically used to inhibit the voltage-dependent Na$^+$ channels. The effects of lidocaine on these slow Na$^+$ channels were investigated using current- and voltage-clamped oocytes. Lidocaine reversibly blocked the channels when they were in their open configuration, but not when the channels were in their closed state. The concentration of lidocaine required for half-inhibition of the slow inward current was 270 ± 67 μmol/l. The current/voltage relationships indicated that lidocaine blocked the sodium current (inward as well as outward) for all the potentials investigated. At a concentration of 0.3 mmol/l, lidocaine caused a shift of 5 ± 1 mV of the activation curve. This suggests that the gating properties of the channels were altered. The effect of lidocaine was found to be non-selective since at least two other channels were affected by the drug, namely the voltage-dependent calcium channels and the monovalent non-selective channels.

Key words: *Xenopus* oocytes — Sodium channel — Lidocaine — Electrophysiology — Pharmacology

Introduction

Two main subtypes of voltage-dependent sodium channels have been superficially classified through pharmacological studies. Firstly, channels which are highly sensitive to tetrodotoxin (TTX) in the order of the nanomolar such as the brain or adult

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skeletal muscle isoforms. Secondly, so-called TTX-resistant channels which are sensitive to micromolar concentrations of the toxin such as cardiac isoforms (Fozzard and Hanck 1996). These two main subtypes each have a distinct sensitivity to divalent cations and to a widely used anesthetic (lidocaine). The TTX-resistant channels from heart cells are blocked by a micromolar concentration of divalent cations from group IIB such as Zn^{2+} (Frelin et al. 1986). On the other hand, TTX-sensitive channels from neuronal cells require at least a millimolar concentration of Zn^{2+} to be half inhibited (Frelin et al. 1986). TTX-resistant channels are blocked by micromolar concentrations of lidocaine, while TTX-sensitive channels are inhibited by higher concentrations (see French et al. 1998).

The membrane of immature *Xenopus* oocytes contains a large variety of channels (Weber 1999). Two of them are voltage-dependent Na^+ channels displaying distinct electrophysiological and pharmacological properties. One type is rarely found in the oocytes, and is a fast activated, inactivating channel, sensitive to TTX and insensitive to lidocaine concentrations up to 100 μmol/l (Parker and Miledi 1987; Bourinet et al. 1992; Krafte and Volberg 1992). The other type is a slow activating, non-inactivating channel, which requires a millimolar concentration of TTX to be blocked (Baud et al. 1982) and is inhibited by micromolar concentrations of divalent cations (Quinteiro-Blondin and Charpentier 2001). Thus, the opening of these channels requires to be first induced. Indeed, numerous studies show that during a long-lasting depolarization of the membrane produced by injected current, a conductance slowly develops which did not exist before the depolarization (Kado et al. 1979; Kado and Baud 1981; Baud et al. 1982; Baud and Kado 1984). This new property of the oocyte membrane is established to be due to voltage-gated sodium channels, referred to here as "slow Na^+ channels" (sNa^+ channels). The most interesting behavior of these channels is that the membrane has to be depolarized for some time before the channels become voltage-sensitive. The sensitivity of the sNa^+ channels to TTX and to divalent cations is apparently related to that of heart cell channels (Quinteiro-Blondin and Charpentier 2001). Therefore, the present experiments were undertaken to study the possible blocking effect of lidocaine. Our data shows that lidocaine exerted a non-selective, reversible block of the channels.

Materials and Methods

a) Isolation, culture of oocytes and treatments

Experiments were performed using *Xenopus laevis* oocytes of stage-V or -VI according to Dumont's classification (1972). The females were purchased from the Centre de Recherches de Biochimie Macromoléculaire (Montpellier, France). Oocytes were treated with dispase II (0.5 mg/ml for 2 hours). Selected oocytes were maintained in an OR2 medium for up to 6 days at 16 $\pm$ 1 °C. The composition of the OR2 medium was (in mmol/l): 82.5 NaCl; 2.5 KCl; 1 $MgCl_2$; 1 $CaCl_2$; 1 Na_2HPO_4; 5 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES); pH 7.4 adjusted

with NaOH. No antibiotics were added. The incubation medium was renewed daily. Chemicals were purchased from Sigma (St. Quentin Fallavier, France) and were applied externally by addition to the superfusate.

b) Electrophysiological measurements

Electrophysiological recordings were performed using a two-electrode voltage clamp amplifier (Dagan Instruments, Minneapolis, MN, USA). Generation of clamp control voltages, data acquisition and analysis were performed using the pCLAMP software (vers. 5.5, Axon Instruments, Burlingame, CA, USA). Oocytes were impaled with 3 mol/l KCl-filled microelectrodes (1–1.5 MΩ for current electrodes and 5–15 MΩ for voltage electrodes) in a 250 μl recording chamber. All experiments were conducted at room temperature, 21–23 °C.

After a stable resting potential was achieved, the oocytes were voltage-clamped at a holding potential at -60 or -20 mV. Baud and Kado (1984) showed that the induction of the sNa$^+$ channels could not proceed if the membrane is depolarized to potentials less positive than $+20$ mV, even if depolarizing pulses last several minutes. Therefore, with potentials ranging from $+30$ to $+80$ mV, induction can occur. In our experiments, induction was obtained either by using a single long-lasting depolarization of 30 s duration or by using iterative short depolarizing steps of 4 s. When the Na$^+$ currents were elicited, some contaminations by outward going Cl$^-$ and K$^+$ currents were expected (Barish 1983; Baud and Kado 1984; Arellano et al. 1995). This source of error was minimized by using depolarizing pulses of $+50$ mV for 4 s duration as reported by Bossi et al. (1998). In this case, membrane currents were recorded with a strip chart recorder. The time for half-maximal inward current ($t_{1/2}$) was measured and used to compare induction rates. Since successive inductions do not show the same kinetics, unless they are separated by at least 1 h (Baud and Kado 1984), each oocyte was used for only one induction, unless specified otherwise. Current amplitude was measured as the peak value of the current from the level of the holding current. Measured currents of a large number of oocytes were expressed as means $\pm$ S.E.M. Significance between the means was tested using an unpaired Student's *t*-test. Values of p smaller than 0.05 were considered significant. The dose-response curve was fitted with equation (1), where $1 - y$ is the fraction of free receptor at equilibrium, K_d is the dissociation constant, C the drug concentration and n_H the Hill coefficient (see Hille 1992):

$$1 - y = \cfrac{1}{1 + \left(\cfrac{C}{K_d}\right)^{n_H}} \qquad (1)$$

The Current/Voltage (I/V) relationships were obtained on oocytes with and without induced Na$^+$ channels from a holding potential at -60 mV. In the non-induced oocytes, currents were elicited by 500 ms test pulses from -90 to $+90$ mV with 10 mV increments at 30 s intervals, to avoid inducing the Na$^+$ channels. I/V curves on the same oocyte were spaced out by a period of at least 15 min, during

which the oocyte was current clamped at zero current. In oocytes with induced Na^+ channels, test pulse duration was 4 s and the interval between two steps was 1 s. The conductance g was calculated as:

$$g = \frac{I_{Na}}{V_m - E_{Na}} \tag{2}$$

in which I_{Na} was the sodium current, V_m the membrane potential and E_{Na} the measured reversal potential. The conductance-voltage curve was fitted with a Boltzmann function of the form:

$$g = \frac{g_{max}}{1 + \exp \dfrac{V_m - V_{0.5}}{k}} \tag{3}$$

where g_{max} was the maximum conductance, $V_{0.5}$ the half activation voltage, k a slope factor (see Hille 1992).

Results

Effect of lidocaine on current clamp membrane responses

The injection of iterative depolarizing currents resulted initially in purely passive changes of the oocyte membrane potential, which corresponded to the membrane's input resistance and capacitance. Successive current pulses produced almost superimposable potential shifts, as shown for the first pulses in Figure 1A. The later current pulses gave rise to a depolarization that increased to +67 mV. This behavior of the potential was equivalent to increasing the intensity of the current pulse. Since the current injected remained constant, this behavior of the potential can only be explained by the recruitment of another source of depolarizing current, with a reversal potential more depolarized than +30 mV. The opening of sNa^+ channels could account for this effect. At some point, depending on the oocyte, the potential remained depolarized for up to several minutes before returning to its resting level. During the sustained depolarization, the potential could be depolarized anywhere from +60 to +85 mV (Kado et al. 1979; Kado and Baud 1981; Baud et al. 1982). The establishment of the plateau indicated that a sufficient number of channels were open and remained in a permeable state for a long time. It also showed that these channels apparently did not inactivate.

To investigate the effect that lidocaine could exert on the sNa^+ channels, the drug was superfused on the oocytes while the membrane potential was depolarized, and this during the plateau phase. The superfusion of an OR2 medium supplemented with lidocaine (1 mmol/l) produced a drop to negative values of the membrane potential ($n = 25$) (Fig. 1B). The subsequent injection of depolarizing current pulses of larger intensities (200 nA) caused transient depolarizations, but neither a plateau nor a shouldering. To compare the effect of increased lidocaine concentrations, the same oocyte was used as its own control. However, to obtain
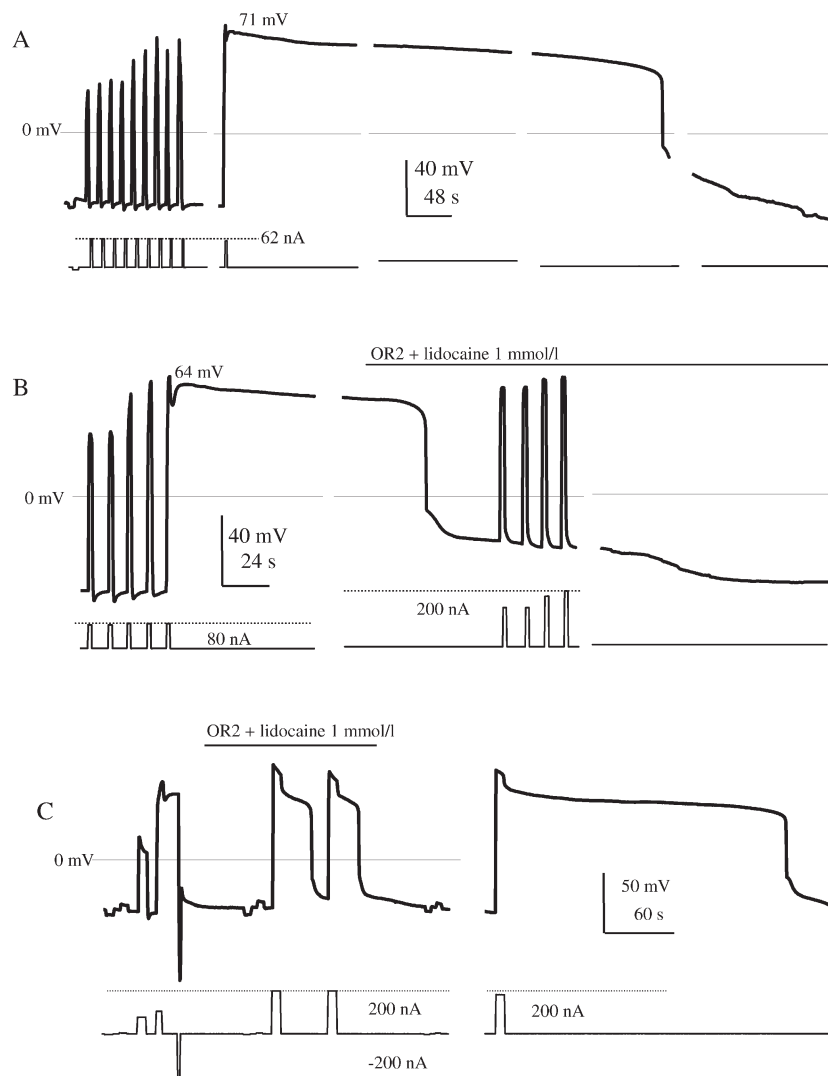
Figure 1. Effect of lidocaine on membrane potential. **A.** In a control oocyte, the injection of successive depolarizing currents, which are shown below the potential traces, resulted in the generation of a sustained membrane depolarization which lasted for about 5 min. Note that the first pulse (a hyperpolarizing current) was used to measure the resistance of the oocyte (0.8 MΩ). **B.** In another oocyte, the superfusion of lidocaine, while the potential was depolarized, caused a drop of the membrane potential to more hyperpolarized values in 32 s, and subsequent current injections of increasing intensity resulted in passive membrane responses. **C.** In another cell, the sNa$^+$ channels were first induced by the injection of successive depolarizing currents and then closed by a brief hyperpolarizing pulse. During the subsequent depolarizing pulses, the membrane responses presented a delayed return to the resting potential. After lidocaine being washed, a sustained depolarization was elicited.

similar inductions, the oocyte was allowed to recover for at least 1 h between two induction procedures (Baud and Kado 1984). With lidocaine (1 mmol/l), the potential dropped within 35 ± 4 s ($n = 5$); whereas with lidocaine (3 mmol/l), the drop occurred after a significantly shorter delay (10 ± 4 s; $p = 0.011$). This data indicates that lidocaine exerted a reversible and concentration-dependent block of the sNa^+ channels.

In order to investigate the possible binding effect of lidocaine on closed channels, the sNa^+ channels were first induced to initiate the plateau response (Fig. 1C). At this time, a hyperpolarizing pulse was delivered to close the channels. An OR2 medium supplemented with lidocaine (1 mmol/l) was superfused on the oocytes for 1 min when the sNa^+ channels were in their closed but induced state. Then, depolarizing currents were injected. The membrane potential depolarized and presented a shouldering which lasted about 25 s after the end of the depolarizing pulses. Afterwards, the potential returned to negative values. Lidocaine was washed out for about 2 min and, in response to a depolarizing current, the cells presented a long-lasting depolarization ($n = 8$).

Effect of lidocaine on membrane currents in voltage clamp experiments

The current/voltage (I/V) relationships were performed on oocytes with either non-induced or induced sNa^+ channels, and also before and after lidocaine exposure. Figure 2A shows the membrane and the leak currents elicited during the voltage steps in a control cell. The leak current was measured in response to 10 mV steps between -90 and -30 mV and extrapolated. In control oocytes with non-induced sNa^+ channels, the current followed the leak up to -10 mV. Then, it increased to peak at $+20$ mV and again superimposed with the leak at about $+50$ mV. Above $+60$ mV, the current increased more than the leak. Once the sNa^+ channels were induced, a large inward current was activated. The activation threshold was about -10 mV, and the current peaked at $+50$ mV and reversed direction at potentials more positive than $+70$ mV (Fig. 2A).

When an OR2 medium supplemented with lidocaine (1 mmol/l) was superfused on the oocytes before the induction of the sNa^+ channels (Fig. 2B), the currents were almost identical, and this up to about -20 mV to that recorded in the control medium. However, at more depolarized potentials, the outward current was reduced. The I/V curves were again almost superimposed for depolarizations ranging between $+50$ and $+70$ mV. For depolarizations greater than $+70$ mV, the outward current was also reduced. This data suggests that lidocaine (1 mmol/l) could bind to other voltage-dependent channels.

When the sNa^+ channels were induced, in the presence of lidocaine (0.3 and 1 mmol/l), the sodium current was reduced (Fig. 2C). Both the inward and outward currents were decreased. The activation curve (Fig. 2D) showed that lidocaine produced a shift of 5 ± 1 mV ($n = 15$) towards more positive voltages. However, neither the equilibrium potential for sodium ions nor the steepness factor were significantly changed.
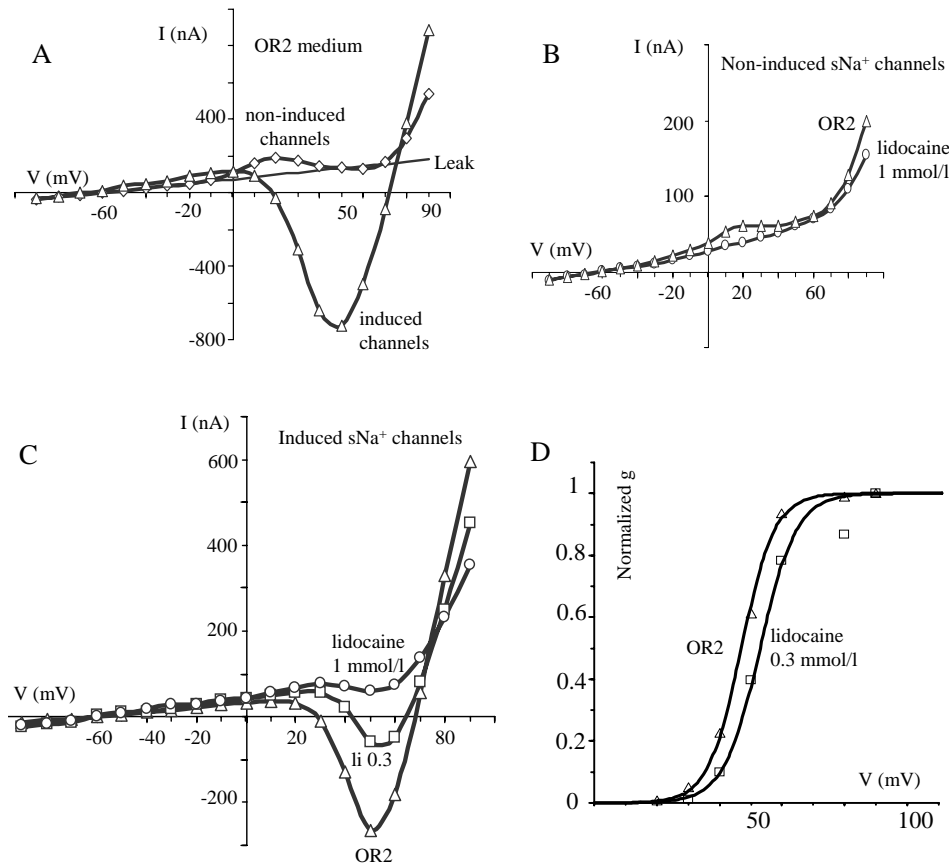
Figure 2. Oocyte voltage clamped currents. Typical current/voltage relationships were obtained according to the different conditions indicated in each of these panels. The same oocyte was used for the experiments shown in each panel. To avoid inducing the sNa⁺ channels, the voltage step was 500 ms. After having first induced the sNa⁺ channels with a long-lasting depolarization of 30 s, the duration of the voltage step was 4 s. **A.** Currents activated before and after the induction of the sNa⁺ channels in a control medium. **B.** Effect of lidocaine 1 mmol/l (open circles) on the currents activated before the induction of the sNa⁺ channels (open triangles). **C.** Effect of 0.3 mmol/l (open squares) and 1 mmol/l (open circles) lidocaine on the control sodium current (open triangles). **D.** Conductance-voltage activation curves generated before and after lidocaine exposure from data in C, and fitted with equation (3). In an OR2 medium, the values for $V_{0.5}$ and k were 47 and -5.3 mV, respectively. In the presence of lidocaine, $V_{0.5}$ was 53 mV and k was -5.8.

Concentrations of lidocaine ranging from 10 μmol/l to 3 mmol/l were tested on the oocytes and the dose-response curve was drawn (Fig. 3). The blocking effect of lidocaine was concentration-dependent. For example, with 0.3 mmol/l of lidocaine, the mean peak current was -139 ± 26 nA ($n = 23$) compared with controls:
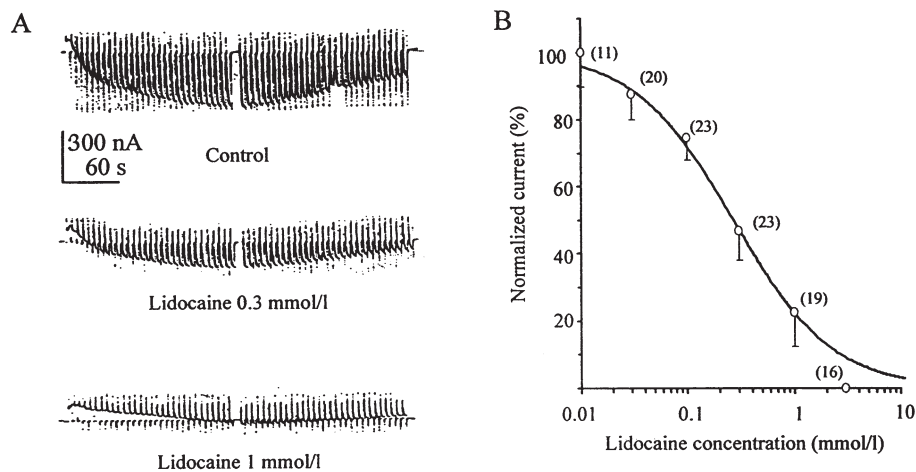
Figure 3. Concentration-dependence of lidocaine on voltage clamped currents. **A.** The traces are strip chart recorded currents from three different oocytes bathed in media with different lidocaine concentrations. The Na^+ currents were elicited by depolarization steps to $+50$ mV from a holding potential at -20 mV using 4 s pulses at 1.6 s intervals. The currents for the first few depolarizations were outward. Their magnitudes were usually equal to the voltage step amplitude divided by the input resistance. Each oocyte was only used for one induction. **B.** The data represents the mean ($-$ S.E.M.) from 11 to 23 oocytes as indicated for each concentration. The dose-response relationship was fitted to the data using equation (1) where K_d was 270 μmol/l and n_H was 0.95.

-296 ± 19 nA ($n = 47$; $p < 0.001$) (Fig. 3A). The time course of the inward current reflects the time course of the induction of the channels (Baud and Kado 1984). Apparently, in the presence of lidocaine, the time for a half-maximal inward current ($t_{1/2}$) was increased (67 ± 8 s), compared with controls (52 ± 6 s). While the mean $t_{1/2}$ was somewhat larger, the difference was not significant ($p = 0.18$). The concentration required to cause a 50% inhibition of the Na^+ current was 270 $\pm$ 67 μmol/l (Fig. 3B).

Discussion

The membrane of immature *Xenopus laevis* oocytes is not excitable in the basal state, however it can be made to produce a long-lasting depolarization in response to the injection of iterative depolarizing currents (Kado et al. 1979). During the sustained depolarization, the potential could be depolarized as high as $+71$ mV (see Fig. 1A), which is close to the equilibrium potential of sodium ions (Barish 1983). This was shown to be due to the opening of sodium-selective, electrically gated channels, which were not activatable before the membrane had been depolarized for some time (Kado and Baud 1981; Baud et al. 1982). The process by which the

channels become potentially gatable has been referred to as induction. It includes the transition of the sNa$^+$ channels in three different states: a closed and non-induced state before the cell has been depolarized; an induced and closed state after the cell has been depolarized; and an open state at positive potentials (Baud and Kado 1984).

The occurrence of a long-lasting depolarization indicates that a sufficient number of sNa$^+$channels are open, remain in a permeable state for a long time, and do not apparently inactivate. Lidocaine, when superfused on the oocytes during the establishment of the plateau, produced a drop of the membrane potential within about 30 s. This suggests that lidocaine blocked most of the sNa$^+$ channels while they were in their open state. The subsequent injection of large depolarizing currents resulted in passive membrane responses because the number of unblocked, permeable sNa$^+$ channels was not sufficient to allow the membrane potential to remain depolarized. On the other hand, the superfusion of lidocaine, when the channels were induced but closed, failed to produce an immediate block of the channels. When depolarizing currents were injected, the potential responses presented a delayed return to the resting level; the opening of the sNa$^+$ channels could account for this effect. Therefore, very few channels may have been blocked in their closed configuration. The fact that the plateau could not be maintained can be explained by the blockage of the channels by the drug as the channels acquired their open configuration. The effect of lidocaine was reversible, since the superfusion of a drug-free medium allowed the long-lasting depolarization to occur in response to a depolarizing current.

The study of the current/voltage relationships, obtained before the induction of the sNa$^+$ channels, revealed at least two conductances: one was activated at about -20 mV, peaked at $+20$ mV and declined for more depolarized potentials; it is a chloride conductance activated by the entry of calcium through voltage-dependent channels (Miledi 1982; Barish 1983; Miledi and Parker 1984; Charpentier and Kado 1999). With an OR2 medium containing calcium (1 mmol/l), the calcium influx is too small to elicit an inward current, but it can activate a transient chloride conductance of variable amplitude depending on the oocytes. The other is a monovalent non-selective cationic conductance, activated by sustained depolarizations of the membrane and by the absence of extracellular divalent cations. It was activated at about $+50$ mV and was mainly carried by potassium ions (Arellano et al. 1995). After the superfusion of lidocaine on the oocytes, the curves were not superimposed for potentials comprised between -20 and $+50$ mV, and above $+70$ mV. This suggests that lidocaine affects other endogenous channels such as the voltage-dependent Ca^{2+} channels (or the Ca^{2+} dependent Cl^- channels) and the non-selective cationic channels. Indeed, the interaction of lidocaine with membrane proteins other than the Na^+ channels is well known and likely to account for the neurotoxic effect of the drug in therapeutics (Johnson 2000). The possible blockage of the Ca^{2+} and K^+ channels by lidocaine was not further investigated. When the sNa$^+$ channels were induced, superfusion of lidocaine resulted in a decreased sodium current inwardly and outwardly directed. However, the interaction

of lidocaine with the non-selective cationic conductance could account for part of the effect on the outward current. In the presence of 300 μmol/l lidocaine, the activation curve was shifted by 5 mV towards more positive voltages, which suggests an alteration of the gating properties of the sNa$^+$ channels. A shift of 10 mV of the I/V curve also towards positive voltages was obtained in isolated ventricular cells of rats superfused with 20 μmol/l lidocaine (Lee et al. 1981). More recent data on Purkinje cell Na$^+$ channels reported altered channel kinetics due to the binding of lidocaine (Baumgarten et al. 1991).

Increasing the concentration of lidocaine in the superfusion medium caused a decrease in the sodium current. Nonetheless, part of the block might be attributed to the shift of the activation curve of the sNa$^+$ channels towards more positive potentials. The dose-dependent effect is illustrated in Fig. 3 and the concentration required to cause a 50 % inhibition of the Na$^+$ current was 270 ± 67 μmol/l. From the data in the literature, the concentration of drug necessary for half inhibition of the Na$^+$ channels in excitable tissues varies from 15–30 μmol/l for cardiac Na$^+$ channels to concentrations about ten times higher for neuronal Na$^+$ channels (Courtney and Strichartz 1987). The lidocaine sensitivity of the sNa$^+$ channels takes place at concentration levels similar to that of the neuronal channels. The effect of lidocaine on the *Xenopus* oocyte membrane proteins might be of interest to further understand the interaction between this drug and the ionic channels.

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