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Expression of cardiac Na channels with appropriate physiological and pharmacological properties in *Xenopus* oocytes

(tetrodotoxin/lidocaine/DPI 201-106/ion channels/heart)

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ABSTRACT **The objective of this study was to determine whether the *Xenopus laevis* oocyte can express an exogenous cardiac Na channel that retains its normal physiological and pharmacological properties. Cardiac Na channels were expressed in oocytes following injection of RNA from guinea pig, rat, and human heart and detailed analysis was performed for guinea pig cardiac Na channels. Average current amplitudes were −351 ± 37 nA with peak current observed at −8 ± 1 mV. Steady-state inactivation was half-maximal at −49 ± 0.6 mV for the expressed channels. All heart Na currents were resistant to block by tetrodotoxin compared to Na currents expressed from brain RNA with IC_{50} values for guinea pig, rat, and human heart of 651 nM, 931 nM, and 1.3 μM, respectively. In contrast, rat brain Na channels were blocked by tetrodotoxin with an IC_{50} value of 9.1 nM. In addition, the effects of the cardiac-selective agents lidocaine and DPI 201-106 were examined on Na currents expressed from brain and heart RNA. Lidocaine (10 μM) blocked cardiac Na current in a use-dependent manner but had no effect on brain Na currents. DPI 201-106 (10 μM) slowed the rate of cardiac Na channel inactivation but had no effect on inactivation of brain Na channels. These results indicate the *Xenopus* oocyte system is capable of synthesizing and expressing cardiac Na channels that retain normal physiological and pharmacological properties.**

The *Xenopus* oocyte expression system has been widely applied to the study of neurotransmitter receptors and ion channels (1). Cardiac Ca channels, which are sensitive to modulation by dihydropyridines and β-adrenergic agonists, have been studied with this system (2, 3) as have cardiac Na channels (4, 5). However, although Na channels in native cardiac membranes are blocked by micromolar concentrations of tetrodotoxin (TTX), those expressed in oocytes have been reported to be blocked by nanomolar concentrations of this neurotoxin. This sensitivity to TTX is similar to that reported for neuronal Na channels (4).

We have examined the properties of cardiac Na channels expressed in the *Xenopus* oocyte system. The purpose was to determine whether the oocyte can express a cardiac Na channel with pharmacological and physiological properties similar to those observed in ventricular tissue. Recently, Na channels have been cloned from cardiac tissue (6) and denervated skeletal muscle (7, 8), tissues that are known to express TTX-resistant channels (blocked by low micromolar TTX concentrations) as opposed to TTX-sensitive channels (blocked by low nanomolar TTX concentrations). One of the denervated skeletal muscle clones has been shown to express a TTX-sensitive channel when expressed in oocytes (7). The question remains, can the *Xenopus* oocyte express an exogenous TTX-resistant Na channel?† Resolution of this question is particularly important if the oocyte system is to be used for structure–function studies of cardiac as well as denervated skeletal muscle Na channels. Our results demonstrate the *Xenopus* oocyte is capable of expressing TTX-resistant Na currents. In addition, the cardiac channel is more sensitive than the neuronal channel to block by lidocaine and modulation of inactivation by DPI 201-106. Therefore, the *Xenopus* oocyte will be useful for studies of cardiac as well as other TTX-resistant Na channels.‡

MATERIALS AND METHODS

Xenopus laevis were purchased from Xenopus I (Ann Arbor, MI) or Nasco (Ft. Atkinson, WI). Frogs were anesthetized with 0.17% Tricaine (Sigma) and oocytes were surgically removed. Follicles were removed by treating the oocytes with 0.2% collagenase (Boehringer Mannheim type A) for 2–3 hr in OR-2 (11). Defolliculated stage V–VI oocytes were selected and placed in normal saline supplemented with 50 μg of gentamicin per ml (see ref. 11 for solution compositions). RNA (50–100 nl) was injected into individual oocytes using a Drummond microdispenser (Drummond Scientific, Broomall, PA).

Adult guinea pigs were anesthetized with sodium pentobarbital (30 mg/kg i.p.) and 10- to 14-day-old rats were anesthetized with methoxyflurane (Pitman–Moore, Washington Crossing, NJ). All animal care and use procedures were reviewed and approved by the Institutional Animal Care and Use Committee and were compliant with the Animal Welfare Act (Public Law 89-544, as amended) and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Pub. 86-23, 1985). Hearts and/or brains were removed and the atria and major blood vessels were cut away from ventricular tissue. Whole brains or ventricles were placed directly into homogenization buffer. Human heart samples were supplied by R. Joho and A. Brown (Baylor College of Medicine, Houston). Total RNA was extracted using a modified LiCl/urea method (12), and RNA was resuspended at 3–5 mg/ml in sterile water for oocyte injections.

Following RNA injections two oocyte incubation protocols were used: (*i*) 21°C for 5–10 days or (*ii*) 30°C for 0–24 hr, 21°C for 24–48 hr, 30°C for 48–52 hr, and 21°C for the remaining life of the oocyte. Electrophysiological recording was performed at 21–23°C using a Dagan 8500 two-microelectrode voltage clamp (Dagan Instruments, Minneapolis). Current and voltage electrodes were filled with 3 M KCl and had resistances of <1 MΩ. All recordings were made in physiological buffer containing (in mM) NaCl, 96; KCl, 2.0; $MgCl_2$, 1.0; $CaCl_2$ 1.8; Hepes, 5.0 (pH 7.4). Data were filtered at 3

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Abbreviation: TTX, tetrodotoxin.

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†Following submission of this manuscript cloned rat heart Na channels have also been shown to be resistant to block by TTX when expressed in *Xenopus* oocytes (9).

‡Some of these data have been presented in abstract form (10).

kHz and digitized at 10 kHz. Data acquisition and analysis were performed with the pCLAMP software suite (Axon Instruments, Foster City, CA) on an AST Premium 286 computer equipped with a LabMaster 125-kHz direct memory access board.

Passive and non-Na channel currents were eliminated by subtracting records in the presence of 20–50 μM TTX (CalBiochem). To confirm the activity of the TTX solutions used during acquisition of cardiac Na current data, the same 300 nM solution that gave partial block of heart current was applied to an oocyte injected with rat brain RNA. The brain Na currents were completely blocked by the 300 nM TTX solution (data not shown). Time constants for inactivation and steady-state voltage dependence of inactivation were determined as described (11). Fits to dose–response data were performed using a Simplex algorithm (13).

All chemicals were from Sigma unless otherwise specified. DPI 201-106 was a gift from Sandoz Pharmaceutical.

RESULTS

Higher Incubation Temperatures Speed Up Expression of Cardiac Na Channels. Incubation protocol *i* (see *Materials and Methods*) led to expression of cardiac Na channels with properties comparable to those following protocol *ii*. Incubation periods of 10 days were often necessary to achieve large currents with protocol *i*, however, and oocyte viability was problematic. Protocol *ii* required shorter incubation times and allowed more reliable use of the system to study cardiac Na channels. In some cases, Na currents could be recorded after 48 hr using protocol *ii*. Protocol *ii* was not used for brain currents since the warmer incubation times led to changes in inactivation rates compared to oocytes incubated under protocol *i* (data not shown). Inactivation rates were not protocol dependent for cardiac Na channels.

Electrophysiological Properties of Guinea Pig Cardiac Na Channels Expressed in *Xenopus* Oocytes. Voltage-clamp recording of membrane currents 2 or more days following injection revealed expression of cardiac Na channels. Fig. 1 illustrates the voltage dependence of peak Na current in a representative oocyte. Na currents activated upon step depolarizations to potentials greater or equal to −50 mV and peaked near −10 mV (−8 mV ± 1.1 mV, n = 20). Peak current amplitude was −351 ± 37 nA with values ranging from −65 nA to −927 nA. Absolute current amplitudes varied among batches of oocytes. In most cases recording was completed after ≈20 min, but in certain instances oocytes were stable for >1 hr with very little diminution of current amplitude.

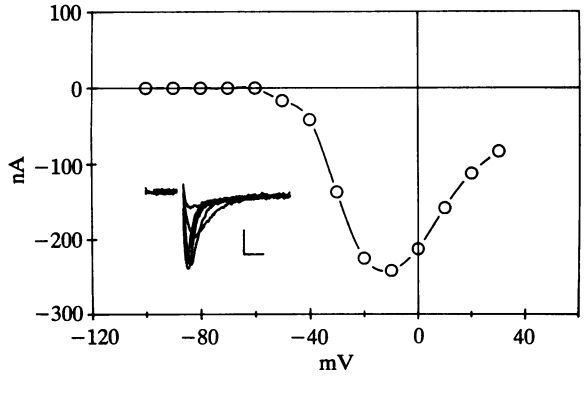

FIG. 1. Current–voltage relationship for cardiac Na channels. Oocytes injected with guinea pig heart RNA were voltage clamped to −100 mV and depolarizing voltage steps were given to various test potentials. Peak inward current is plotted as a function of test potential. (*Inset*) Na currents elicited by voltage steps from −100 mV to potentials ranging from −40 mV to 0 mV in 10-mV increments. Passive and non-Na currents were eliminated by subtracting currents in the presence of 20 μM TTX. (Bars = 200 nA, 5 ms.)

In addition to the voltage dependence of peak currents, we also examined steady-state inactivation properties. Inactivation was assessed using a prepulse protocol and typical results are shown in Fig. 2. Channel availability had a sigmoidal dependence on membrane potential that was well described by a two-state Boltzmann distribution (see legend to Fig. 2). Half-maximal inactivation ($V_{1/2}$) was −49 ± 0.6 mV with a slope factor (k) of 7.3 ± 0.2 mV (n = 20). Inactivation was assessed using 50-ms prepulses; therefore, the above values describe the voltage dependence of the fast inactivation process. Although we did not quantitatively examine the properties of slow inactivation (14, 15) in this preparation, slow inactivation of Na channels was clearly present. Following impalements, current amplitude increased slightly for 10–20 s upon hyperpolarization of the membrane potential to −100 mV, indicating recovery from slow inactivation. Consequently, oocytes were allowed to stabilize for several minutes at −100 mV prior to acquiring data.

Rates of inactivation were measured for currents during a test pulse to various voltages and increased as the membrane was depolarized (Fig. 3). At all voltages the majority of the inactivation phase was well fit by a single exponential component. For comparison, the time-constant values for oocytes injected with guinea pig brain RNA are shown at the same test potentials. At voltages less than or equal to −20 mV cardiac Na channels showed slower inactivation rates than those expressed from brain RNA.

Na Current Block by TTX. To examine the sensitivity of expressed channels to block by TTX we exposed oocytes to the toxin at varying concentrations and measured peak current amplitude. The results from these experiments are illustrated in Fig. 4. Na currents from rat and guinea pig brain as well as guinea pig, rat, and human heart were examined. A 70- to 140-fold difference in sensitivity to TTX block was observed for cardiac vs. neuronal Na currents, with the cardiac channels being less sensitive. A single-site binding isotherm (see legend to Fig. 4) was fit to the data and gave an IC_{50} value for guinea pig heart currents of 651 nM. A fit to the dose–response curve for the rat brain currents gave an IC_{50} value of 9.1 nM. Complete block was obtained with 500 nM TTX for guinea pig brain Na currents, indicating a high

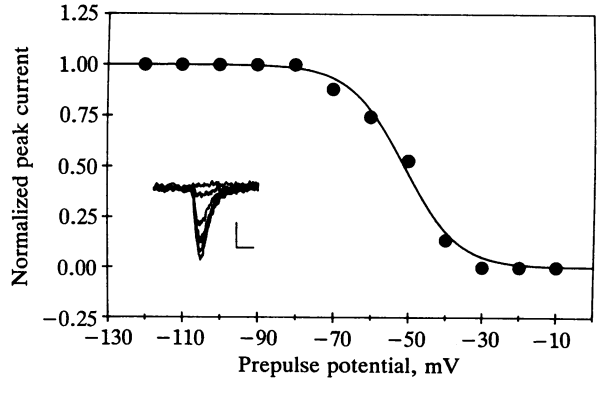

FIG. 2. Steady-state voltage dependence of inactivation for cardiac Na currents. Membrane potential was held at −100 mV and test pulses to −10 mV were given to determine Na channel availability. The test pulse was preceded by a 100-ms prepulse to various potentials. Normalized peak current is plotted as a function of prepulse potential. The smooth curve is a fit to the data of the function $I = \{1 + \exp[(V_m - V_{1/2})/k]\}^{-1}$, where V_m = membrane potential, $V_{1/2}$ is the point of half-maximal inactivation, and k is a slope factor. $V_{1/2}$ = −51 mV and k = 7.16. (*Inset*) Currents during test potential following prepulses from −80 mV to −30 mV. Currents were obtained as in Fig. 1. (Bars = 100 nA, 5 ms.)

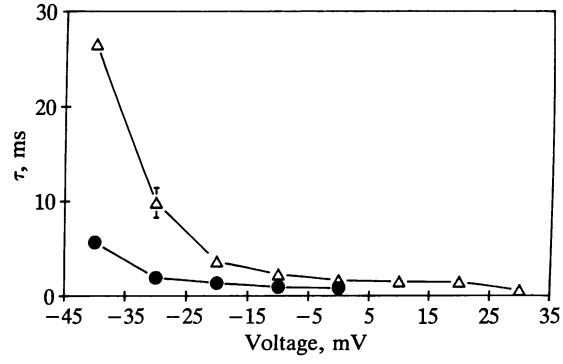

FIG. 3. Time constant of inactivation vs. test potential. Time constants were determined by fitting a single exponential plus a baseline to the inactivating phase of the Na currents. The time constant (τ) is plotted vs. test potential for Na currents induced by guinea pig heart (△) or brain (●) RNA.

sensitivity. Rat heart and human heart Na channels were blocked with IC_{50} values of 931 nM and 1.3 μM, respectively.

Block of Na Currents by Lidocaine. Fig. 5 summarizes the effects of application of 10 μM lidocaine to Na channels expressed from rat brain or guinea pig heart RNA. A 10-Hz train of depolarizing pulses was used to assess use-dependent block by lidocaine. This train of pulses resulted in 10–20% reductions in current amplitude in the absence of drug during this series of experiments. When oocytes expressed a brain Na channel, 10 μM lidocaine produced no additional block during the train. In oocytes expressing guinea pig cardiac Na channels, however, the same lidocaine concentration produced 50% block of peak current by the end of the 3-s pulse train. No tonic block was present at this concentration for either brain or heart Na channels when the membrane was held at −100 mV.

Effects of DPI 201-106 on Na Currents from Heart and Brain. Fig. 6 shows current records from oocytes injected with guinea pig heart RNA (Fig. 6 *A* and *B*) or rat brain RNA (Fig. 6 *C* and *D*). Control records prior to exposure to drug are shown in Fig. 6 *A* and *C* and records following exposure to 10 μM DPI 201-106 for 10 min are shown in Fig. 6 *B* and *D*. Inward current remains at the end of the pulse (Fig. 6*B*), indicating cardiac Na channel inactivation is slowed and/or

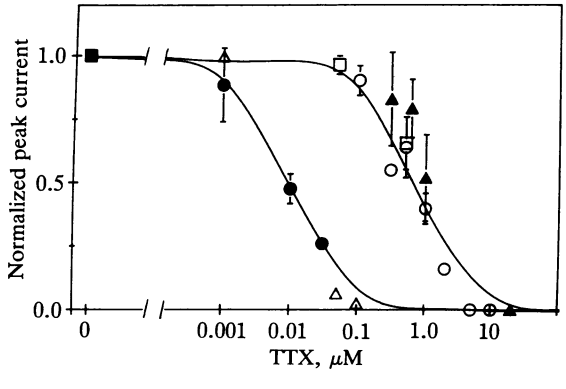

FIG. 4. Dose–response curves for TTX block of heart and brain channels. Peak current amplitude, determined as in Fig. 1, was measured, normalized to control values, and plotted as a function of TTX concentration in the bath. Dose–response curves are fits to the data of the equation $I = 1/[1 + ([TTX]/IC_{50})]$. Fitted IC_{50} values for guinea pig, rat, human heart, and rat brain Na currents were 651 nM, 931 nM, 1.3 μM, and 9.1 nM, respectively. Fitted curves are shown for guinea pig heart and rat brain data only. ●, Rat brain; △, guinea pig brain; ○, guinea pig heart; □, rat heart; ▲, human heart. Data are presented as means ± SEM. The 300 nM guinea pig heart value is a single point. All others are means of 2–10 values. Where SEMs are not shown the error bars are smaller than the symbol size.

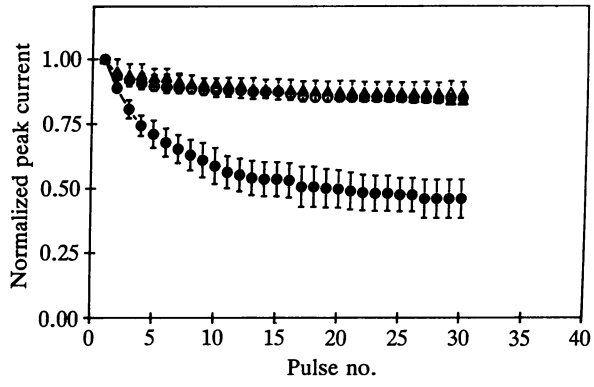

FIG. 5. Effects of lidocaine on heart and brain Na currents. Use-dependent block by 10 μM lidocaine was assessed for currents induced by either guinea pig brain (△, ▲) or heart RNA (○, ●). Open symbols are for control data and filled symbols indicate the presence of 10 μM lidocaine. A 10-Hz train of pulses from −100 mV to −10 mV was applied, and current amplitude as a function of pulse number was plotted. Data are presented as means ± SEM (n = 5).

removed by DPI 201-106. Inactivation rates of brain currents were not affected by DPI 201-106, but peak current amplitudes were slightly reduced (Fig. 6*D*).

DISCUSSION

The major finding of this work is that the *Xenopus* oocyte system can be used to express either neuronal or cardiac Na channels that retain the physiological and pharmacological properties exhibited by Na channels in their native cell membranes. This assumption is supported by four different experimental results, as follows.

A hallmark of cardiac Na channels is a markedly reduced sensitivity to blockade by TTX (16–18) compared to neuronal channels. Our IC_{50} values for blockade by TTX of 651 nM to 1.3 μM in oocytes agree quite well with the reported value of 820 nM in rabbit cardiac tissue (16). Recently, in contrast to this study, cardiac Na channels expressed in oocytes from

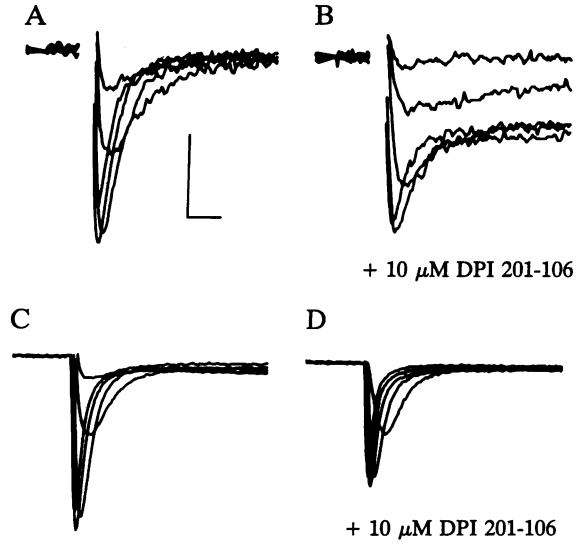

FIG. 6. Effects of DPI 201-106 on brain and heart RNA-induced Na currents. (*A* and *B*) Current records in a guinea pig heart RNA-injected oocyte before (*A*) and after (*B*) a 10-min application of DPI 201-106 (10 μM). (*C* and *D*) An analogous experiment in a rat brain RNA-injected oocyte. In all panels voltage steps were from a holding potential of −100 mV to test potentials of −40 mV to +10 mV in 10-mV increments. [Bars = 200 nA (*A* and *B*), 1500 nA (*C* and *D*), 5 ms.]

rat, rabbit (4), and human heart RNA (5) have been reported to have neuronal-type sensitivities to block by TTX, a result that casts doubt upon the ability of the *Xenopus* oocyte to express a pharmacologically normal Na channel. Our results indicate that the oocyte is certainly capable of synthesizing a Na channel that is relatively insensitive to TTX, however. At present the reason for the difference between our results and those previously reported is unclear. It remains possible that various batches of oocytes process exogenous proteins differently and this batch-to-batch variability could explain the results. We have, however, consistently observed cardiac-type properties following injection of heart RNA among batches (at least 10) of oocytes from different donors. Another possibility is that endogenous currents, which appear in a small percentage of oocytes, complicated analysis in these previous studies.

A second difference between cardiac and neuronal Na channels is that the cardiac channel has been reported to inactivate more slowly than its neuronal counterpart (19). Fig. 3 showed that, at voltages more negative than −20 mV, this is also the case for the channels expressed in oocytes. It is important to note that a generalization of fast inactivation rates to brain channels must be made with caution. We have observed species-dependent differences in the rate of inactivation for RNA-induced Na channels, with chicken brain RNA giving the most slowly inactivating channels and rat brain RNA giving the fastest rates of inactivation (D.S.K., unpublished observations). Comparisons of neuronal vs. cardiac inactivation rates are appropriate, though, when made within a given species as was done in this study.

In addition to the TTX resistance of cardiac Na channels, other pharmacological agents are known to be cardiac specific. Cardiac Na channels have been shown to be blocked by lidocaine in a use-dependent manner (20) at lower concentrations than that which affect neuronal channels (21). Our data indicate use-dependent block of cardiac Na channels occurs at concentrations of lidocaine as low as 10 μM, whereas no such block was observed in oocytes with neuronal channels. Higher concentrations of lidocaine (e.g., 100 μM) did decrease neuronal Na current, however. The greater sensitivity of cardiac Na channels to lidocaine block may reflect differences in steady-state inactivation at the holding potential compared to brain channels. Alternatively, there may be a difference in the affinity of lidocaine for cardiac vs. neuronal channels. A more systematic examination of lidocaine block of Na channels expressed in oocytes, utilizing various holding potentials and pulse durations, will determine the extent of these differences. Our data clearly indicate, however, a cardiac selectivity to lidocaine block of Na channels expressed in oocytes when compared at a concentration of 10 μM under our experimental conditions.

DPI 201-106 is an organic molecule known to slow the rate of cardiac Na channel inactivation (22). Consistent with the cardiac selectivity noted previously (22), DPI 201-106 (10 μM) selectively slowed cardiac Na channel inactivation but lacked an effect on brain Na channel inactivation in the oocyte expression system. This cardiac vs. neuronal specificity suggests the presence of a specific receptor on the cardiac channel that does not exist, or is not accessible, on the neuronal channel. There are, however, examples of DPI 201-106 affecting inactivation of noncardiac Na channels at concentrations similar to those used in this study (23, 24). Our oocyte data must, therefore, be cautiously extrapolated.

Structure–function studies of voltage-dependent Na channels, directed toward understanding permeation, gating, and toxin interactions, will yield more information about subtype and function of these membrane proteins. Mutagenesis of a rat brain Na channel has identified a specific glutamic acid residue that may be involved in binding the positively charged TTX molecule (25). Replacement of this glutamic acid with a glutamine eliminated the sensitivity to TTX block at toxin concentrations up to 10 μM. In the rat cardiac Na channel (6) and a second Na channel cloned from a denervated skeletal muscle library (8), the negatively charged glutamic acid is flanked by a positively charged arginine. If the interaction with TTX and its receptor is electrostatic in nature, then it is possible the arginine residue could account for the reduced TTX sensitivity of cardiac and denervated skeletal muscle Na channels as previously suggested (25). Since we have shown the *Xenopus* oocyte is capable of synthesizing a TTX-resistant channel, this system should prove a useful one in examining these types of structure–function relationships for cardiac as well as denervated skeletal muscle Na channels.

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