The newborn rabbit sino-atrial node expresses a neuronal type I-like Na^+ channel

Mirko Baruscotti, Ruth Westenbroek*, William A. Catterall*, Dario DiFrancesco† and Richard B. Robinson‡

*Department of Pharmacology, Columbia University, New York, NY, USA, *Department of Pharmacology, University of Washington, Seattle, WA, USA and †Dipartimento di Fisiologia e Biochimica Generali, Elettrofisiologia, Università di Milano, Milano, Italy*

1. Newborn rabbit sino-atrial node (SAN) myocytes were recently found to express a tetrodotoxin (TTX)-sensitive Na^+ current. We now report that the dose–response relation indicates that this SAN Na^+ channel has unusually high TTX sensitivity, with half-maximal inhibition (26 ± 5 nM) which is more typical of neuronal than cardiac tissue.

2. Additional characterization used μ-conotoxin GIIIA and Cd^{2+} as relatively selective blockers of the skeletal and cardiac isoforms, respectively. μ-Conotoxin GIIIA had no effect on the current recorded from SAN myocytes, but the Cd^{2+} sensitivity was unexpectedly high for a neuronal isoform (half-maximal inhibition = 185 ± 8 μM).

3. Analysis of the time constant of inactivation did not reveal evidence of multiple inactivation processes, with the data well fitted by a single, relatively rapid exponential (inactivation time constant = $0{\cdot}58 \pm 0{\cdot}03$ ms at 0 mV).

4. *In situ* hybridization with anti-sense cDNA probes was used to test for expression of neuronal type I, II and III Na^+ channel isoforms. Myocardial cells in newborn SAN tissue exhibited clear hybridization to the type I, but not the type II or III probes. No hybridization was observed in adult SAN tissue with any of the three probes.

5. It is concluded that the newborn SAN expresses a neuronal type I-like Na^+ channel isoform, and that this probably accounts for the unusual characteristic of high sensitivity to both TTX and Cd^{2+}.

The sino-atrial node (SAN) is the site of origin of the heartbeat, and as such exhibits specialized electrophysiological and morphological characteristics. While the normal adult SAN action potential lacks a Na^+-dependent upstroke, strikingly different characteristics were recently reported in cells from the neonatal rabbit SAN. In the case of the newborn, the cells exhibit a prominent fast Na^+ current (I_{Na}) that contributes to the action potential upstroke and overshoot as well as to the spontaneous rate (Baruscotti, DiFrancesco & Robinson, 1996). This current is blocked by the Na^+ channel-specific blocker tetrodotoxin (TTX). However, one surprising observation in this earlier study was that 3 μM TTX almost completely blocked (~95%) the current, despite the fact that typical K_d values for TTX action in cardiac tissue are in the micromolar range (Cohen, Bean, Colatsky & Tsien, 1981; Satin *et al.* 1992). In contrast, skeletal and neuronal-specific isoforms of the Na^+ channel are blocked by TTX in the nanomolar range (Satin *et al.* 1992).

It has previously been reported, using poly(A)$^+$ RNA or total RNA from heart, that mRNAs encoding Na^+ channel types I, II and III are present at low levels in postnatal day 7 and adult cardiac tissue (Suzuki *et al.* 1988; Beckh, 1990). mRNA encoding type I was the most abundant, and this mRNA is also represented in cardiac cDNA libraries (Rogart, Cribbs, Muglia, Kephart & Kaiser, 1989). In these studies, the specific cell type(s) within the heart tissue responsible for the results was not determined. Given the fact that other neuronal-specific proteins have been reported to be localized in the SAN and specialized conducting tissue of the heart (Gorza, Schiaffino & Vitadello, 1988; Gorza & Vitadello, 1989), we hypothesized that the neonatal SAN cells were expressing a neuronal-like rather than a cardiac-like isoform of the Na^+ channel.

‡ To whom correspondence should be addressed.

METHODS

Protocols employed in these experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Columbia University.

Isolation of the SAN region of rabbits less than 20 days old was performed according to a standard procedure, as previously described (Baruscotti *et al.* 1996). Briefly, animals were anaesthetized by I.M. injection of a mixture of xilazine (4·6 mg kg^{-1}; Fermenta Animal Health Co., Kansas City, MO, USA) and ketamine (60 mg kg^{-1}; Fort Dodge Laboratories Inc., Fort Dodge, IA, USA). The heart was removed and placed in prewarmed (37 °C) normal Tyrode solution (mM: NaCl, 140; KCl, 5·4; $CaCl_2$, 1·8; $MgCl_2$, 1; Hepes–NaOH, 5; glucose, 5·5) containing 0·5 ml heparin (1000 U ml^{-1}). The ventricles were then cut away and the internal part of the right atrium exposed. The thin layer of nodal tissue was separated from surrounding tissue, using as reference points for SAN anatomical definition the interatrial septum, crista terminalis and the orifices of the inferior and superior vena cava. Care was taken to exclude more peripheral areas of the node. Single cells were then isolated by a collagenase dissociation as previously described (Baruscotti *et al.* 1996). As previously reported, the current is uniformly present in all cells isolated from animals up to 20 days of age, even when distinct subregions of the SAN are used for cell isolation.

In most experiments, the external solution used to dissect the Na^+ current from other ionic components was nominally Ca^{2+} free, and with Na^+ lowered to 50 mM to improve the quality of the voltage clamp. The solution contained (mM): TEA-Cl, 90; NaCl, 50; CsCl, 5; Hepes, 10; $MgCl_2$, 0·5; glucose, 5·5, pH = 7·4. In some experiments Ca^{2+} was increased to 0·6 or 1·8 mM without in any way modifying the results. The pipette solution contained (mM): aspartic acid, 80; CsOH, 75; Hepes, 10; EGTA, 10; NaCl, 10; $MgCl_2$, 1; CsCl, 30; Na_2ATP, 5, pH = 7·2. TTX (Calbiochem), μ-conotoxin GIIIA (μ-CTX; Bachem California, Torrance, CA, USA) or Cd^{2+} were added at the desired concentrations to the external solution. Control and test solutions were delivered to the cells through a fast perfusion system that completely exchanged the solution around the cell in less than 3 s.

Currents were recorded by the whole-cell patch clamp technique at room temperature (20–22 °C). Pipettes were obtained by pulling borosilicate capillaries with a horizontal puller (Sutter Instruments), and when filled with the internal solution had resistances measuring 1–4 MΩ. On-line series resistance compensation (40–80%) and capacitance correction were employed. Current measurements were calculated by subtracting the value of the current after complete inactivation from the amplitude at the peak. All drug effects were measured at steady state. Experimental data were filtered at a corner frequency of 10 kHz and stored on a PC-compatible computer at an acquisition frequency of 50 kHz. Data analysis was performed using pCLAMP (Axon Instruments) and Origin (MicroCal, North Hampton, MA, USA) software.

High resolution *in situ* hybridization methods with anti-sense cDNA probes to the 3′-untranslated region of the mRNA of neuronal type I, II and III Na^+ channels were used to test for and localize the expression of different Na^+ channel isoforms. The probes are expected to be highly specific for the three Na^+ channel types. The probes were generated by polymerase chain reaction, labelled with digoxigenin-11-UTP, and used to label cryostat sections. The probes for the types I, II and III Na^+ channels correspond to nucleotides 6314–6613, 5997–6450, and 5956–6283 respectively. Following dissection of the SAN, the tissue was either frozen on crushed dry ice or fixed in 4% paraformaldehyde for 10 min, sunk in 30% sucrose (in 0·1 M phosphate buffer, pH 7·4), and frozen. Cryostat sections (20 μm) were collected on glass slides and stored at −80 °C. Slides were warmed to room temperature, placed in 4% paraformaldehyde for 5 min, rinsed in PBS for 5 min, and then processed for *in situ* hybridization as described previously (Qu *et al.* 1995), beginning with a rinse in 0·1 M triethanolamine, pH 8·0 for 2 min.

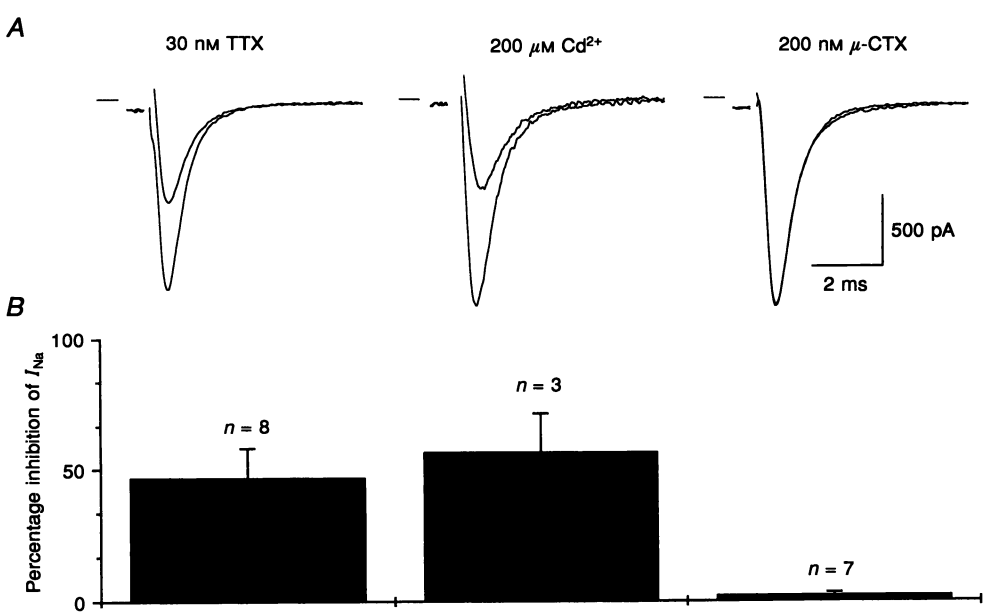

Figure 1. Pharmacological profile of the SAN I_{Na}

Representative tracings (*A*) and average results (*B*) of the effect of TTX, Cd^{2+} and μ-CTX. Mean block was, respectively, 47 ± 11, 57 ± 15 and 2·2 ± 0·9%. In the traces in *A*, the first 200–500 μs of the capacitative transient has been blanked, and the zero current level is indicated by the initial horizontal line.

RESULTS

We first defined the pharmacological profile of the newborn SAN Na^+ current by using a series of different agents at fixed concentrations. These experiments took advantage of the fact that while both skeletal and neuronal (type II) isoforms are sensitive to TTX in the nanomolar range, only the skeletal, and not the neuronal or cardiac isoform, is sensitive to μ-CTX (reported skeletal $K_d = 50$–140 nM: Moczydlowski, Olivera, Gray & Strichartz, 1986). In addition, the cardiac isoform is relatively sensitive to block by Cd^{2+}, with a K_d of 182 μM (DiFrancesco, Ferroni, Visentin & Zaza, 1985), whereas the skeletal and neuronal isoforms are not ($K_d = 5$–41 mM: Tanguy & Yeh, 1988; Ravindran, Schild & Moczydlowski, 1991). We therefore tested the effect of 200 nM μ-CTX, 200 μM Cd^{2+} and 30 nM TTX on the Na^+ current of the newborn SAN. Figure 1*A* provides representative current traces illustrating the extent of blockade by each of these agents. Mean values are illustrated in Fig. 1*B*. At 30 nM, TTX caused 47 ± 11% block ($n = 8$), indicating a higher sensitivity than would be expected for the cardiac isoform. μ-CTX did not cause any block at the concentration employed ($n = 5$), arguing against the presence of the skeletal isoform. Cd^{2+}, which should be relatively ineffective on either the skeletal or neuronal type II Na^+ channel, in fact caused 57 ± 15% block ($n = 3$). Because the initial experiments were performed in one-third of the normal Na^+ and 0 Ca^{2+} (to avoid contamination by Ca^{2+} currents), we first considered the possibility that the high Cd^{2+} or TTX sensitivity might be an artifact of the Ca^{2+}-free environment. We therefore did additional experiments in one-third of the normal Ca^{2+} (thereby maintaining the same Na^+/Ca^{2+} ratio as in normal Tyrode solution) in the presence of 10 μM nifedipine. In five cells, 100 nM TTX caused 76 ± 9% block (compared with 74 ± 10% in 0 Ca^{2+}) and in three cells, 200 μM Cd^{2+} caused 50 ± 7% block. In seven additional cells, 200 μM Cd^{2+} was tested in the presence of normal external Ca^{2+} and caused 52 ± 5% block. None of these results differed significantly from the corresponding results in Ca^{2+}-free solution.

While these results argue against the current in the newborn SAN arising from a skeletal isoform, they differ from what would be expected from a typical cardiac (Cd^{2+} sensitive and TTX insensitive) or neuronal type II (TTX sensitive and Cd^{2+} insensitive) channel. We therefore conducted additional experiments to determine the full dose–response relation for inhibition of the current by Cd^{2+} and TTX. The full dose–response relation for inhibition of the current by Cd^{2+} is shown in Fig. 2. The concentration for half-maximal inhibition (EC_{50}) is 185 ± 8 μM, confirming a sensitivity comparable to that reported in cardiac Purkinje fibres (DiFrancesco *et al.* 1985) and in reconstitution assays with the cardiac isoform of the Na^+ channel (Ravindran *et al.* 1991; Satin *et al.* 1992). A slope factor of 0·82 ± 0·3 suggests that Cd^{2+} ions block the channel with a 1:1 binding ratio. Subsequent experiments also confirmed the high TTX sensitivity suggested by Fig. 1. These results are illustrated in Fig. 3, which depicts the full dose–response curve for TTX block of the Na^+ channel in the neonatal SAN. The EC_{50} value for TTX was 26 ± 5 nM, markedly lower than that typically reported in cardiac preparations (Cohen *et al.* 1981; Satin *et al.* 1992). While an EC_{50} value in the nanomolar range clearly distinguishes these cells from other

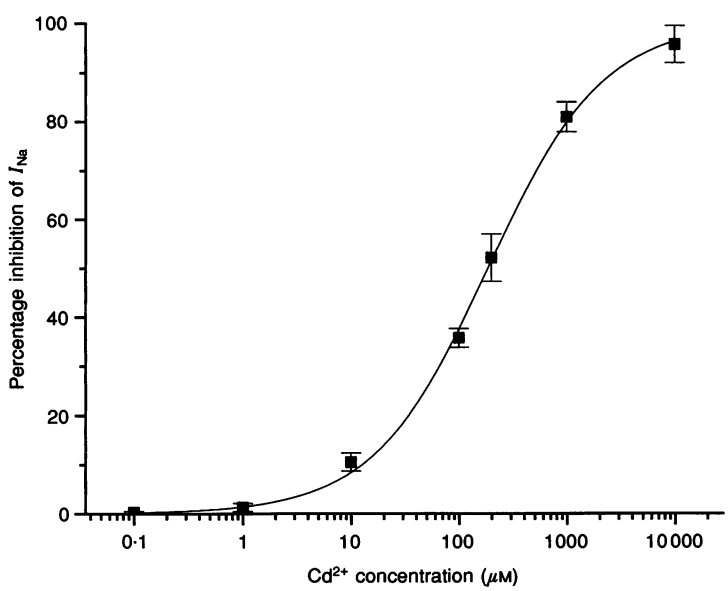

Figure 2. Cd^{2+} sensitivity of I_{Na} in newborn SAN myocytes

The dose–response curve shows the percentage inhibition of the control current as a function of Cd^{2+} concentration. Currents were elicited (in 1·8 mM Ca^{2+} external solution) by stepping to 0 mV from a holding potential of −90 mV every 2 s. The experimental points ($n = 3$–9) are fitted by a form of the Hill equation (continuous line): $1/(1 + (EC_{50}/Cd^{2+})^p)$. EC_{50} and the parameter p were, respectively, 185 ± 8 μM and 0·82 ± 0·03.

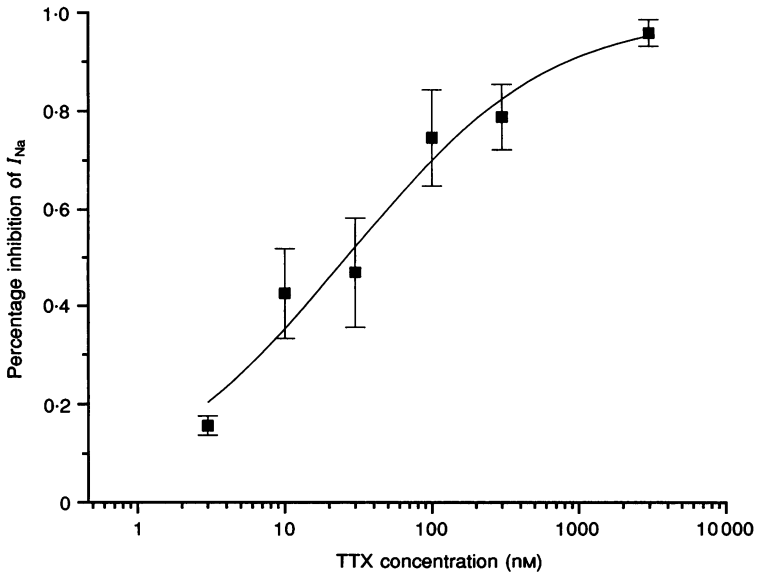

Figure 3. TTX sensitivity of I_{Na} in newborn SAN myocytes

The dose–response curve for TTX-induced block of peak I_{Na}. Currents were evoked (in Ca^{2+}-free external solution) by stepping to 0 mV from a holding potential of −90 mV every 2 s. Each point represents the mean of 6–9 values. The continuous line is drawn according to the fit obtained with a Hill equation as described in the legend for Fig. 2: $EC_{50} = 26 \pm 5$ nM; $p = 0{\cdot}63 \pm 0{\cdot}09$.

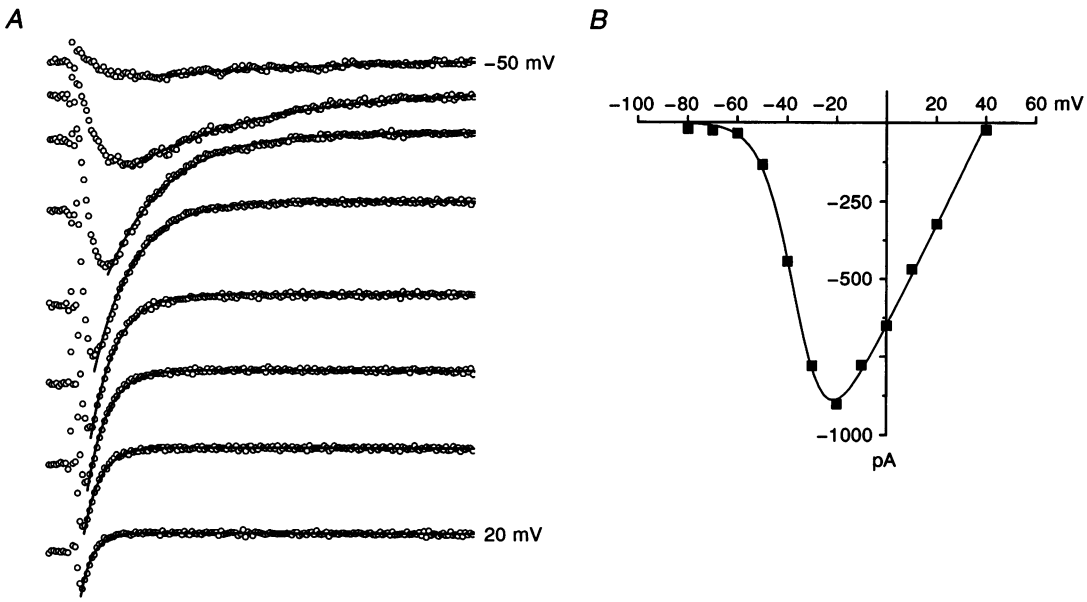

Figure 4. Representative records of inactivation time course and corresponding *I–V* curve

A, family of current traces obtained by stepping to different test potentials (−50 to +20) from a holding potential of −90 mV. The current records are depicted by the open circles and the calculated best fits to a single exponential function are depicted by the continuous lines. For clarity, only every third data point is shown. *B*, *I–V* relation from the same cell as in the left panel. The first 10 ms of the experimental points were fitted according to the equation, $I = g_{Na,max}(1/(1 + \exp(-(E - E_h)/s))(E - E_{Na})$, to give the smooth curve. E_h, mid-point of activation curve; $g_{Na,max}$, maximal conductance; s, inverse slope factor; E_{Na}, reversal potential for Na^+ current. E_{Na} was determined by fitting the linear part of the *I–V* curve and calculating the *x*-axis intercept.

cardiac preparations, the relatively shallow slope of the TTX dose–response relation (slope factor of 0·63 ± 0·09) suggests some contribution to the whole-cell current by a TTX-resistant Na^+ channel isoform. However, as previously reported (Baruscotti *et al.* 1996), approximately 95% block was achieved by 3 μM TTX, indicating that the contribution of any TTX resistant Na^+ channel to the total whole-cell Na^+ current is relatively modest.

We further reasoned that if the shallow slope arose from a significant cardiac isoform contribution then evidence of that would be apparent from an analysis of the time course of inactivation. This time course tends to be slower, and is often biexponential, in cardiac preparations (Sakakibara *et al.* 1992; Fozzard & Hanck, 1996). However, in the newborn SAN the inactivating current time course is best fitted by a monoexponential function. A family of current traces and the calculated best-fit single exponential curves are presented in Fig. 4*A*; the traces are offset along the *y*-axis to allow a clearer visualization of the individual current records (○) and the calculated fits (lines). It is apparent that the inactivating current traces are well fitted by a single exponential function at all test voltages. Figure 4*B* shows the *I–V* relation obtained from the traces. Figure 5 provides the mean value of the inactivation time constants at all test voltages between −50 and +40 mV. The mean values range from 5·3 ± 0·5 ms at −50 mV to 0·3 ± 0·1 ms at +40 mV. These results indicate that a single, relatively rapid, component dominates the inactivation kinetics at all voltages. While these data suggest that a single isoform dominates, they do not rule out the possibility of multiple isoforms (one TTX sensitive and the other Cd^{2+} sensitive) with relatively similar inactivation kinetics. However, in this case by using Cd^{2+} and TTX to preferentially block one or the other component we should accentuate any small difference in kinetics. This possibility was tested in experiments using a test voltage of 0 mV. Regardless of whether TTX (30 nM) or Cd^{2+} (200 μM) was used to partially inhibit the current, the resulting inactivation time constant was not modified by the partial blockade. In the case of TTX exposure, the measured values were 0·55 ± 0·06 ms (before TTX) and 0·61 ± 0·08 ms (during TTX; $n = 8$). In the Cd^{2+} experiments the measured values were 0·64 ± 0·11 ms (before Cd^{2+}) and 0·63 ± 0·11 ms (during Cd^{2+}; $n = 3$). The differences were not significant in either case ($P > 0·05$).

The insensitivity of I_{Na} to μ-CTX indicates that the skeletal Na^+ channel isoform is not functionally expressed in the newborn SAN to any significant extent. In addition, the low EC_{50} value for TTX block of the current (~80% block at 300 nM) suggests that any cardiac isoform present probably contributes no more than 20% of the total whole-cell current under our recording conditions. However, such a minor contribution could not account for our observation of ~50% block by 200 μM Cd^{2+} – a concentration that should be partially effective on any current arising from the cardiac isoform but virtually ineffective against a neuronal (type II) isoform. This indicates that the TTX-sensitive isoform in SAN must have a significantly lower K_d for Cd^{2+} than that reported for TTX-sensitive channels in other tissues (Tanguy & Yeh, 1988; Ravindran *et al.* 1991). This raised the

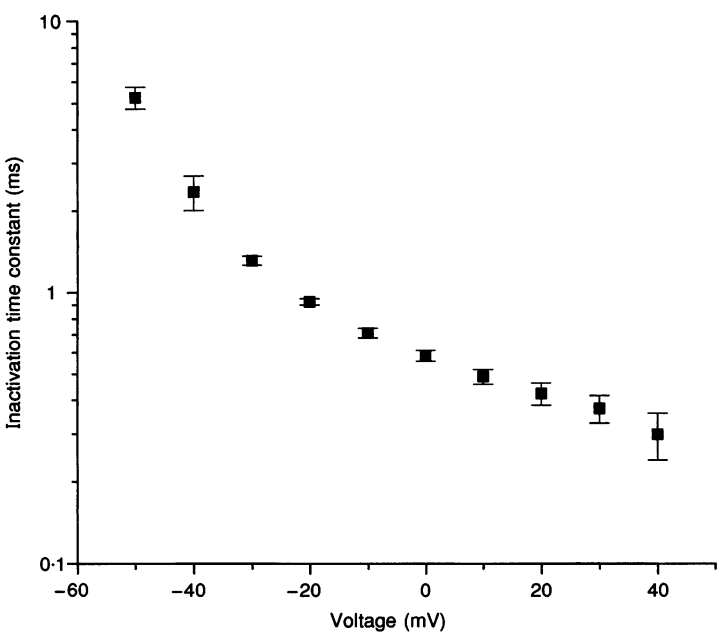

Figure 5. Voltage dependence of inactivation kinetics of the SAN I_{Na}

Cells were held at a potential of −90 mV and stepped to different test potentials. The time course of current inactivation was well fitted by a single exponential function for all voltages tested. Each point represents the mean of 3–6 values.

possibility that one of the other neuronal isoforms, which has not yet been fully characterized pharmacologically, could account for the unique pharmacological profile in the newborn SAN myocytes.

To investigate whether any of the three neuronal isoforms previously reported in whole heart assays (Suzuki *et al.* 1988; Beckh, 1990) might be responsible for the TTX-sensitive Na^+ currents observed electrophysiologically in the SAN of young rabbits, we examined their expression using high resolution *in situ* hybridization methods. No hybridization was observed with probes specific for the type II or type III Na^+ channels in either 4-day-old or adult SAN tissue (data not shown). However, we found clear hybridization of the anti-sense probe to type I-like Na^+ channel mRNA located in the SAN myocytes of young rabbits (Fig. 6*A*). No hybridization was observed with the corresponding sense probe (Fig. 6*D*), and no hybridization was observed in adult SAN with either sense or anti-sense

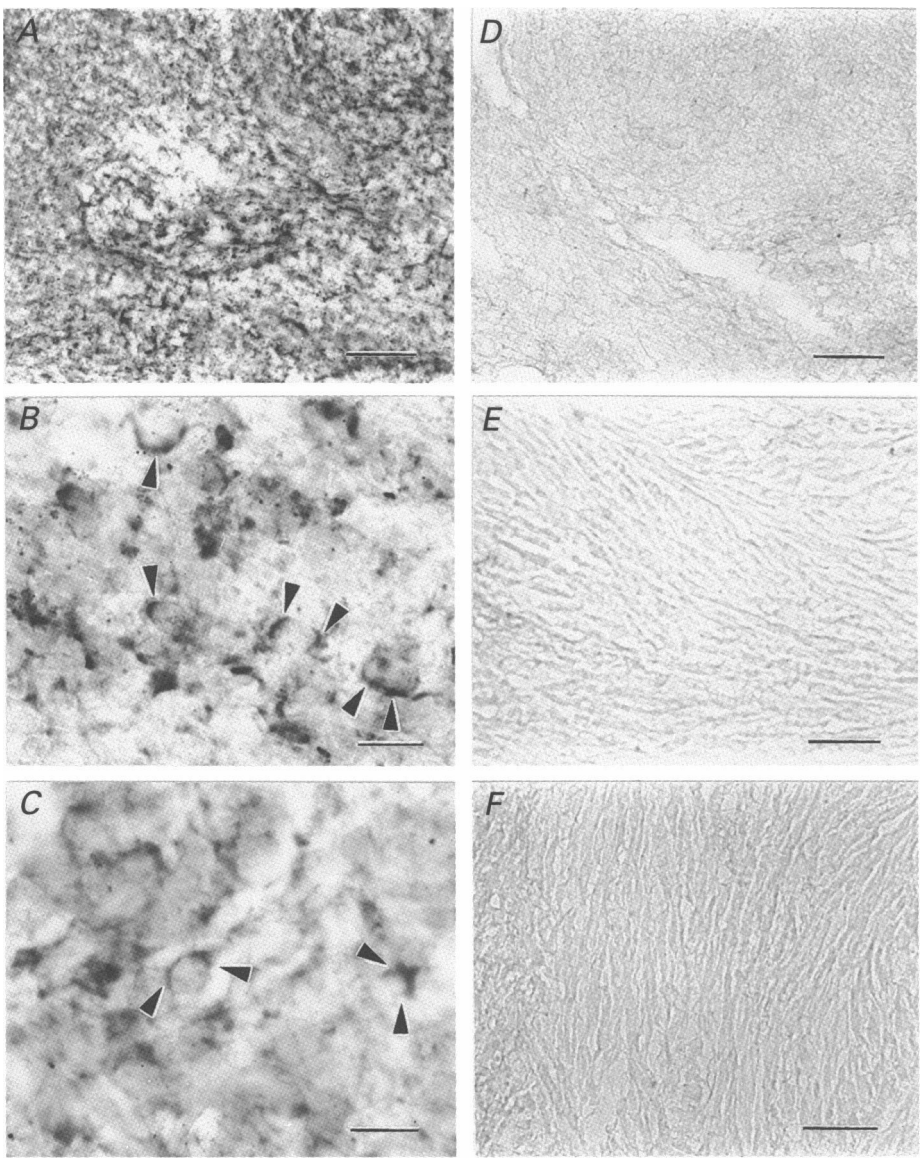

Figure 6. *In situ* hybridization of type I Na^+ channels in the SAN

A, SAN from 4-day-old rabbit hybridized with type I Na^+ channel anti-sense probe demonstrating extensive localization in the tissue. *B* and *C*, higher magnifications of SAN hybridized with type I anti-sense probe illustrating labelling present in the cytoplasm surrounding the nuclei of muscle cells. Arrowheads outline reaction product surrounding nuclei. *D*, SAN of 4-day-old rabbit hybridized with type I Na^+ channel sense probe. *E*, SAN tissue from adult rabbit hybridized with type I Na^+ channel anti-sense probe. *F*, SAN from adult rabbit hybridized with type I Na^+ channel sense probe. Scale bars in *A*, *D*, *E* and *F* represent 100 μm; scale bars in *B* and *C* represent 20 μm.

probes (Fig. 6*E* and *F*). At higher magnification, there is positive staining of mRNA for type I Na^+ channels in regions of the cytoplasm surrounding the nucleus where the endoplasmic reticulum and Golgi complex are located (Fig. 6*B* and *C*), as expected for mRNA encoding an integral membrane protein.

DISCUSSION

In the adult SAN, Na^+ current appears to play a role primarily in the periphery (Nikmaram, Kodama, Boyett, Suzuki & Honjo, 1996), and is relatively resistant to TTX (Muramatsu, Zou, Berkowitz & Nathan, 1996), as would be expected for the cardiac isoform of the channel (Brown, Lee & Powell, 1981; Satin *et al.* 1992). In contrast, our previous studies indicated that newborn rabbit SAN cells express a Na^+ current which has a high degree of relevance for spontaneous activity, and the results we present here indicate that these cells predominantly express a Na^+ channel isoform which is similar to the neuronal type I isoform, rather than the cardiac isoform. This finding is based on the characteristic pharmacological profile, which has not been described previously, and on *in situ* hybridization experiments. We used TTX, μ-CTX and Cd^{2+} in order to match the affinities of these Na^+ channel blockers to those already described for known isoforms. The unusually high Cd^{2+} and TTX sensitivity can be interpreted in two different ways: as an intrinsic characteristic of the Na^+ channels expressed in the newborn, or as the result of the existence of two separate populations: one typically cardiac, with a low affinity for TTX and a high affinity for Cd^{2+}, and the other neuronal type II-like, with opposite sensitivities. We tend to exclude this second hypothesis on the basis of several arguments. First, we show that relatively low concentrations of TTX are enough to block the current almost completely (3 μM, 95% block) and this would not be expected if there was any significant expression of a cardiac-like Na^+ channel. Second, analysis of time constants of current inactivation conducted at several test potentials revealed that the inactivation time course is dominated by a single exponential process. Using Cd^{2+} or TTX to partially block the current did not alter the inactivation time course or unmask a second component. Finally, even though the TTX and Cd^{2+} sensitivities of Na^+ channel isoforms have generally been used as a primary tool to discriminate between different proteins, some of the isoforms (George, Knittle & Tamkun, 1992; Lalik, Krafte, Volberg & Ciccarelli, 1993; Felipe, Knittle, Doyle & Tamkun, 1994) have been identified only in terms of sequence but have not been functionally expressed, making the comparison with these isoforms more difficult. However, our *in situ* hybridization studies demonstrate the presence of mRNA encoding a type I-like Na^+ channel in the SAN myocytes of young rabbits but not in those of adults. This differential expression in young *versus* adult rabbits parallels the presence of TTX-sensitive Na^+ currents and suggests that these Na^+ currents may be mediated by type I Na^+ channels, or possibly an alternatively spliced variant similar to those reported to occur by Schaller, Krzemien, McKenna & Caldwell (1992).

Although mRNA encoding type I Na^+ channels has previously been shown to be represented in cardiac cDNA libraries (Rogart *et al.* 1989), these libraries contain cDNAs originating from the resident neurons of the heart as well as from myocytes. Our results demonstrate directly for the first time a neuronal-like Na^+ channel isoform in the myocytes in a specific region of the heart. The presence of neuronal-like proteins in the SAN is intriguing, given earlier suggestions, based on the presence of neurofilaments in SAN tissue (Gorza, Schiaffino & Vitadello, 1988; Gorza & Vitadello, 1989), that the SAN may be of neural crest origin. Our observation of transient expression of a neuronal Na^+ channel in the SAN is compatible with this hypothesis and supports the idea that the SAN is under distinct developmental regulation relative to other regions of the heart. The actual sequence of the isoform expressed in the newborn SAN remains to be determined, and definitive association of the pharmacological sensitivity reported here with this type I-like isoform will require reconstitution studies with a defined sequence. However, it is apparent from the loss of the current with age (Baruscotti *et al.* 1996) that the developmental regulation of the channel is different from that of the cardiac isoform expressed elsewhere in the heart. It also is apparent, from the data presented here, that the potential exists for differential pharmacological control of the newborn SAN Na^+ current and the Na^+ current expressed in the working myocardium and Purkinje fibres.

Baruscotti, M., Difrancesco, D. & Robinson, R. B. (1996). A TTX-sensitive inward sodium current contributes to spontaneous activity in newborn rabbit sino-atrial node cells. *Journal of Physiology* **492**, 21–30.

Beckh, S. (1990). Differential expression of sodium channel mRNAs in rat peripheral nervous system and innervated tissues. *FEBS Letters* **262**, 317–322.

Brown, A. M., Lee, K. S. & Powell, T. (1981). Sodium current in single rat heart muscle cells. *Journal of Physiology* **318**, 479–500.

Cohen, C. J., Bean, B. P., Colatsky, T. J. & Tsien, R. W. (1981). Tetrodotoxin block of sodium channels in rabbit Purkinje fibers. *Journal of General Physiology* **78**, 383–411.

Difrancesco, D., Ferroni, A., Visentin, S. & Zaza, A. (1985). Cadmium-induced blockade of the cardiac fast Na channels in calf Purkinje fibers. *Proceedings of the Royal Society* B **223**, 475–484.

Felipe, A., Knittle, T. J., Doyle, K. L. & Tamkun, M. M. (1994). Primary structure and differential expression during development and pregnancy of a novel voltage-gated sodium channel in the mouse. *Journal of Biological Chemistry* **269**, 30125–30131.

Fozzard, H. A. & Hanck, D. A. (1996). Structure and function of voltage-dependent sodium channels: comparison of brain II and cardiac isoforms. *Physiological Reviews* **76**, 887–926.

George, A. L. Jr, Knittle, T. J. & Tamkun, M. M. (1992). Molecular cloning of an atypical voltage-gated sodium channel expressed in human heart and uterus: Evidence for a distinct gene family. *Proceedings of the National Academy of Sciences of the USA* **89**, 4893–4897.

Gorza, L., Schiaffino, S. & Vitadello, M. (1988). Heart conduction system: a neural crest derivative? *Brain Research* **457**, 360–366.

Gorza, L. & Vitadello, M. (1989). Distribution of conduction system fibers in the developing and adult rabbit heart revealed by an anti-neurofilament antibody. *Circulation Research* **65**, 360–369.

Lalik, P. H., Krafte, D. S., Volberg, W. A. & Ciccarelli, R. B. (1993). Characterization of endogenous sodium channel gene expressed in Chinese hamster ovary cells. *American Journal of Physiology* **264**, C803–809.

Moczydlowski, E., Olivera, B. M., Gray, W. R. & Strichartz, G. R. (1986). Discrimination of muscle and neuronal Na-channel subtypes by binding competition between [^{3}H]saxitoxin and μ-conotoxins. *Proceedings of the National Academy of Sciences of the USA* **83**, 5321–5325.

Muramatsu, H., Zou, A., Berkowitz, G. A. & Nathan, R. D. (1996). Characterization of a TTX-sensitive Na^+ current in pacemaker cells isolated from the rabbit sinoatrial node. *American Journal of Physiology* **270**, H2108–2119.

Nikmaram, M. R., Kodama, I., Boyett, M. R., Suzuki, R. & Honjo, H. (1996). The Na^+ current plays an important role in pacemaker activity in the periphery, but not the centre, of the rabbit sinoatrial node. *Journal of Physiology* **491.P**, 154*P*.

Qu, Y., Isom, L. L., Westenbroek, R. E., Rogers, J. C., Tanada, T. N., McCormick, K. A., Scheuer, T. & Catterall, W. A. (1995). Modulation of cardiac Na^+ channel expression in *Xenopus* oocytes by β1 subunits. *Journal of Biological Chemistry* **270**, 25696–25701.

Ravindran, A., Schild, L. & Moczydlowski, E. (1991). Divalent cation selectivity for external block of voltage-dependent Na^+ channels prolonged by Batrachotoxin. *Journal of General Physiology* **97**, 89–115.

Rogart, R., Cribbs, L. L., Muglia, L. K., Kephart, D. D. & Kaiser, M. W. (1989). Molecular cloning of a putative tetrodotoxin-resistant rat heart sodium channel isoform. *Proceedings of the National Academy of Sciences of the USA* **86**, 8170–8174.

Sakakibara, Y., Wasserstrom, J. A., Furukawa, T., Jia, H., Arentzen, C. E., Hartz, R. S. & Singer, D. H. (1992). Characterization of the sodium current in single human atrial myocytes. *Circulation Research* **71**, 535–546.

Satin, J., Kyle, J. W., Chen, M., Bell, P., Cribbs, L. L., Fozzard, H. A. & Rogart, R. B. (1992). A mutant of TTX-resistant cardiac sodium channels with TTX-sensitive properties. *Science* **256**, 1202–1205.

Schaller, K. L., Krzemien, D. M., McKenna, N. M. & Caldwell, J. H. (1992). Alternatively spliced sodium channel transcripts in brain and muscle. *Journal of Neuroscience* **12**, 1370–1381.

Suzuki, H., Beckh, S., Kubo, H., Yahagi, N., Ishida, H., Kayano, T., Noda, M. & Numa, S. (1988). Functional expression of cloned cDNA encoding sodium channel III. *FEBS Letters* **228**, 195–200.

Tanguy, J. & Yeh, J. Z. (1988). Divalent cation block of normal and BTX-modified sodium channels in squid axons. *Biophysical Journal* **53**, 229a (abstract).

Acknowledgements

The authors thank Ms Patricia McLaughlin for technical assistance during the cell isolation procedures. This work was supported by NIH NHLBI grants P01 HL-28958 and P01 HL-44948.

Author's present address

M. Baruscotti: Dipartimento di Fisiologia e Biochimica Generali, Elettrofisiologia, Università di Milano, Milano, Italy.

Author's email address

R. B. Robinson: RBR1@columbia.edu

Received 21 August 1996; accepted 28 October 1996.