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Amitriptyline modulation of Na^+ channels in rat dorsal root ganglion neurons

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Abstract

The effects of amitriptyline, a tricyclic antidepressant, on tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ currents in rat dorsal root ganglion neurons were studied using the whole-cell patch clamp method. Amitriptyline blocked both types of Na^+ currents in a dose-and holding potential-dependent manner. At the holding potential of -80 mV, the apparent dissociation constants (K_d) for amitriptyline to block tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels were 4.7 and 105 μM, respectively. These values increased to 181 and 193 μM, respectively, when the membrane was held at a potential negative enough to remove the steady-state inactivation. Amitriptyline dose-dependently shifted the steady-state inactivation curves in the hyperpolarizing direction and increased the values of the slope factors for both types of Na^+ channels. The voltage dependence of the activation of both types of Na^+ channels was shifted in the depolarizing direction. It was concluded that amitriptyline blocked the two types of Na^+ channels in rat sensory neurons by modulating the activation and the inactivation kinetics.

Keywords: Amitriptyline; Antidepressant; Tetrodotoxin-sensitive; Tetrodotoxin-resistant; Na^+ channel; Dorsal root ganglion

1. Introduction

The pharmacological action of tricyclic antidepressants is thought to block the reuptake of norepinephrine and/or serotonin at the synaptic cleft. In addition, they inhibit various receptors such as muscarinic receptors, α-adrenoceptors and histamine receptors. They also inhibit various ion channels. For example, tricyclic antidepressants inhibit $P2X_2$ purinoceptor/channels expressed in *Xenopus* oocytes (Nakazawa et al., 1999), transient outward K^+ channels in rat ventricular myocytes (Casis and Sanchez-Chapula, 1998), the large conductance Ca^{2+}-activated K^+ channels in rat cortical neurons (Lee et al., 1997), nicotinic acetylcholine receptor/channels in rat hippocampus (Hennings et al., 1999), NMDA receptor/channels expressed in *Xenopus* oocytes (Tohda et al., 1995), L-type Ca^{2+} channels in murine dorsal root ganglia (Choi et al., 1992), and Na^+ channels in cardiac myocytes and neurons (Ogata and Narahashi, 1989; Ogata et al., 1989; Pancrazio et al., 1998).

Tricyclic antidepressants show analgesic effects regardless of the presence of depression and are useful for the control of pain associated with various diseases such as postherpetic neuralgia, chronic low back pain, chronic tension-type headache, chronic orofacial pain, diabetic or nondiabetic polyneuropathy (Pettengill and Reisner-Keller, 1997; Vrethem et al., 1997; Atkinson et al., 1998; Cerbo et al., 1998; Watson et al., 1998). The analgesic mechanism of tricyclic antidepressants is unclear, but their ability to block Na^+ channels is considered to play a certain role in relieving pain.

Most Na^+ channels are selectively blocked by a potent neurotoxin, tetrodotoxin. Some Na^+ channels, however, are relatively resistant to the blocking action of tetrodotoxin (Yoshida, 1994). In dorsal root ganglion neurons, tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels are distinguished by their relative sensitivity to tetrodotoxin (Kostyuk et al., 1981; Ogata and Tatebayashi, 1993). In an experimental pain model, the expression and the function of these channels are changed to cause a variety of electrophysiological abnormalities (Waxman et al., 1999). This suggests that the blockade of Na^+ channels in sensory neurons may underlie the analgesia induced by tricyclic antidepressants. The present study was under-

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taken to elucidate the action of amitriptyline, one of the tricyclic antidepressants, on tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels in rat dorsal root ganglion neurons.

2. Materials and methods

2.1. Cell preparation

Dorsal root ganglion neurons were isolated as described previously (Roy and Narahashi, 1992). Rats (2–6 days postnatal) were anesthetized with isoflurane and the spinal column was removed and cut longitudinally. Ganglia were plucked from between the vertebrae of the spinal column and incubated in phosphate-buffered saline solution (Sigma, St. Louis, MO) containing trypsin (2.5 mg/ml, Type IX, Sigma) at 37°C for 30 min. After enzyme treatment, ganglia were rinsed with Dulbecco's Modified Eagle Medium (GibcoBRL, Grand Island, NY) supplemented with horse serum (10%, v/v, Sigma). Single cells were mechanically dissociated by trituration with a fire-polished Pasteur pipette and plated on poly-L-lysine-coated glass cover slips (12 mm, Warner Instruments, Hamden, CT). Cells were incubated for 2–7 h before patch clamp experiments.

2.2. Electrophysiological recording

Cells attached to cover slips were transferred into a recording chamber on the stage of an inverted microscope. Ion currents were recorded under voltage-clamp conditions by the whole-cell patch clamp technique (Hamill et al., 1981). Suction pipettes (0.8–1.1 MΩ) were made of borosilicate glass capillary tubes (TW150F-4, World Precision Instrument, Sarasota, FL) using a two-step vertical puller (PP-83, Narishige, Tokyo, Japan) and heat-polished with a microforge (MF-83, Narishige). The pipette solution contained (in mM): CsCl 65, CsF 70, NaCl 10, HEPES 10. The pH was adjusted to 7.2 with CsOH and the osmolarity was 280 mosM/l on average. The external solution contained (in mM): NaCl 25, choline chloride 120, tetraethylammonium chloride 20, D-glucose 5, HEPES 5, $MgCl_2$ 1, $CaCl_2$ 1. Lanthanum ($LaCl_3$, 10 μM) was used to block the Ca^{2+} channel current. The solution was adjusted to pH 7.4 with tetraethylammonium hydroxide and the osmolarity was 295 to mOsm/l on average. An Ag–AgCl pellet/3 M KCl-agar bridge was used for the reference electrode. Membrane currents were recorded using an Axopatch-1D amplifier (Axon Instruments, Foster City, CA). Signals were digitized by a 12-bit analog-to-digital interface (Digidata 1200A, Axon Instruments), filtered with a lowpass Bessel filter at 5 kHz and sampled at 50 kHz using pCLAMP6 software (Axon Instruments) on an IBM-compatible PC. Series resistance was compensated 60–70%. Capacitative and leakage currents were subtracted by using a P + P/4 procedure (Bezanilla and Armstrong, 1977). The liquid junction potential between internal and external solution was on average −4 mV. The data shown in this paper were corrected for the liquid junction potential. All experiments were performed at 22–24°C. Stock solutions of amitriptyline were made in dimethylsulfoxide at concentrations of 10 to 500 mM and aliquots were stored at −20°C until used. They were diluted in the external solution to the desired concentrations just before experiments. The dimethylsulfoxide concentration in the perfusate was less than 0.1% (v/v), which had no effect on Na^+ currents (data not shown). All chemicals were purchased from Sigma.

A period of 5–10 min was allowed after the establishment of the whole-cell recording configuration to ensure adequate equilibration between the internal pipette solution and the cell interior, and to obtain a stable membrane current.

2.3. Data analysis

Data were analyzed by a combination of pCLAMP6 (Axon Instruments) and SigmaPlot (Jandel Scientific, San Rafael, CA) programs. Results are expressed as means ± S.E.M. and n represents the number of cells examined. The statistical significance of the amitriptyline effect was determined using Student's t-test with $P < 0.05$ considered significant.

3. Results

3.1. Effects of amitriptyline on the Na^+ current amplitude

Two types of Na^+ currents were distinguished in rat dorsal root ganglion neurons on the basis of their sensitivity to tetrodotoxin. Tetrodotoxin-sensitive Na^+ currents were completely blocked by tetrodotoxin at 100 nM (data not shown). For the study of tetrodotoxin-sensitive Na^+ channels, cells that expressed only tetrodotoxin-sensitive currents were used. Tetrodotoxin-sensitive Na^+ currents were completely inactivated within 2 ms when currents were evoked by depolarizing steps to 0 mV (Fig. 1A). Other cells expressed both tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ currents in variable proportions. In these cells, tetrodotoxin-sensitive currents were blocked by tetrodotoxin 100 nM and the remaining currents were used for the study of tetrodotoxin-resistant Na^+ channels. Tetrodotoxin-resistant Na^+ currents showed considerably slower activation and inactivation kinetics compared to tetrodotoxin-sensitive Na^+ currents (Fig. 1B).

Amitriptyline reduced the current amplitude of both types of Na^+ channels within 3 min after bath application of the drug and the current recovered within 3 min after washout with drug-free external solution. The degree of block was dependent on the holding potential. Tetro-

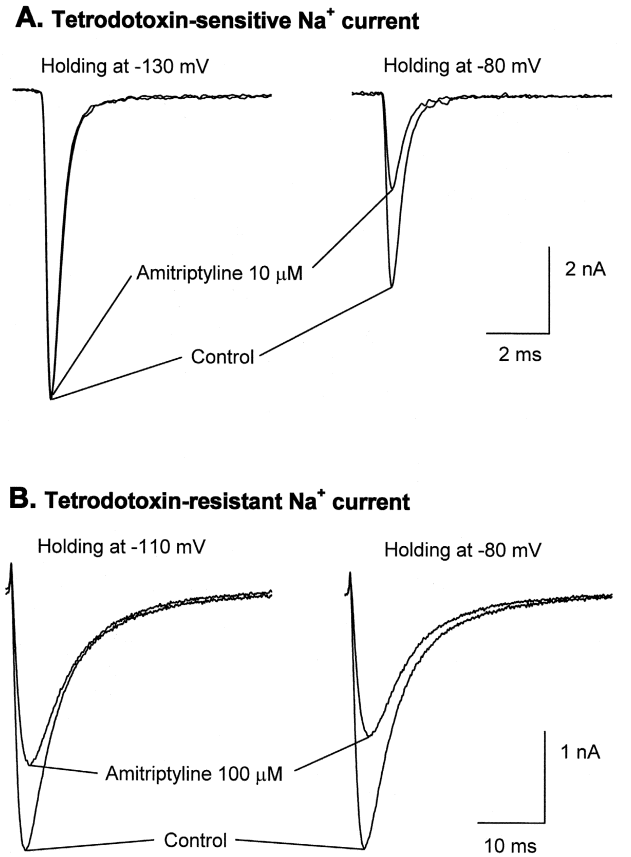

Fig. 1. Holding potential-dependent inhibition of tetrodotoxin-sensitive (A) and tetrodotoxin-resistant (B) Na^+ currents by amitriptyline. Currents were elicited by step depolarizations to 0 mV from holding potentials as indicated. Cells were exposed to amitriptyline for 3 min.

dotoxin-sensitive Na^+ currents were elicited by depolarizing steps to 0 mV from holding potentials of -130 or -80 mV (Fig. 1A). Amitriptyline at 10 μM blocked the currents by $6 \pm 3\%$ and $63 \pm 4\%$, respectively ($n = 7$). Tetrodotoxin-resistant Na^+ currents were elicited by step depolarizations to 0 mV from holding potentials of -110 or -80 mV (Fig. 1B). Amitriptyline at 100 μM blocked the currents by $36 \pm 4\%$ and $45 \pm 3\%$, respectively ($n = 7$).

The dose-dependent effect of amitriptyline on Na^+ current amplitude is shown in Fig. 2. The fraction of control current remaining after amitriptyline treatment is plotted as a function of the amitriptyline concentration. The lines were obtained by curve fitting of the following Hill equation to the data points: $I_{\text{Amitriptyline}}/I_{\text{Control}} = 1/\{1 + ([\text{Amitriptyline}]/K_d)^h\}$, where I_{Control} is control current amplitude, $I_{\text{Amitriptyline}}$ is current amplitude remaining after amitriptyline treatment, [Amitriptyline] is the concentration of amitriptyline, K_d is the apparent dissociation constant for amitriptyline block of Na^+ currents, and h is the Hill coefficient. Two different sets of dose–response curves were plotted to evaluate the holding potential-dependent effect of amitriptyline. At a holding potential of -80 mV, the K_d values and the Hill coefficients were approximately 4.7 (± 0.5) μM and 0.88 (± 0.08) for the tetrodotoxin-sensitive Na^+ current, and 105 (± 12) μM and 0.92 (± 0.11) for the tetrodotoxin-resistant Na^+ current, respectively. The maximum current amplitude was obtained by eliciting a current from a holding potential sufficiently negative to remove the steady-state inactivation of Na^+ channels. The curves were best fitted to the dose–response data on the maximum current amplitude when the K_d values and the Hill coefficients were 181 (± 41) μM and 0.98 (± 0.23) for the tetrodotoxin-sensitive Na^+ current, and 193 (± 27) μM and 0.88 (± 0.14) for the tetrodotoxin-resistant Na^+ current, respectively.

3.2. Effects of amitriptyline on Na^+ channel inactivation

The effects of amitriptyline on the steady-state inactivation curves of tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels are shown in Fig. 3A and B, respectively. The holding potential was changed to various levels for 20 s, and was immediately followed by a step depolar-

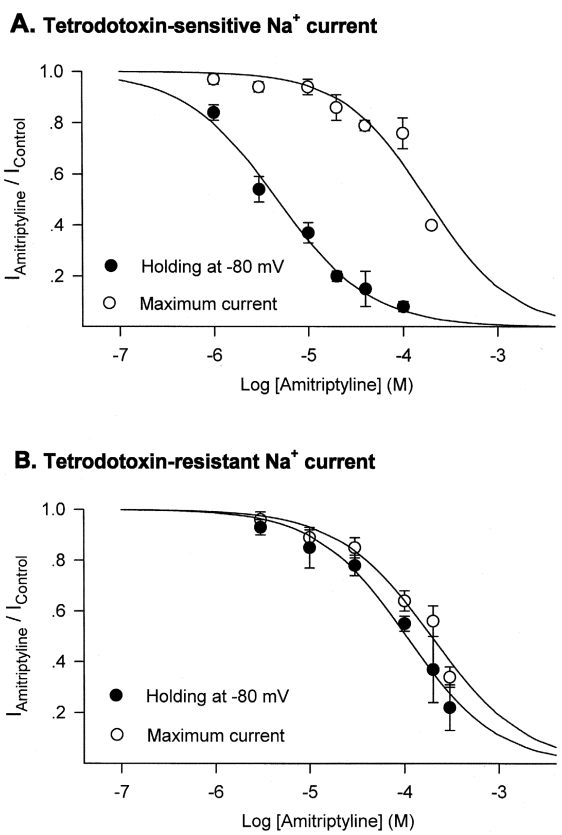

Fig. 2. Dose–response relationships for the amitriptyline block of tetrodotoxin-sensitive (A) and tetrodotoxin-resistant (B) Na^+ currents. Currents were elicited by step depolarizations to 0 mV from a holding potential of -80 mV (●), or large negative-holding potentials ranging from -160 to -120 mV (A) and from -120 to -90 mV (B) when maximum currents were generated (○). I_{Control} is control-current amplitude and $I_{\text{Amitriptyline}}$ is current amplitude remaining after amitriptyline treatment for 3 min. Curves were drawn according to the Hill equation (see text). n is 7 for each data point.

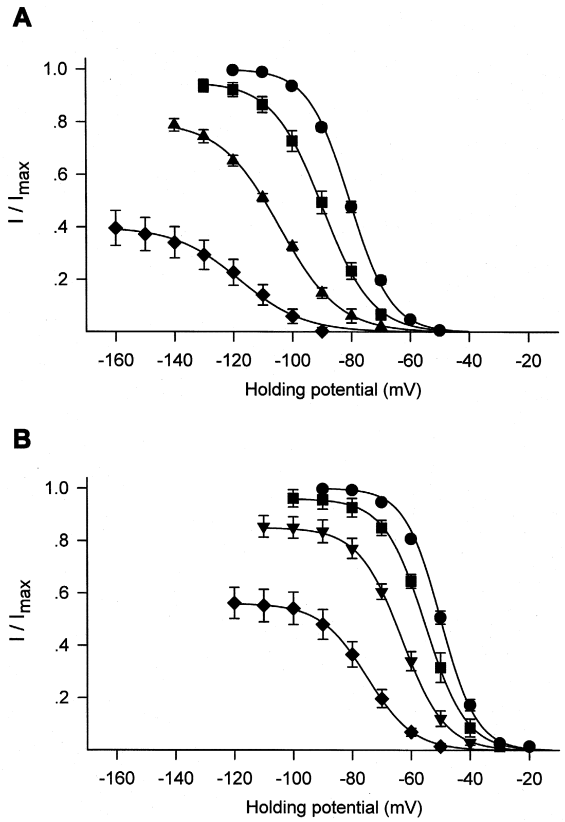

Fig. 3. Dose-dependent effects of amitriptyline on the steady-state inactivation curves for tetrodotoxin-sensitive (A) and tetrodotoxin-resistant (B) Na^+ currents. The membrane potential was held at various levels for 20 s, and then the current was evoked by a step depolarization to 0 mV. The current amplitude (I) is normalized to the maximum control current amplitude (I_{max}) and plotted as a function of the holding potential. The curves were drawn according to the Boltzmann equation (see text). ●, control ($n = 49$ (A) and 35 (B)); ■, amitriptyline 3 μM; ▼, amitriptyline 30 μM; ▲, amitriptyline 40 μM; ♦, amitriptyline 200 μM. n is 7 for each concentration of amitriptyline.

ization to 0 mV. This protocol was run in the absence and in the presence of amitriptyline for 3 min. For each concentration of amitriptyline, a different cell was used. The current amplitudes measured were normalized to the maximum control current amplitude, and plotted as a function of the holding potential. The voltage dependence of the Na^+ channel inactivation was shifted in the hyperpolarizing direction and the maximum current amplitude was decreased with increasing concentrations of amitriptyline. The curves were drawn according to the Boltzmann equation, $I/I_{max} = A/\{1 + \exp[(V_h - V_{h_{0.5}})/k_h]\}$, where I is current amplitude, I_{max} is maximum control current amplitude, A is maximum value of I/I_{max} in each set of experiment, V_h is holding potential, $V_{h_{0.5}}$ is the potential at which I is 0.5 A, and k_h is the slope factor (potential required for an e-fold change). In the control experiment the curve for tetrodotoxin-sensitive Na^+ channels was best fitted when $V_{h_{0.5}}$ and k_h were -80.5 ± 0.6 and 6.91 ± 0.14 mV, respectively ($n = 49$). For tetrodotoxin-resistant Na^+ channels, $V_{h_{0.5}}$ and k_h were -49.9 ± 0.7 and 6.06 ± 0.15 mV, respectively ($n = 35$). The spontaneous shifts of $V_{h_{0.5}}$ and k_h for 3 min were -0.9 ± 0.3 and $+0.07 \pm 0.11$ mV ($n = 5$), respectively, for tetrodotoxin-sensitive Na^+ channels, and -3.2 ± 0.5 and $+0.13 \pm 0.23$ mV ($n = 6$), respectively, for tetrodotoxin-resistant Na^+ channels. With both types of Na^+ channels amitriptyline caused a shift in $V_{h_{0.5}}$ in the hyperpolarizing direction and increased the k_h value in a dose-dependent manner (Table 1).

3.3. Use-dependent block

Amitriptyline caused a profound use-dependent block of the Na^+ current during repetitive stimulation (Fig. 4). Currents were evoked by 20 consecutive 25-ms pulses to 0

Table 1
Effects of amitriptyline on Boltzmann parameters of Na^+ channel inactivation

	Amitriptyline (μM)	$V_{h_{0.5}}$ shift (mV)	k_h change (mV)
Tetrodotoxin-sensitive Na^+ channel	0 ($n = 5$)	-0.9 ± 0.3	$+0.07 \pm 0.11$
	1 ($n = 7$)	-2.2 ± 0.5	$+0.04 \pm 0.08$
	3 ($n = 7$)	-7.2 ± 0.8^a	$+0.77 \pm 0.14^b$
	10 ($n = 7$)	-12.1 ± 1.4^a	$+0.76 \pm 0.20^c$
	20 ($n = 7$)	-17.0 ± 1.3^a	$+1.02 \pm 0.13^a$
	40 ($n = 7$)	-24.8 ± 1.5^a	$+1.81 \pm 0.23^a$
	100 ($n = 7$)	-30.9 ± 1.9^a	$+2.88 \pm 0.34^a$
	200 ($n = 7$)	-37.4 ± 2.1^a	$+3.98 \pm 0.53^a$
Tetrodotoxin-resistant Na^+ channel	0 ($n = 6$)	-3.2 ± 0.5	$+0.13 \pm 0.23$
	3 ($n = 7$)	-3.6 ± 0.5	$+0.92 \pm 0.10^b$
	10 ($n = 7$)	-6.1 ± 0.7^b	$+1.22 \pm 0.28^c$
	30 ($n = 7$)	-11.7 ± 1.2^a	$+1.62 \pm 0.12^a$
	100 ($n = 7$)	-17.0 ± 1.1^a	$+1.77 \pm 0.32^a$
	200 ($n = 7$)	-23.6 ± 2.2^a	$+2.17 \pm 0.49^b$

$V_{h_{0.5}}$ is the membrane potential for the half-maximum steady-state Na^+ channel inactivation, and k_h is the slope factor.
[a] Statistically significantly different from the spontaneous shifts in the absence of amitriptyline ($P < 0.001$).
[b] Statistically significantly different from the spontaneous shifts in the absence of amitriptyline ($P < 0.01$).
[c] Statistically significantly different from the spontaneous shifts in the absence of amitriptyline ($P < 0.05$).

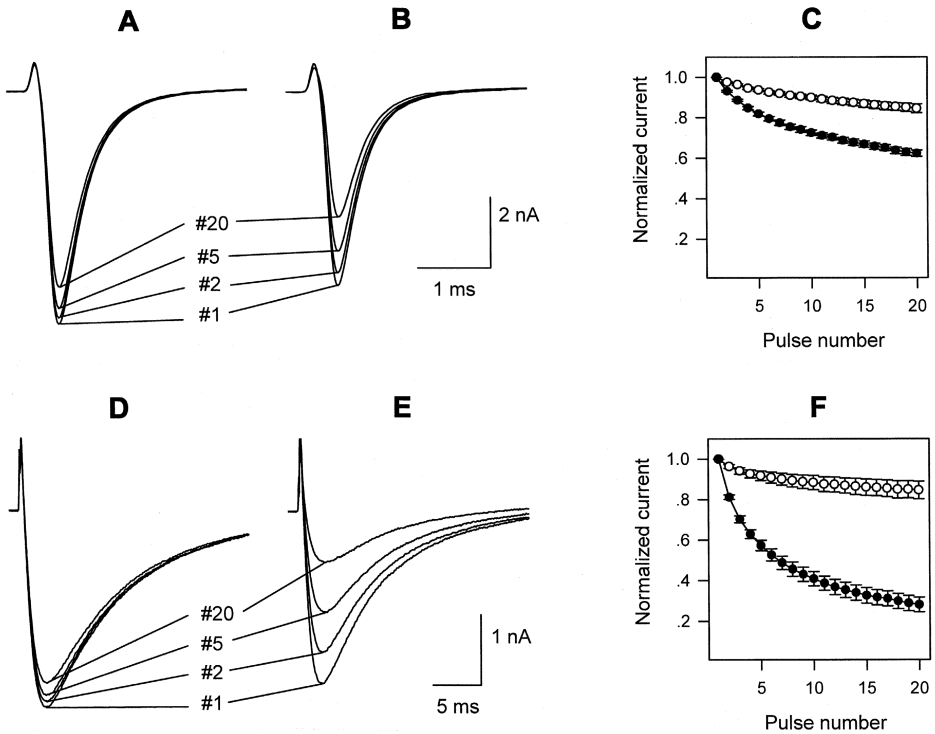

Fig. 4. Use-dependent block of Na^+ currents by amitriptyline. Twenty consecutive pulses to 0 mV for 25 ms from a holding potential of −80 mV were delivered at 0.5 Hz. P + P/4 procedure was not used. (A and B) Representative tetrodotoxin-sensitive currents in the absence and in the presence of amitriptyline 3 μM for 3 min, respectively. (D and E) Representative tetrodotoxin-resistant currents in the absence and in the presence of amitriptyline 10 μM for 3 min, respectively. # denotes the pulse number. (C and F) The amplitudes of tetrodotoxin-sensitive and tetrodotoxin-resistant currents, respectively, were normalized to the initial amplitudes and plotted as a function of the pulse number. ○, control; ●, amitriptyline. n is 7 for each type of Na^+ current.

mV from a holding potential of −80 mV at a frequency of 0.5 Hz. Even under control conditions, the current amplitude progressively decreased with increasing pulse number due to the accumulation of channel inactivation. The ratio, I_{20}/I_1 (current amplitude at 20th pulse/current amplitude at 1st pulse), was 0.84 ± 0.02 ($n = 7$) for the tetrodotoxin-sensitive current and it was 0.85 ± 0.04 ($n = 7$) for the tetrodotoxin-resistant current. The decrement was markedly enhanced by amitriptyline. In the presence of amitriptyline for 3 min, the ratio was 0.62 ± 0.02 for the tetrodotoxin-sensitive current (amitriptyline 3 μM) and it was 0.28 ± 0.04 for the tetrodotoxin-resistant current (amitriptyline 10 μM).

3.4. Effects of amitriptyline on Na^+ channel activation

In addition to its effect on the inactivation kinetics, amitriptyline also affected the activation kinetics of Na^+ channels. In Fig. 5A and C, typical current–voltage relationship curves for tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels, respectively, are shown. Na^+ currents were evoked by step depolarizations to various levels in 5-mV increments from a holding potential of −80 mV. The current amplitude was plotted as a function of depolarizing potential. Tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels started to open at around −45 and −30 mV, respectively, and the current polarity reversed at around +25 mV, which was near the calculated equilibrium potential for Na^+ ions. Amitriptyline reduced the current amplitude for both types of Na^+ channels over the entire range of test potentials. To evaluate the effect of amitriptyline on the voltage-dependence of Na^+ channel activation, the conductance–voltage relationship curves for the two types of Na^+ channels were plotted as shown in Fig. 5B and D. The curves were plotted according to the Boltzmann equation, $g/g_{max} = 1/\{1 + \exp[(V_{g_{0.5}} - V_g)/k_g]\}$, where g is conductance, g_{max} is maximum conductance, V_g is test potential, $V_{g_{0.5}}$ is the potential at which g is $0.5g_{max}$, and k_g is the slope factor. Conductance was calculated by using the equation, $g = I/(V_g - V_{rev})$, where I is current amplitude and V_{rev} is the reversal potential. In the absence of amitriptyline, the conductance–voltage data for tetrodotoxin-sensitive Na^+ channels were best fitted when $V_{g_{0.5}}$ was -29.9 ± 1.2 mV and k_g was 5.8 ± 0.4 mV ($n = 7$). Amitriptyline at 10 μM for 3 min increased $V_{g_{0.5}}$ by 4.7 ± 1.3 mV and k_g by 1.01 ± 0.34 mV, which were significantly different from the spontaneous changes of -1.7 ± 0.6 mV ($P < 0.01$) and -0.28 ± 0.30 mV ($P < 0.05$) ($n = 5$), respectively. For tetrodotoxin-resistant Na^+ channels, $V_{g_{0.5}}$ was -14.3 ± 0.9 mV and k_g was 4.39 ± 0.31 mV ($n = 7$). Amitriptyline at 100 μM for 3 min increased $V_{g_{0.5}}$ by 2.8 ± 1.0 mV

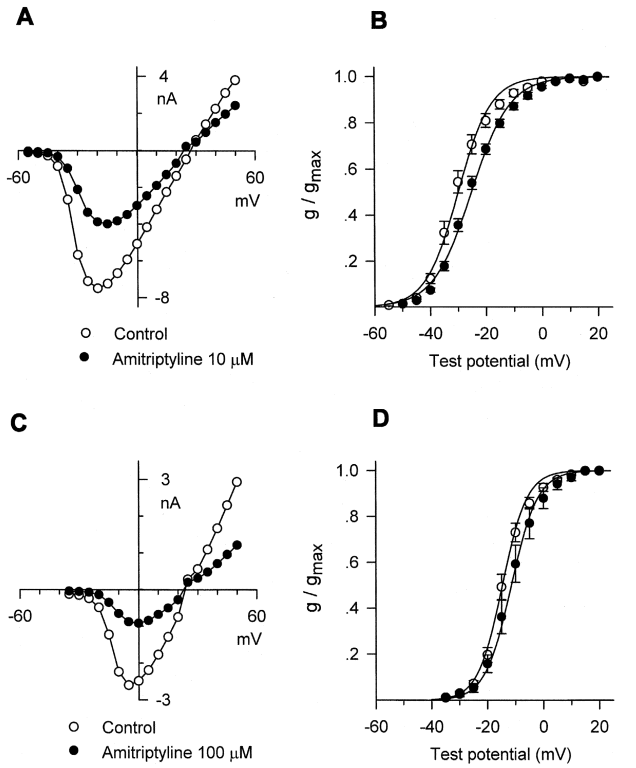

Fig. 5. Effects of amitriptyline on Na^+ channel activation. (A and C) Representative current–voltage relationship curves for tetrodotoxin-sensitive and tetrodotoxin-resistant currents, respectively. Currents were elicited by depolarizing steps to various test potentials from a holding potential of −80 mV. Current amplitude was plotted as a function of the test potential. (B and D) The conductance–voltage curves for tetrodotoxin-sensitive and tetrodotoxin-resistant currents, respectively. The curves were drawn according to the Boltzmann equation (see text). n is 7 for each type of Na^+ current.

and k_g by 0.31 ± 0.25 mV. The spontaneous shifts in $V_{g_{0.5}}$ and k_g for the time period of 3 min were -3.3 ± 0.7 and -0.09 ± 0.12 mV ($n = 5$), respectively. The difference between the values of $V_{g_{0.5}}$ was statistically significant ($P < 0.01$) but that of k_g was not.

4. Discussion

The analgesic mechanism of tricyclic antidepressants is not completely understood. Since tricyclic antidepressants affect so many biological systems, a complex interplay between them may be important for their analgesic action. One of the possible mechanisms for tricyclic antidepressant-induced analgesia is their action on α_2-adrenoceptors. Clonidine, an α_2-adrenoceptor agonist, has been used for pain control. The analgesia induced by tricyclic antidepressants as well as clonidine was abolished by an α_2-adrenoceptor antagonist (Gray et al., 1999). It has been suggested that potentiation of the opioidergic system may be involved in tricyclic antidepressant-induced analgesia. Opioid antagonists, naloxone and naltrindole, attenuated the analgesic effect of tricyclic antidepressants in experimental pain models. Moreover acetorphan, an enkephalin catabolism inhibitor, potentiated the tricyclic antidepressant-induced analgesia (Gray et al., 1998). Ion channels are also involved in tricyclic antidepressant-induced analgesia. An antisense oligonucleotide to mKv 1.1, a mouse Shaker-like K^+ channel, abolished tricyclic antidepressant-induced analgesia in mice (Galeotti et al., 1997). Anticonvulsants (carbamazepine, phenytoin) and antiarrhythmic agents (lidocaine, mexiletine) with Na^+ channel blocking activity have been used for pain relief in various conditions. In this context, the inhibitory action of tricyclic antidepressants on Na^+ channels may play a role in their analgesic action.

The cardiac toxicity of tricyclic antidepressants, such as electrocardiographic changes and conduction disturbances, is known to be caused by their ability to block cardiac Na^+ channels (Ogata and Narahashi, 1989). Amitriptyline reduced the cardiac Na^+ current in a use-dependent manner by acting on the same binding site for lidocaine but not on the binding site for phenytoin (Barber et al., 1991). Neuronal Na^+ channels are also blocked by tricyclic antidepressants. Amitriptyline-inhibited veratridine- or scorpion toxin evoked Na^+ influx in rat striatal slices (Ishii and Sumi, 1992). In bovine adrenal chromaffin cells, amitriptyline inhibited the Na^+ current, with no change in the rate or voltage dependence of Na^+ channel activation, and caused a hyperpolarizing shift of the steady-state inactivation curve (Pancrazio et al., 1998). The same article also showed the amitriptyline block of Na^+ currents in rat dorsal root ganglion neurons for reference and implied that Na^+ channels from two different tissues would be modulated by amitriptyline in a similar fashion. However, there are two types of Na^+ channels, tetrodotoxin-sensitive and tetrodotoxin-resistant, in rat dorsal root ganglion neurons, and they differ in many respects, such as channel activation/inactivation kinetics and pharmacological profiles. So we decided to evaluate the differential action of amitriptyline on the two types of Na^+ channels in rat dorsal root ganglion neurons.

Multiple components of tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ currents are known to be expressed in rat dorsal root ganglion neurons. For instance, Van den Berg et al. (1996) reported the existence of three tetrodotoxin-sensitive Na^+ current components and two tetrodotoxin-resistant Na^+ current components, which were distinguished by differences in $V_{h_{0.5}}$. Others, however, recorded a Na^+ current composed of single components of tetrodotoxin-sensitive and tetrodotoxin-resistant currents (Roy and Narahashi,1992; Ogata and Tatebayashi, 1993; Scholz et al., 1998). The discrepancy may arise from the different experimental settings used. In fact, the emergence of multiple components is known to be affected by many factors, such as the inclusion of serum or nerve growth factor in the culture media, culture duration, and the developmental stages of rat (Omri and Meiri, 1990;

Schwartz et al., 1990; Caffrey et al., 1992; Van den Berg et al., 1996). In our experimental setting, the tetrodotoxin-sensitive and the tetrodotoxin-resistant Na^+ currents exhibited only a single component and, thus, the discussion is confined to these currents.

Amitriptyline reduced the two types of Na^+ currents in rat dorsal root ganglion neurons in a dose-dependent manner. At a holding potential of −80 mV, which is near the resting membrane potential, the K_d values for amitriptyline inhibition of tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ currents were estimated to be 4.7 and 105 μM, respectively. The K_d value for amitriptyline blockade of Na^+ channels in bovine chromaffin cells and that for imipramine (another tricyclic antidepressant) blockade of Na^+ channels in guinea-pig cardiac myocytes are 20.2 μM (holding potential at −80 mV) and 0.35 μM (holding potential at −90 mV), respectively (Ogata and Narahashi, 1989; Pancrazio et al., 1998). Thus, at a resting membrane potential, tetrodotoxin-resistant Na^+ channels in rat dorsal root ganglion neurons are least sensitive to tricyclic antidepressants. The Hill coefficients were estimated to be 0.88 and 0.92 for tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels, respectively, suggesting a single binding site for amitriptyline. This result is consistent with data for bovine chromaffin cells treated with amitriptyline, but different from the result observed in guinea-pig cardiac myocytes, where two imipramine molecules interacted with one Na^+ channel.

In both types of Na^+ channels in dorsal root ganglion neurons, amitriptyline caused a shift of the steady-state inactivation curve in the hyperpolarizing direction in a dose-dependent manner. The shift was more pronounced for tetrodotoxin-sensitive than tetrodotoxin-resistant Na^+ channels. Amitriptyline at 20 μM shifted the curve for the tetrodotoxin-sensitive Na^+ channels by −16 mV, which is comparable to the −15 mV shift observed in Na^+ channels of bovine chromaffin cells. Amitriptyline increased the values for the slope factor in the two types of Na^+ channels in dorsal root ganglion neurons while no effect was observed in Na^+ channels of bovine chromaffin cells.

When cells were held at a more negative potential, which was enough to remove the effect of amitriptyline on the steady-state inactivation, K_d values for amitriptyline blockade of tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels were increased to 181 and 193 μM, respectively. Therefore, the affinity of amitriptyline for both types of Na^+ channels is almost the same if the channels are free of inactivation. The large difference in K_d values between the two types of channels observed at the holding potential of −80 mV is due to the fact that the steady-state inactivation curve for tetrodotoxin-sensitive Na^+ channels lies at more negative potentials than the curve for tetrodotoxin-resistant Na^+ channels.

The shift of the inactivation curve and the increased K_d value at a large negative holding potential suggest a higher affinity of amitriptyline for the inactivated state of the channel than for the resting state, as postulated in the modulated receptor theory (Hille, 1977). The apparent dissociation constant for amitriptyline to block Na^+ channels in the inactivated state, K_I, was estimated from K_R (K_d in the resting state; 181 μM for tetrodotoxin-sensitive channel, 193 μM for tetrodotoxin-resistant channel) and the shift in $V_{h_{0.5}}$ ($\Delta V_{h_{0.5}}$), by the following equation assuming 1 to 1 stoichiometry for drug-receptor binding (Bean et al., 1983): $\Delta V_{h_{0.5}} = k_h \ln\{(1 + [\text{Amitriptyline}]/K_R)/(1 + [\text{Amitriptyline}]/K_I)\}$, where k_h is the slope factor for the inactivation curve and [Amitriptyline] is the drug concentration. The K_I values for tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels were estimated to be 3 and 10 μM, respectively. Thus, compared to the resting state, amitriptyline has a much higher affinity for the inactivated state of the channel by a factor of 60 for tetrodotoxin-sensitive channels and 19 for tetrodotoxin-resistant channels. In line with this, the use-dependent block of Na^+ currents by amitriptyline can be attributable to its slow dissociation from the inactivated channel (Ragsdale et al., 1994).

Amitriptyline also affected Na^+ channel activation. The conductance–voltage relationship curves for both types of Na^+ channels were shifted in the depolarizing direction by amitriptyline. The result is in contrast to that for Na^+ channels of bovine chromaffin cells, where amitriptyline had no effect on the activation process of the channel.

Na^+ channels in dorsal root ganglion neurons are implicated in pain perception. After nerve injury, which causes neuropathic pain, downregulation of tetrodotoxin-resistant Na^+ channels and upregulation of rapidly repriming tetrodotoxin-sensitive Na^+ channel isoform are observed (Cummins and Waxman, 1997). The decrease in tetrodotoxin-resistant Na^+ channel density is explained by the translocation of the channels from neuronal somata to peripheral axons, with subsequent accumulation at the site of injury (Novakovic et al., 1998). The involvement of tetrodotoxin-resistant Na^+ channels in nociception is further supported by the fact that they are expressed predominantly in the capsaicin-sensitive small neurons, and that hyperalgesic agents increase tetrodotoxin-resistant Na^+ currents (Arbuckle and Docherty, 1995; Gold et al., 1996). A null-mutant mouse for tetrodotoxin-resistant Na^+ channels showed analgesia to noxious mechanical stimuli and inflammatory hyperalgesia (Akopian et al., 1999). The present study showed that amitriptyline inhibited two types of Na^+ channels by acting preferentially on the inactivated state of the channels, and by causing a depolarizing shift in the activation voltage. The effect was more pronounced in tetrodotoxin-sensitive than in tetrodotoxin-resistant Na^+ channels and was in many aspects similar to the action of phenytoin and carbamazepine on these channels (Song et al., 1996). Thus, the block of tetrodotoxin-sensitive Na^+ channels may contribute to amitriptyline-induced analgesia. However, it should be noted that the concentration of amitriptyline to block Na^+ channels is higher than the

usual therapeutic plasma concentration, which is less than 1 μM (Baldessarini, 1996). Thus, Na^+ channel blockade does not seem to be the sole contributor to amitriptyline-induced analgesia.

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