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Differential Action of Riluzole on Tetrodotoxin-Sensitive and Tetrodotoxin-Resistant Sodium Channels[1]

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ABSTRACT

The effects of riluzole, a neuroprotective drug, on tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels in rat dorsal root ganglion neurons were studied using the whole-cell patch clamp technique. At the resting potential, riluzole preferentially blocked TTX-S sodium channels, whereas at more negative potentials, it blocked both types of sodium channels almost equally. The apparent dissociation constants for riluzole to block TTX-S and TTX-R sodium channels in their resting state were 90 and 143 μM, respectively. Riluzole shifted the voltage dependence of activation of TTX-R sodium channels in the depolarizing direction more than that of TTX-S sodium channels. The voltage dependence of the fast inactivation of both types of sodium channels was shifted in the hyperpolarizing direction in a dose-dependent manner, and the apparent dissociation constants for riluzole to block the inactivated channels were estimated to be 2 and 3 μM for the TTX-S and TTX-R sodium channels, respectively, indicating a much higher affinity for the inactivated channels than for the resting channels. Riluzole was equally effective in blocking both types of sodium channels in their slow inactivated state. Since more TTX-S channels are inactivated than TTX-R channels at the resting potential, riluzole blocks TTX-S sodium channels more potently than TTX-R sodium channels. It was concluded that one of the mechanisms by which riluzole exerts its neuroprotective action is to preferentially block the inactivated sodium channel of damaged or depolarized neurons under ischemic conditions, thereby suppressing excess stimulation of the glutamatergic receptors and massive influx of Ca^{++}.

Riluzole is a neuroprotective drug that has antiischemic, sedative and antiepileptic properties (Malgouris *et al.*, 1989; Romettino *et al.*, 1991; Pratt *et al.*, 1992; Bryson *et al.*, 1996), and is effective in slowing the progression of amyotrophic sclerosis (Bensimon *et al.*, 1994; Couratier *et al.*, 1994; Bryson *et al.*, 1996). Riluzole is thought to exert its neuroprotective effects by blocking both voltage-gated sodium channels and NMDA-receptor-mediated responses thereby preventing excess calcium influx into neurons (Hubert *et al.*, 1994; Malgouris *et al.*, 1994). Various other sodium channel blocking agents such as phenytoin, carbamazepine and lamotrigine are known to protect neurons from cerebral ischemia, hypoxia or head trauma (Taylor and Meldrum, 1995). Riluzole inhibits the glutamate-induced release of aspartate from cerebellar granule cells via a pertussis toxin-sensitive GTP-binding protein, and reduces the release of glutamate in caudate nucleus and hippocampal slices (Cheramy *et al.*, 1992; Doble *et al.*, 1992; Martin *et al.*, 1993). Riluzole does not interact with any of the known ligand recognition sites on either kainate or NMDA receptor in radioligand binding studies, but blocks the activity of these receptor-channels in a noncompetitive manner (Debono *et al.*, 1993).

The effects of riluzole in blocking sodium channel currents have been found in frog nodes of Ranvier and cloned rat brain IIA sodium channel α subunits expressed in *Xenopus* oocytes (Benoit and Escande, 1991; Hebert *et al.*, 1994). In these preparations, riluzole specifically blocks inactivated sodium channels without affecting the time course of inactivation nor the current-voltage relationship.

Most sodium channels can be blocked by TTX with a K_d ranging from 1 to 10 nM. The sodium channels that are resistant to TTX block have been found in various tissues and in different animal species (see review by Yoshida, 1994). DRG neurons are endowed with both TTX-S and TTX-R sodium channels (Kostyuk *et al.*, 1981; Roy and Narahashi, 1992; Elliott and Elliott, 1993; Ogata and Tatebayashi, 1993). Besides their differences in TTX sensitivity, they differ in pharmacological profiles. For example, TTX-R channels are less sensitive to lidocaine, but much more sensitive to lead, cadmium and pyrethroid insecticides than are TTX-S channels (Roy and Narahashi, 1992; Tatebayashi and Narahashi, 1994). These two types of sodium channels exhibit different activation and inactivation kinetics as well. Notably, TTX-R sodium channels have slower kinetics of activa-

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ABBREVIATIONS: TTX-S, tetrodotoxin-sensitive; TTX-R tetrodotoxin-resistant; NMDA, N-methyl-D-asparate; DRG, dorsal root ganglion; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; DMSO, dimethylsulfoxide; RZ, riluzole; GTP, guanosine 5′-triphosphate.

tion and inactivation and activate and inactivate at more positive potentials than TTX-S sodium channels.

Our study was undertaken to examine the differential effect of riluzole on TTX-S and TTX-R sodium channels using rat DRG neurons as a model. This preparation was chosen because the differential sensitivity of TTX-S and TTX-R sodium channels to riluzole provided us with an excellent model with which detailed mechanisms of action of riluzole could be elucidated. Riluzole has a much higher affinity for channels in the inactivated state than in the resting state of either TTX-S or TTX-R sodium channels. This difference in affinity accounts for the higher potency of riluzole to block TTX-S sodium channels than TTX-R sodium channels as TTX-S sodium channels are inactivated more at resting membrane potentials than TTX-R sodium channels.

Material and Methods

Cell preparations. DRG neurons were isolated as described previously (Roy and Narahashi, 1994; Tatebayashi and Narahashi, 1994). Rats (2–6 days postnatal, either sex) were anesthetized with methoxyflurane 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 (GIBCO BRL, Grand Island, NY) containing trypsin (2.5 mg/ml, type XI, Sigma Chemical Co., St. Louis, MO) at 37°C for 25 min. After enzyme treatment, ganglia were rinsed with Dulbecco's modified Eagle medium (GIBCO BRL) supplemented with newborn calf serum (10%, v/v, GIBCO BRL) and gentamicin (80 μg/ml, Northwestern University Lurie Cancer Center). Single cells were mechanically dissociated with a fire-polished Pasteur pipette and plated on poly-L-lysine-coated glass coverslips. Cells were incubated for 2 to 7 hr before patch clamp experiments.

TTX (0.2 μM) was used to separate TTX-R sodium currents from TTX-S sodium currents. For the study of TTX-S sodium channels, cells that expressed only TTX-S sodium currents were used. TTX-S sodium currents were completely inactivated at the end of a 5-msec depolarizing pulse to 0 mV, although TTX-R currents were still present. Thus, the difference in kinetics was used to identify the type of sodium current.

Electrophysiological recording. Currents were recorded using the whole-cell patch clamp technique (Hamill *et al.*, 1981). Suction pipettes (0.6–1.2 MΩ) were made of borosilicate glass capillary tubes (1.5–1.8 mm inner diameter, Kimble, Vineland, NJ) using a two-step vertical puller (Narishige, Tokyo, Japan). The pipette solution contained (in mM): CsF 135, NaCl 10 and HEPES 5. The pH was adjusted to 7.0 with CsOH and the osmolarity was 275 mOsmol. The external solution contained (in mM): NaCl 25, tetramethylammonium chloride 75, tetraethylammonium chloride 20, CsCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1.0, D-glucose 25, HEPES-acid 5. Lanthanum ($LaCl_3$, 3 μM) was used to block calcium channel current. The solution was adjusted to pH 7.4 with tetraethylammonium-OH and 290 mOsmol with sucrose. An Ag-AgCl pellet/3M KCl-agar bridge was used for the reference electrode. Membrane currents were recorded using an Axopatch 200 amplifier (Axon Instruments, Foster City, CA). Signals were digitized by a 14-bit analog-to-digital converter, filtered with a Bessel filter at 5 kHz and stored on a PDP 11/73 computer (Digital Equipment Corporation, Pittsburgh, PA). Series resistance was compensated 70 to 75%. Capacitative and leakage currents were digitally subtracted by using the P + P/4 procedure (Bezanilla and Armstrong, 1977). The liquid junction potential between internal and external solution was −4.7 mV on average. Our data shown in this paper were compensated for the liquid junction potential. All experiments were performed at 22 to 24°C.

Stock solutions of riluzole were made in DMSO at concentrations of 1 to 100 mM and diluted with external solution to the desired concentrations. The DMSO concentration in the perfusate was less than 0.1% (v/v), except when the riluzole concentration in the external solution was 300 μM (DMSO 0.3%). DMSO (up to 0.3%) had no effect on sodium currents when applied externally (data not shown).

Data analysis. Results are expressed as means ± S.E.M., and n represents the number of the cells examined. All figures represent typical examples from at least four independent experiments. Analyses of currents were achieved by using REV, a locally developed FORTRAN/IV program on the PDP 11/73 computer and SigmaPlot (Jandel Scientific, San Rafael, CA) on PC.

Results

Effects of riluzole on sodium channel currents. As has been reported previously, two types of sodium channels were found in rat DRG neurons (Kostyuk *et al.*, 1981; Roy and Narahashi, 1992; Elliott and Elliott, 1993; Ogata and Tatebayashi, 1993). TTX-S sodium currents activated and inactivated quickly and were completely blocked by 200 nM TTX, whereas the TTX-R sodium currents activated and inactivated slowly and were not blocked by 200 nM TTX. When the membrane was held at −80 mV, which was near the resting membrane potential (Song and Narahashi, 1995), TTX-S sodium currents were more sensitive to the blocking action of riluzole than TTX-R sodium currents. At 3 μM, riluzole blocked 50% of the TTX-S sodium current (fig. 1Aa), whereas it took 30 μM for riluzole to produce a similar block of the TTX-R sodium currents (fig. 1Ab). Both types of sodium currents were blocked within 3 min after bath application of the drug and the currents recovered within a few minutes after washout with drug-free external solution. When the peak current amplitude in the presence of riluzole was normalized to the control value, the activation and inactivation kinetics were not changed by riluzole in TTX-S sodium current (fig. 1Ba), whereas the time course of inactivation of TTX-R sodium current was greatly accelerated by riluzole (fig. 1Bb).

The voltage dependence of the steady-state inactivation is

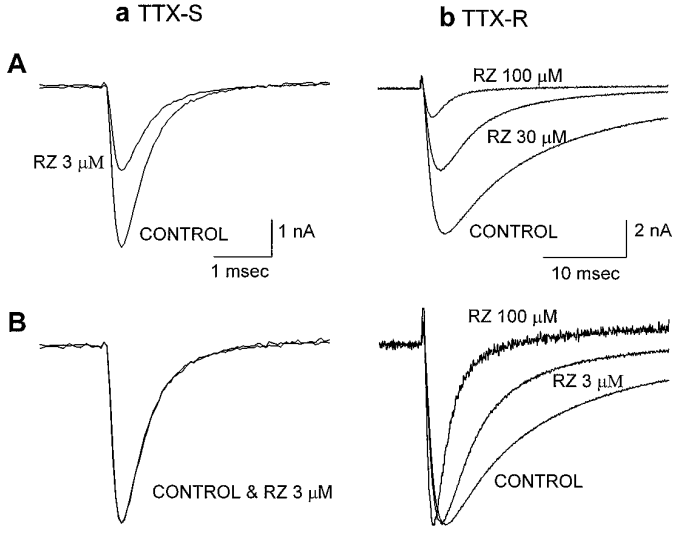

Fig. 1. Effects of riluzole (RZ) on sodium channel currents of rat DRG neurons. Currents were evoked by depolarizing steps to 0 mV from a holding potential of −80 mV. a, TTX-S sodium channel. b, TTX-R sodium channel. A, Riluzole blocks TTX-S sodium channel currents more potently than TTX-R sodium channel currents when the membrane was held at −80 mV. B, Peak current amplitude in the presence of riluzole is normalized to the control current.

different between the two types of sodium channels (Roy and Narahashi, 1992; Tatebayashi and Narahashi, 1994). Because the steady-state inactivation for TTX-S sodium channels occurs at more negative potentials than that for TTX-R sodium channels, and because riluzole is known to have a higher affinity for the inactivated state than for the resting state of TTX-S sodium channels in other preparations (Benoit and Escande, 1991; Hebert *et al.*, 1994), the apparent difference in the potency of riluzole block of TTX-S and TTX-R sodium channels in DRG neurons could be due to their different inactivation characteristics.

To examine this hypothesis, the membrane was held at large negative potentials, *i.e.*, −120 mV for TTX-S and −100 mV for TTX-R channels. Under these conditions, riluzole blocked both types of sodium channels to almost the same degree (figs. 2A and 3A). Riluzole had no effect on the time course of TTX-S sodium currents evoked at 0 mV, as evidenced from superimposed currents in the presence and absence of drug (fig. 2Bb). These results suggest that riluzole does not alter either activation or inactivation of TTX-S sodium channel currents at 0 mV (fig. 2Bb). However, it slightly accelerated the inactivation time course of TTX-S sodium currents at −20 mV (fig. 2Ba). The time course of TTX-R sodium currents was greatly accelerated by riluzole at all membrane potentials tested; this effect was more pronounced at negative potentials than at positive potentials (fig. 3Ba, b and c). The small outward currents seen in riluzole (fig. 3, a and b) may de due to an artifact of the P+P/4 procedure since the potassium channel was blocked by internal cesium.

Effect of riluzole on the time constant of inactivation. The decay of sodium currents was fitted to a single exponential function and the time constants are plotted as a function of membrane potential in figure 4. In TTX-S sodium channels, riluzole reduced the time constant of inactivation at potentials more negative than −25 mV but had little or no effect at potentials more positive than −25 mV (fig. 4A).

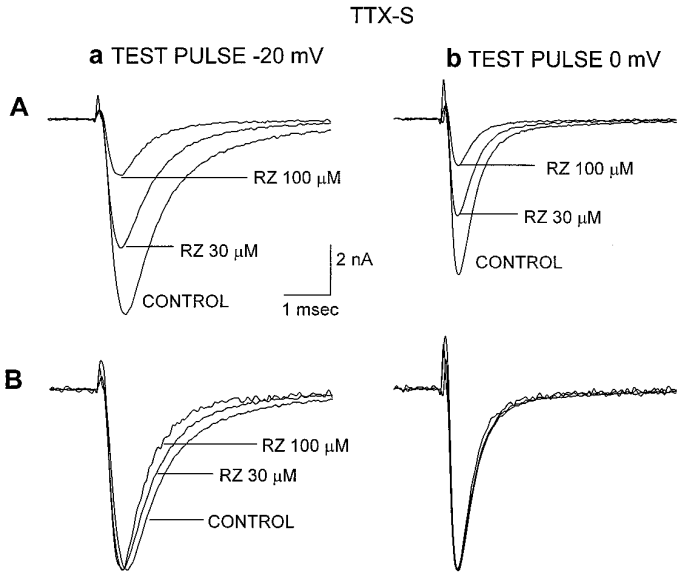

Fig. 2. Effects of riluzole on TTX-S sodium channel currents evoked by depolarizing steps to either −20 mV (a) or 0 mV (b) from a holding potential of −120 mV. A, Riluzole at 30 and 100 μM blocks the TTX-S sodium currents dose dependently. B, Peak current amplitude in the presence of riluzole is normalized to the control current.

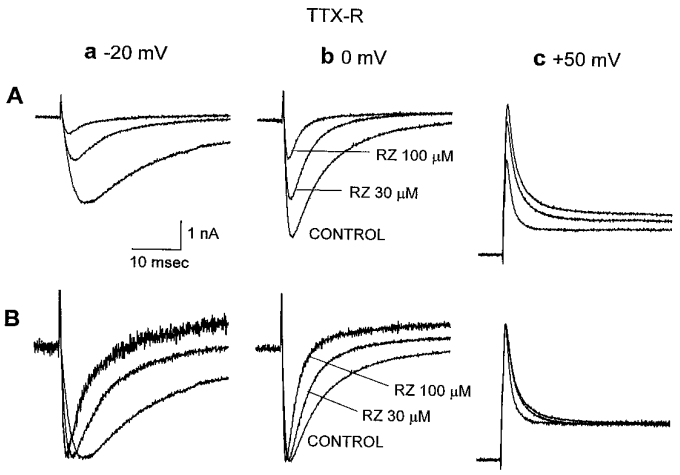

Fig. 3. Effects of riluzole on TTX-R sodium channel currents evoked by depolarizing steps to −20 mV (a), 0 mV (b), or +50 mV (c) from a holding potential of −100 mV. A, Riluzole at 30 and 100 μM blocks the TTX-R sodium currents dose dependently. B, Peak current amplitude in the presence of riluzole is normalized to the control current.

By contrast, the time constant of inactivation of TTX-R sodium channels was reduced by riluzole at potentials more negative than +20 mV, being more pronounced at more negative potentials (fig. 4B). The voltage dependence of inactivation time constant of TTX-R sodium channels appears to be shifted in the hyperpolarizing direction by riluzole. However, the difference between the effects on the two types of channels may arise from the differences in the inherent voltage dependence of the channels; as the rate of inactivation increases, the effectiveness of the drug diminishes.

Effects of riluzole on the kinetics of sodium channel activation. Effects of riluzole on the current-voltage relationship and the conductance-voltage curve are illustrated in figure 5 for TTX-S sodium channels and figure 6 for TTX-R sodium channels. As can be seen from the current-voltage curve, riluzole blocked TTX-S sodium currents to the same degree in the entire membrane potential range (fig. 5A). The membrane potential corresponding to half-maximum conductance ($Vg_{0.5}$) was −27.9 ± 1.4 mV (n = 4) for TTX-S channels. Riluzole at 30 and 100 μM shifted $Vg_{0.5}$ of TTX-S channels by 1.3 ± 1.5 and 5.0 ± 1.8 mV (n = 4), respectively, in the depolarizing direction (fig 5B; table 1). The slope factor (kg) for the conductance-voltage curve was increased by riluzole or the slope became less steep after application of riluzole (table 1).

Riluzole blocked TTX-R sodium currents more in the negative voltage range than in the positive voltage range (fig. 6A), resulting in a great shift in the conductance-voltage curve in the depolarizing direction (fig. 6B). The half-maximum activation of TTX-R sodium channels occurred at −16.6 ± 1.3 mV (n = 4). Riluzole at 30 and 100 μM shifted $Vg_{0.5}$ by 8.7 ± 0.1 and 18.5 ± 0.1 mV (n = 4), respectively, in the depolarizing direction (fig. 6B, table 1). The slope factor (kg) for the conductance-voltage curve was also affected by riluzole, becoming larger (table 1).

The apparent dissociation constant for riluzole block of sodium channels in the resting state. To estimate the apparent dissociation constant for riluzole to block sodium channels in the resting state (K_R), the membrane was held at −120 mV for TTX-S sodium channels and −100 mV

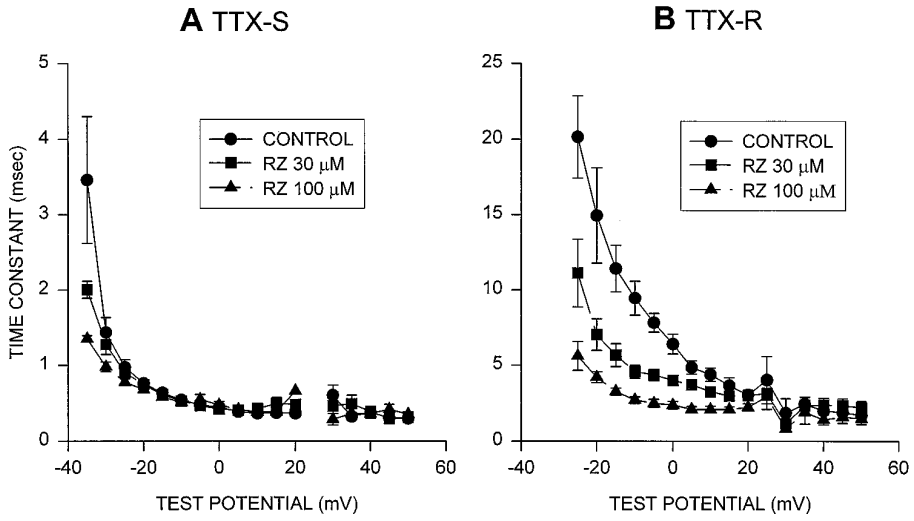

Fig. 4. Effect of riluzole on the time course of sodium channel inactivation. The decaying phases of current traces were fitted by a single exponential function, and the time constants in the absence (●) and presence of 30 μM (■) or 100 μM (▲) riluzole are plotted as a function of test potentials. A, TTX-S sodium channels. Currents were evoked by 10-msec step depolarizations to various levels (ranging from −35 to +50 mV in 5 mV increments) from a holding potential of −120 mV (n = 4). B, TTX-R sodium channels. Currents were evoked by 40 msec step depolarizations to various levels (ranging from −25 to +50 mV in 5 mV increments) from a holding potential of −100 mV (n = 4).

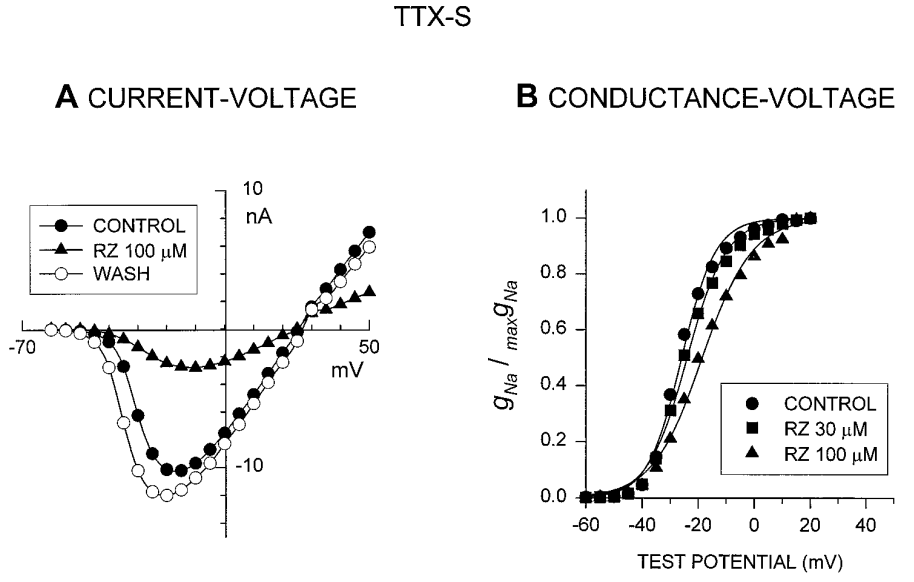

Fig. 5. A, The current-voltage relationship for TTX-S sodium channels in the absence (●) and presence (▲) of 100 μM riluzole, and after washout with riluzole-free solution (○). Currents were evoked by 10-msec depolarizing steps to various levels from a holding potential of −120 mV. Test potentials ranged from −60 to +50 mV in 5-mV increments and were delivered at a frequency of 0.2 Hz. B, The conductance-voltage relationship for TTX-S sodium channels in the absence (●) and presence of 30 μM (■) and 100 μM (▲) riluzole. The conductance (g_{Na}) was calculated according to the equation, $g_{Na} = I_{Na}/(Vg\text{-}V_r)$, where I_{Na} is the peak amplitude of sodium current, Vg is the test potential, and V_r is the reversal potential for sodium. The curves are drawn according to the equation, $g_{Na}/_{max}g_{Na} = 1/\{1 + \exp[(Vg_{0.5} - Vg)/kg]\}$, where $_{max}g_{Na}$ is the maximum value for g_{Na} $Vg_{0.5}$ is the potential at which g_{Na} is 0.5 $_{max}g_{Na}$, *and kg* is the slope factor (potential required for an *e*-fold change) (n = 4).

TABLE 1

Effects of riluzole on Boltzmann parameters of sodium channel activation

	Riluzole (μM)	kg (mV)	$Vg_{0.5}$ (mV)	$\Delta Vg_{0.5}$ (mV)
	0	5.8 ± 0.4	−27.9 ± 1.4	
TTX-S	30	7.0 ± 0.2	−26.6 ± 1.1	1.3 ± 1.5
	100	8.0 ± 0.3	−22.9 ± 1.6	5.0 ± 1.8
	0	6.0 ± 0.6	−16.6 ± 1.3	
TTX-R	30	9.6 ± 0.8	−7.9 ± 1.4	8.7 ± 0.1
	100	11.9 ± 0.5	1.9 ± 0.5	18.5 ± 0.1

Values are means ± S.E.M. (n = 4). kg is the slope factor (potential required for an *e*-fold change), $Vg_{0.5}$ is the membrane potential for the half-maximum conductance and $\Delta Vg_{0.5}$ *is the shift in* $Vg_{0.5}$ relative to control value.

for TTX-R sodium channels. At these potentials sodium channel inactivation was completely removed. To minimize errors due to the shift in conductance-voltage curve by riluzole, currents were elicited by depolarizing steps to +50 mV for both types of sodium channels to elicit the maximum conductance. The percentage of current inhibition is plotted as a function of riluzole concentration in figure 7. The dose-response data were fitted to the Hill equation with K_R values of 90 and 143 μM, and Hill coefficients of 1.12 and 1.15, for the TTX-S and TTX-R sodium channels, respectively.

Effects of riluzole on the fast sodium channel inactivation. The effects of riluzole on the fast steady-state inactivation curves of both TTX-S and TTX-R sodium channels are shown in figure 8. Prepulses of 150-msec duration were used. Riluzole greatly shifted the inactivation curves of both types of sodium channels in the hyperpolarizing direction. In control experiments, the half-maximum inactivation potential ($Vh_{0.5}$) was estimated to be −61.2 ± 2.2 mV for TTX-S channels and −27.3 ± 1.0 mV for TTX-R channels (n = 4). Riluzole shifted the curve in the hyperpolarizing direction, with a greater shift occurring in TTX-S channels than TTX-R channels at the same concentration of riluzole. However, riluzole did not change the slope factor, kh, in either type of sodium channels. The riluzole-induced changes in parameters of sodium channel inactivation are given in table 2.

The apparent dissociation constant for riluzole block of sodium channels in the inactivated state. The apparent dissociation constant for riluzole to block sodium channels in the inactivated state, K_I, was estimated from K_R and the shift in $Vh_{0.5}$. As shown in figure 9, the shift in $Vh_{0.5}$ is plotted as a function of riluzole concentration. The data are plotted along with three lines with different parameters ac-

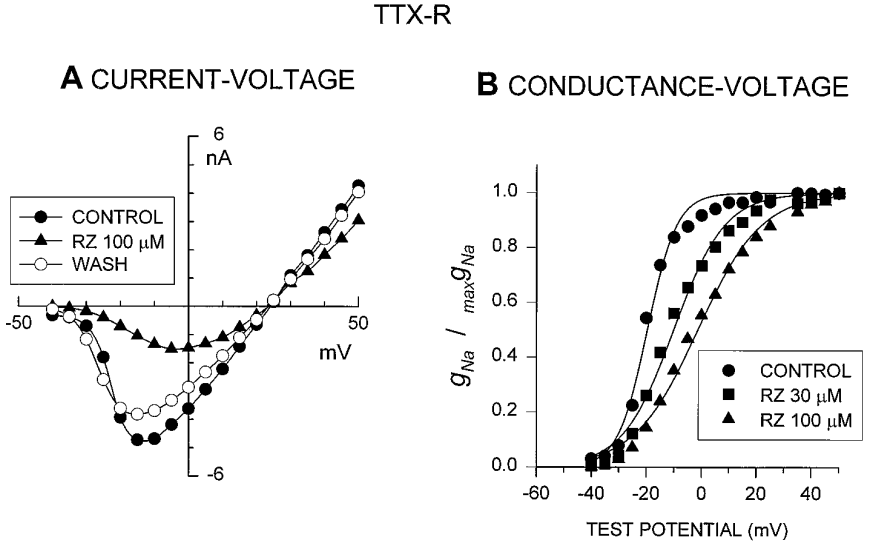

Fig. 6. A, The current-voltage relationship for TTX-R sodium channels in the absence (●) and presence (▲) of 100 μM riluzole, and after washout with riluzole-free solution (○). Currents were evoked by 40-msec depolarizing steps to various levels from a holding potential of −100 mV. Test potentials ranged from −40 to +50 mV in 5 mV increments and were delivered at a frequency of 0.2 Hz. B, The conductance-voltage relationship for TTX-R sodium channels in the absence (●) and presence of 30 μM (■) and 100 μM (▲) riluzole. The method to determine the sodium channel conductance is the same as that for figure 5 (n = 4).

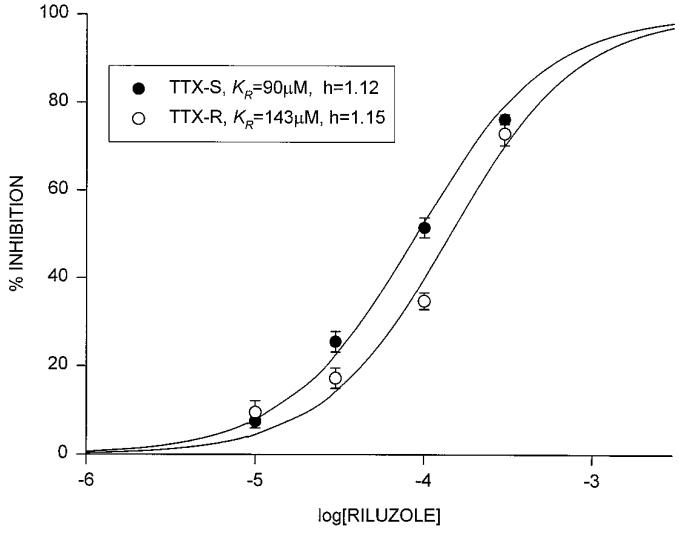

Fig. 7. Dose-response relationships for the riluzole block of both TTX-S (●) and TTX-R (○) sodium channel currents in the resting state. Currents were evoked by depolarizing steps (10 msec for TTX-S and 40 msec for TTX-R) to +50 mV from large negative holding potentials (−120 mV for TTX-S and −100 mV for TTX-R). The percentage of the block is plotted as a function of the riluzole concentration. The dose-response data were fitted to the Hill equation, %inhibition = $100/\{1 + (K_R/[RZ])^h\}$, where [RZ] and K_R represent the concentration of riluzole and apparent dissociation constant for riluzole block of sodium channels in the resting state, respectively, and h represents the Hill coefficient (n = 4).

cording to the equation, $\Delta Vh_{0.5} = kh\ln\{(1 + [RZ]/K_R)/(1 + [RZ]/K_I)\}$ (Bean *et al.*, 1983). Curves were best fitted when K_I values for TTX-S and TTX-R sodium channels are 2.0 and 3.0 μM, respectively. Thus, compared to the resting state, riluzole has a much higher affinity for the inactivated state of both types of sodium channels.

Effect of riluzole on the slow steady-state inactivation of sodium channels. To examine whether different inactivation states of sodium channels affects the riluzole's effect, long pre-pulse duration (20 sec) was given to measure the slow inactivation. In the absence of riluzole, $Vh_{0.5}$ was estimated to be −77.0 ± 1.8 mV (n = 6) and −47.6 ± 1.3 mV (n = 5) for TTX-S and TTX-R sodium channels, respectively. These values were 16 and 20 mV more negative for TTX-S and TTX-R channels, respectively, than those of fast inactivation using 150 msec prepulse. $Vh_{0.5}$ was shifted by −8.5 ± 1.0 mV (n = 6) by 3 μM riluzole in TTX-S channels and 15.6 ± 0.7 mV by 30 μM riluzole in TTX-R channels (fig. 10; table 2). Both shifts were comparable to those obtained for fast inactivation curve.

Discussion

Our study demonstrated that riluzole at low concentrations preferentially blocked both TTX-S and TTX-R sodium channels of rat DRG neurons in their inactivated state although it had much less effect on the channels in the resting state. Riluzole blocked both TTX-S and TTX-R sodium channels to nearly the same extent under the experimental conditions where the inactivation of two types of channels was minimal.

The apparent dissociation constants for riluzole to block sodium channels in their resting states were estimated to be 90 and 143 μM for the TTX-S and TTX-R sodium channels, respectively. These values are in the same order of magnitude as those previously reported. In frog nodes of Ranvier and rat brain IIA sodium channel α-subunits expressed in *Xenopus* oocytes, K_R values were estimated to be 90, and 30 μM, respectively (Benoit and Escande, 1991; Hebert *et al.*, 1994).

Riluzole shifted the steady-state inactivation curves to the same extent with either 150 msec or 20 sec prepulse. Therefore, riluzole does not distinguish between the fast and slow inactivated states of sodium channels, and blocks both inactivated states almost equally. The apparent dissociation constants for riluzole to block the sodium channels in the fast inactivated state were estimated from the concentration-dependent shift in the steady-state inactivation curve. The K_I values were 2 and 3 μM for the TTX-S and TTX-R sodium channels, respectively. These values are considerably smaller than those for blocking the resting channels. However, our estimates of the K_I values are almost 10 times larger than those previously reported in other preparations (Benoit and Escande, 1991; Hebert *et al.*, 1994).

Inasmuch as riluzole does not exhibit much difference in blocking TTX-S and TTX-R sodium channels at either resting

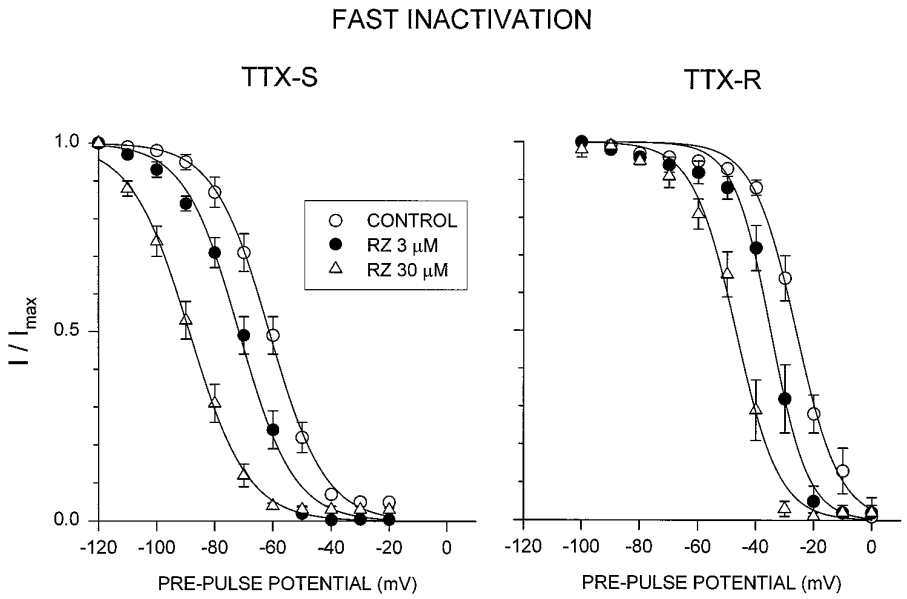

Fig. 8. Effects of riluzole on the fast steady-state inactivation curves for TTX-S and TTX-R sodium channels. The pre-pulse potential was changed to various levels (−120 to −20 mV for TTX-S channel and −100 to 0 mV for TTX-R channel in 10 mV increments) for 150 msec from a holding potential of −100 mV, and was immediately followed by a 5-msec step depolarization to 0 mV. Protocol was run at a frequency of 0.2 Hz. The peak amplitude of sodium current normalized to its respective maximum value is plotted as a function of the pre-pulse potential. The curves are drawn according to the equation $I/I_{max} = 1/\{1 + \exp[(Vh - Vh_{0.5})/kh]\}$, where *Vh* is prepulse potential, $Vh_{0.5}$ is the potential at which I is 0.5 I_{max}, and kh is the slope factor (potential required for an e-fold change) (n = 4).

TABLE 2
Effects of riluzole on Boltzmann parameters of sodium channel inactivation

	Prepulse	Riluzole	*kh*	$Vh_{0.5}$	$\Delta Vh_{0.5}$
		μM	*mV*	*mV*	*mV*
TTX-S	150 msec	0	9.3 ± 0.7	−61.2 ± 2.2	
	(*n* = 4)	1	9.0 ± 0.5	−65.7 ± 2.0	−3.9 ± 0.3
		3	9.7 ± 0.3	−71.5 ± 2.0	−8.3 ± 0.7
		10	10.1 ± 0.1	−78.8 ± 1.8	−14.4 ± 1.3
		30	9.7 ± 0.1	−88.9 ± 2.2	−23.9 ± 1.6
	20 sec	0	8.6 ± 0.5	−77.0 ± 1.8	
	(*n* = 6)	3	7.7 ± 0.3	−85.5 ± 2.3	−8.5 ± 1.0
TTX-R	150 msec	0	6.9 ± 0.7	−27.3 ± 1.0	
	(*n* = 4)	1	6.6 ± 0.7	−31.2 ± 1.8	−2.4 ± 1.0
		3	6.2 ± 0.6	−35.0 ± 2.3	−5.1 ± 1.3
		10	6.1 ± 0.6	−40.0 ± 2.0	−8.8 ± 1.0
		30	7.2 ± 0.7	−47.0 ± 2.4	−15.1 ± 1.4
	20 sec	0	5.5 ± 0.5	−47.6 ± 1.3	
	(*n* = 5)	30	6.3 ± 0.4	−63.2 ± 0.8	−15.6 ± 0.7

Values are means ± S.E.M. *kh* is the slope factor (potential required for an e-fold change), $Vh_{0.5}$ is the membrane potential for the half-maximum steady-state sodium channel inactivation, and $\Delta Vh_{0.5}$ is the shift in $Vh_{0.5}$ relative to control value.

or inactivated state, how can one explain the differential block of TTX-S and TTX-R channels by riluzole near the resting membrane potential? When the membrane is held at −80 mV, 61% of TTX-S channels and 95% of TTX-R channels are available for activation (fig. 10). More TTX-S channels are in the inactivated state than the TTX-R channels. Thus, riluzole preferentially blocks the TTX-S sodium channels. Because the resting membrane potential of DRG neurons with either type of sodium channels is around −80 mV (Song and Narahashi, 1995), it is expected that TTX-S sodium channels experience more block than TTX-R sodium channels by the same concentration of riluzole. The percentage of riluzole block of sodium channels at a given membrane potential can be estimated from the following equation (Hebert *et al.*, 1994):

$$[1-(I_{RZ}/I_{control})] \times 100 = 100/\{1 + (h_\infty \times [RZ]/K_R) + [(1 - h_\infty) \times [RZ]/K_I]\}$$

where I_{RZ} is the current amplitude in the presence of riluzole, $I_{control}$ is the control current amplitude, h_∞ is the availability of sodium channels for activation at a given membrane potential, and [RZ] is the concentration of riluzole. At the resting membrane potential of −80 mV, riluzole at 10 and 30 μM blocks 67 and 86% of TTX-S sodium channels, respectively, although the same concentrations of riluzole block only 19 and 41% of TTX-R sodium channels, respectively.

Riluzole greatly accelerated the time course of inactivation of sodium channels in rat DRG neurons especially in TTX-R sodium channels. This is a unique phenomenon because it is not observed in the TTX-S sodium channels in DRG neurons, frog nodes of Ranvier, or rat brain IIA sodium channel α subunit expressed in *Xenopus* oocytes (Benoit and Escande, 1991; Hebert *et al.*, 1994). The voltage dependence of inactivation time constant of TTX-R sodium channels appears to be shifted in the hyperpolarizing direction by riluzole. However, the difference between the effects on the two types of channels may arise from the differences in the inherent voltage dependence of the channels.

Another difference between two types of sodium channels was found with respect to the riluzole effect on the sodium

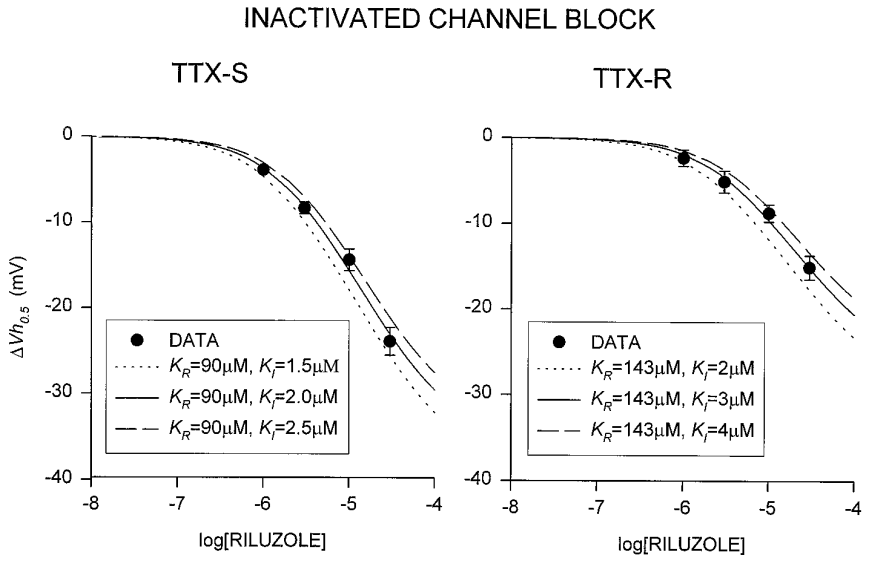

Fig. 9. Determination of K_i, the apparent dissociation constant for riluzole block of sodium channels in the inactivated state. $\Delta Vh_{0.5}$ determined from the fast steady-state inactivation curve is potted as a function of riluzole concentration (n = 4). See the text for explanation.

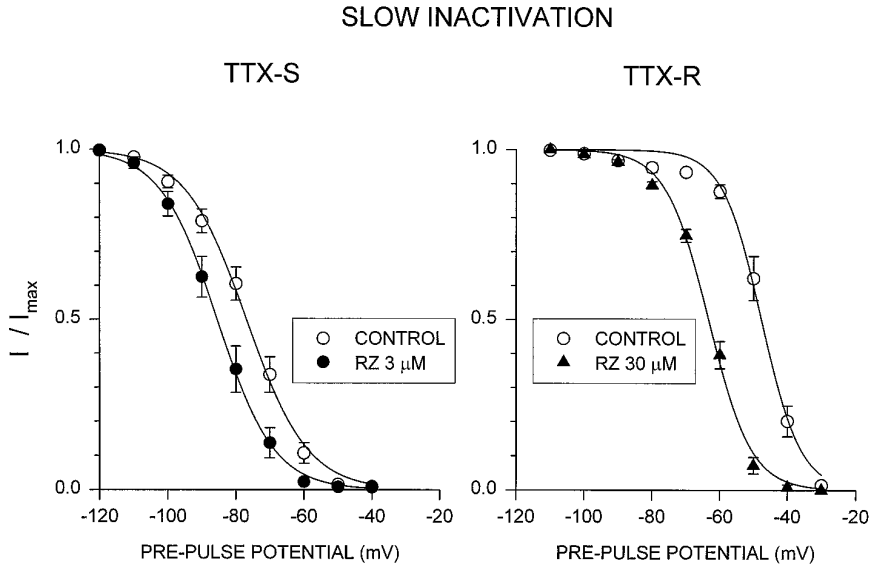

Fig. 10. Effects of riluzole on the slow steady-state inactivation curves for TTX-S and TTX-R sodium channels. The pre-pulse was changed to various levels (−120 to −40 mV for TTX-S channels and −110 to −30 mV for TTX-R channels in 10 mV increments) for 20 sec from a holding potential of −80 mV, and was immediately followed by a 5-msec step depolarization to 0 mV. Protocol was run at a frequency of 0.03 Hz. The peak amplitude of sodium current normalized to its respective maximum values are plotted as a function of the prepulse potential. Curves are drawn according to the same equation as described for figure 8 (n = 6 for TTX-S, n = 5 for TTX-R).

channel activation. Riluzole shifted the conductance-voltage curves for both TTX-S and TTX-R sodium channels in the depolarizing direction. However, the degree of shift was far greater for TTX-R sodium channels than for TTX-S sodium channels. Also the conductance curves became less steep after riluzole treatment and the effect was more pronounced in TTX-R sodium channels. The effects of riluzole on the sodium channel activation kinetics were not observed in other preparations (Benoit and Escande, 1991; Hebert *et al.*, 1994).

Ischemic conditions will cause gradual depolarization of neuronal membranes evoking repetitive discharges and glutamate release from nerve terminals that in turn stimulate the NMDA receptors. Massive calcium influx will ensue through the open NMDA receptor channels causing cell death. Because riluzole blocks the sodium channels much more potently in the inactivated state than in the resting state, it will effectively suppress the sodium channel activity and action potentials in the ischemic conditions, preventing cell death. Riluzole block of high voltage-gated N-type and P/Q-type calcium channels (Huang *et al.*, 1996, submitted for publication) and the NMDA receptor (Debono *et al.*, 1993) also contributes to neuroprotective activity. It should be noted that the plasma concentration of riluzole in healthy human volunteers is estimated to be 1.62 μM 1 hr after administration of 100 mg riluzole, a usual dose (Bryson *et al.*, 1996). This plasma concentration is in the same order of magnitude as the apparent dissociation constants of riluzole to block the TTX-S and TTX-R sodium channels in their inactivated state that are estimated to be 2 and 3 μM, respectively.

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