Intracellular QX-314 Causes Depression of Membrane Potential Oscillations in Lamprey Spinal Neurons During Fictive Locomotion

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Intracellular QX-314 causes depression of membrane potential oscillations in lamprey spinal neurons during fictive locomotion. J Neurophysiol 87: 2676–2683, 2002; 10.1152/jn.00685.2001. Spinal neurons undergo large cyclic membrane potential oscillations during fictive locomotion in lamprey. It was investigated whether these oscillations were due only to synaptically driven excitatory and inhibitory potentials or if voltage-dependent inward conductances also contribute to the depolarizing phase by using N-(2,6-dimethylphenyl carbamoylmethyl)triethylammonium bromide (QX-314) administered intracellularly during fictive locomotion. QX-314 intracellularly blocks inactivating and persistent Na+ channels, and in some neurons, effects on certain other types of channels have been reported. To detail the effects of QX-314 on Na+ and Ca2+ channels, we used dissociated lamprey neurons recorded under whole cell voltage clamp. At low intracellular concentrations of QX-314 (0.2 mM), inactivating Na+ channels were blocked and no effects were exerted on Ca2+ channels (also at 0.5 mM). At 10 mM QX-314, there was, however, a marked reduction of I\textsubscript{Na}. In the isolated spinal cord of the lamprey, fictive locomotion was induced by superfusing the spinal cord with Ringer’s solution containing N-methyl-D-aspartate (NMDA), while recording the locomotor activity from the ventral roots. Simultaneously, identified spinal neurons were recorded intracellularly, while infusing QX-314 from the microelectrode. Patch electrodes cannot be used in the intact spinal cord, and therefore “sharp” electrodes were used. The amplitude of the oscillations was consistently reduced by 20–25% in motoneurons (P < 0.05) and unidentified spinal neurons (P < 0.005). The onset of the effect started a few minutes after impalement and reached a stable level within 30 min. These effects thus show that QX-314 causes a reduction in the amplitude of membrane potential oscillations during fictive locomotion. We also investigated whether QX-314 could affect glutamate currents by applying short pulses of glutamate from an extracellular pipette. No changes were observed. We also found no evidence for a persistent Na+ current in dissociated neurons, but these cells have a much-reduced dendritic tree. The results indicate that there is an inward conductance, which is sensitive to QX-314, during membrane potential oscillations that “boosts” the synaptic drive during fictive locomotion. Taken together, the results suggest that inactivating Na+ channels contribute to this inward conductance although persistent Na+ channels, if present on dendrites, could possibly also contribute to shaping the membrane potential oscillations.

INTRODUCTION

The amplitude of synaptic potentials depends not only on the different synaptic currents but also on the biophysical properties of the neuron. Active conductances in both the soma and dendritic membrane contribute to the amplitude and shape of integrated synaptically driven potentials. It has been shown in cat spinal motoneurons that voltage-activated inward currents in the dendrites potently amplify synaptic potentials (Brownstone et al. 1994; Lee and Heckman 2000). The activation of voltage-dependent Na+ channels in the dendrites may also boost the amplitude of excitatory postsynaptic potentials (EPSPs) (Nettleton and Spain 2000), which is of particular importance if the synapse is located on a distal dendrite. Reticulospinal (RS) synapses produce EPSPs in lamprey motoneurons. These synapses are located far out on the dendrites. In this case, indirect evidence (Buchanan et al. 1992) suggests that the EPSP amplitude from these synapses is amplified to some degree by the activation of TTX-sensitive Na+ channels. However, these results were inferred from electrotonic responses elicited through gap junctions and dealt exclusively with reticulospinal synapses, which are not active during fictive locomotion in the isolated spinal cord because their somata are located in the brain stem.

In our analyses of the intrinsic function of the spinal neuronal network coordinating locomotion in the lamprey (see Grillner et al. 1998), detailed knowledge is available on the synaptic interaction between network neurons. The synaptic drive potentials in motoneurons consist of a depolarizing half cycle, due to activation of excitatory segmental interneurons (Buchanan and Grillner 1987), and an inhibitory half cycle, due to crossed glycinergic interneurons (Buchanan 1982; Parker and Grillner 2000; Russell and Wallén 1983). The inhibitory synapses are thought to be located on soma and proximal dendrites, whereas excitatory synapses may predominantly be located at more distal dendritic sites (Russell and Wallén 1983). Our aim in this study was to investigate if Na+ and other inward-conducting channels play a role in synaptically driven membrane potential oscillations during activity in the locomotor network (studied in the isolated spinal cord). To test the hypothesis that active inward conductances participate in shaping the integrated synaptic activity, it is desirable to selectively block them in a single cell while the network continues to operate. N-(2,6-dimethylphenyl carbamoylmethyl)triethylammonium bromide (QX-314) is a lidocain derivative that...
blocks Na\(^+\) channels when injected intracellularly from the recording electrode (Connors and Prince 1982). It has been used in hippocampal neurons to block voltage-dependent Na\(^+\) channels of different types (Hu 1991; Hu et al. 1992; Nettleton and Spain 2000; Stuart and Sakmann 1994). The specificity of QX-314 has recently been investigated. Talbot and Sayer (1996) showed that high levels of QX-314 can also affect Ca\(^{2+}\) currents. Perkins and Wong (1995) reported that it also depresses hyperpolarization-activated inward currents (I\(_h\)), and QX-314 has been shown to block the G-protein-linked inwardly rectifying K\(^+\) channel (GIRK) (Andrade 1991; Harkins et al. 2000).

In this study, we show that potential oscillations, driven by rhythmic synaptic activity, are reduced in amplitude by 20–25% within 30 min following impalement when QX-314 is present intracellularly.

**METHODS**

**Intracellular recording**

Adult lampreys (*Lampetra fluviatilis* or *Ichthyomyzon unicupis*, depending on seasonal availability) were anesthetized with tricaine methanesulphonate (100 mg/L MS-222, Sandoz) and decapitated caudal to the gills. All procedures were in accordance with the ethical committee and institutional guidelines. The spinal cord/notochord was isolated by removing the surrounding muscle tissue and the meninges. The preparation was pinned down with the dorsal side up in a chamber lined with a silicon elastomer (Sylgard) and perfused with physiological solution maintained at 6–12°C. The physiological solution for *L. fluviatilis* was composed of (in mM) 138 NaCl, 2.1 KCl, 1.8 CaCl\(_2\), 1.2 MgCl\(_2\), 4 glucose, 2 HEPES, and 0.5 l-glutamine and was bubbled with O\(_2\) for 20 min to facilitate equilibration of O\(_2\) in the solution made with de-ionized water. For *I. unicupis*, the composition of the physiological solution was (in mM) 104.5 NaCl, 2 KCl, 2.6 CaCl\(_2\), 1.8 MgCl\(_2\), 4 glucose, and 2NaHCO\(_3\), bubbled with 95% O\(_2\)-5% CO\(_2\) (Wickelgren 1977). When necessary, the solutions were adjusted to pH 7.4 using 1 M NaOH. Fictive locomotion was induced by adding 50–150 μM NMDA to the perfusate (Grillner et al. 1981).

Intracellular sharp electrodes were filled with 10–100 mM QX-314 (Astra, Sigma) in 3 M KAc and 0.1 M KCl and had resistances of 22–66 MΩ. Fictive locomotion was monitored by ventral root recordings using suction electrodes. Motoneurons were identified on the basis of orthodromically elicited phase-locked action potentials in ventral roots or antidromically elicited phase-locked action potentials in spinal gray matter neurons due to ventral root stimulation (Wallén et al. 1985). QX-314 was allowed to passively diffuse from the intracellular electrode into the nerve cell. In unidentified neurons, QX-314 was injected into the neuron by delivering 500-nS depolarizing current pulses (2–4 nA) at 0.5 Hz for a period of 3 min at 10-min intervals. Recordings were made in bridge mode using an Axoclamp 2B amplifier (Axon Instruments). The signals were sampled using a Digidata 1200 digitizer (Axon Instruments) and stored on a PC. Data acquisition and analysis were performed using pClamp software versions 5.5 and 7.0 (Axon Instruments). Amplitudes of membrane potential oscillations were measured from trough to peak for 10–30 consecutive oscillations at the various recording times and means and standard intervals for statistical analysis of these measurements as well as those for whole cell currents described in the following text. Measured values are reported as means ± SD.

**Dissociation of spinal neurons**

Larval lampreys (*Petromyzon marinus*) were anesthetized with tricaine methanesulphonate (100 mg/L MS-222) and decapitated caudal to the gills. The spinal cord was exposed from the dorsal aspect in cooled physiological solution composed of (in mM) 138 NaCl, 2.1 KCl, 1.8 CaCl\(_2\), 1.2 MgCl\(_2\), 4 glucose, 2 HEPES, and 0.5 l-glutamine (bubbled with O\(_2\) for 20 min and adjusted to pH 7.4 with 1 M NaOH). The meninges were removed, and the spinal cord was dissected out. The dissociation (see El Manira and Bussières 1997) was carried out in Liebovitz’s L-15 culture medium (Sigma) supplemented with gentamicin (1 μg/ml) and penicillin/streptomycin (2 μg/ml). The osmolality of the medium was adjusted to 270 mosM with distilled water.

The spinal cord was subsequently treated with collagenase (2 mg/ml, 30 min, Sigma) and protease (4 mg/ml, 45 min, Sigma), then dissociated with gentle trituration through the tip of a 5-ml volumetric pipette. Dissociated cells were plated in 35-mm culture dishes (Falcon) and incubated at 10°C for 1–5 days.

**Whole cell recording from dissociated spinal neurons**

Middle-sized neurons with at least a short dendrite were selected for study. Whole cell voltage-clamp recordings were made from the somata of the neurons using an Axopatch 200A amplifier (Axon Instruments). Patch pipettes had resistances of 4–6 MΩ. For voltage-step experiments, series resistance was compensated by 80–95%. Data acquisition was performed using a TL-1 or Digidata 1320 A/D interface (Axon Instruments), and pClamp software versions 5.5 or 8.01 (Axon Instruments) were used for amplifier control and data analysis.

To record the effects of QX-314 on fast-inactivating sodium currents (I\(_{Na}\)), the pipette was filled with a solution containing (in mM) 112 potassium gluconate, 5 MgCl\(_2\), 1 CaCl\(_2\), 10 EGTA, 10 glucose, and 10 HEPES (pH 7.5). When glutamate-induced current was recorded, MgCl\(_2\) was isosmotically replaced with glucose in the external medium. For recording calcium currents (I\(_{Ca}\)), the pipette solution contained (in mM) 102 CsCl, 5 MgCl\(_2\), 1 CaCl\(_2\), 10 EGTA, 10 glucose, 2 ATP, 0.4 GTP, 5 phosphocreatine, and 10 HEPES (pH 7.5). The extracellular solution contained (in mM) 137 tetraethylammonium chloride (TEA), 1.2 MgCl\(_2\), 5 CaCl\(_2\), 2 KCl, 10 glucose, and 10 HEPES plus 1.5 μM TTX (pH 7.5). Experiments investigating persistent sodium currents (I\(_{Na}\)) were done with 15 mM TEA replacing equimolar NaCl in the extracellular solution and using Cs\(^+\)-filled pipettes as above. Extracellular solutions containing Cd\(^{2+}\) (500 μM) were perfused to block calcium currents. The pipette solutions were adjusted to 268 mosM and the external medium to 270 mosM with glucose or water. When QX-314 was tested, the drug was isosmotically substituted for glucose in the pipette solutions. Solutions containing glutamate or Cd\(^{2+}\) were delivered by a gravity-driven perfusion system with electrically controlled valves. Statistical comparisons for the whole-cell data were made using Student’s t-test and the results given in the figures are reported as means ± SE.

**RESULTS**

**QX-314 blocks fast-inactivating I\(_{Na}\) at low concentrations**

The effect of QX-314 on the Na\(^+\) (I\(_{Na}\)) and Ca\(^{2+}\) (I\(_{Ca}\)) currents were analyzed in dissociated neurons of the lamprey spinal cord using whole cell voltage clamp. In control neurons dialyzed with normal pipette solution (see METHODS), I\(_{Na}\) ran down slightly (less than 10%) within 10 min after whole cell access was gained. However, in the neurons dialyzed with 1 mM QX-314 (n = 5), I\(_{Na}\) totally disappeared within 20–30 s. When the concentration of QX-314 was reduced to 200 μM QX-314 (n = 5), the time course of QX-314 inhibition became clear; peak I\(_{Na}\) was reduced by about 90% within 3–4 min (Fig. 1D) and was abolished almost completely within 10 min (Fig. 1, A and D).
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10 mM QX-314, that with 500 μM QX-314 caused a reduction by about 20% at 10 min, similar to 80% in 10 min (n up to 4 min and then began to decline to reach approximately voltage-activated (HVA) Ca$^{2+}$ much higher than for fast inactivating but a substantial reduction occurred only at concentrations with 500 μM QX-314 in the pipette. C: as in B except with 10 mM QX-314. D: time course of I$_{Na}$ and I$_{Ca}$ reduction at different concentrations of QX-314 over a 10-min period, given as percent of the initial current shortly after rupture. With 0.2 mM QX-314, I$_{Ca}$ was 25% of the initial value at 2 min, falling to 2% at 10 min (n = 5). With 10 mM QX-314, I$_{Ca}$ was 29% of the initial value at 2 min, falling to 14% at 10 min (n = 9). With 0.5 mM QX-314, I$_{Ca}$ was 100% of the initial value at 2 min, falling to 84% at 10 min (n = 4). Note that at 0.5 mM, I$_{Ca}$ is similar to control levels (see text). Error bars represent ±SE. Holding voltages were −60 mV for I$_{Na}$ and −70 mV for I$_{Ca}$, and voltage was stepped from −70 to 20 mV to measure peak currents.

**FIG. 1.** Effect of N-(2,6-dimethylphenyl carbamoylmethyl)triethylammonium bromide (QX-314) on I$_{Na}$ and I$_{Ca}$. A: peak whole cell I$_{Na}$ during voltage steps within the first minute (left) and 5 min after rupture (right) with 0.2 μM QX-314 in the recording pipette. B: superimposed peak I$_{Ca}$ initially (larger response) and after 10 min (smaller response) with 0.5 mM QX-314 in the pipette. C: as in B except with 10 mM QX-314. D: time course of I$_{Na}$ and I$_{Ca}$ reduction at different concentrations of QX-314 over a 10-min period, given as percent of the initial current shortly after rupture. With 0.2 mM QX-314, I$_{Ca}$ was 25% of the initial value at 2 min, falling to 2% at 10 min (n = 5). With 10 mM QX-314, I$_{Ca}$ was 29% of the initial value at 2 min, falling to 14% at 10 min (n = 9). With 0.5 mM QX-314, I$_{Ca}$ was 100% of the initial value at 2 min, falling to 84% at 10 min (n = 4). Note that at 0.5 mM, I$_{Ca}$ is similar to control levels (see text). Error bars represent ±SE. Holding voltages were −60 mV for I$_{Na}$ and −70 mV for I$_{Ca}$, and voltage was stepped from −70 to 20 mV to measure peak currents.

$I_{Ca}$ is blocked with QX-314 at high concentrations

Because it has been reported that QX-314 reduces high-voltage-activated (HVA) Ca$^{2+}$ current (I$_{Ca}$) at 10 mM in dissociated hippocampal neurons (Talbot and Sayer 1996), we also analyzed effects of different concentrations of the drug on HVA I$_{Ca}$ in dissociated spinal neurons. In neurons dialyzed with 500 μM QX-314 (n = 4), I$_{Ca}$ remained at initial levels for up to 4 min and then began to decline to reach approximately 80% in 10 min (n = 3; Fig. 1, B and D). In two neurons, 1 mM QX-314 caused a reduction by about 20% at 10 min, similar to that with 500 μM (data not shown). In neurons dialyzed with 10 mM QX-314, I$_{Ca}$ disappeared within 5 min (Fig. 1, C and D) in most neurons (6/9) and within 10 min in the remaining neurons (Fig. 1C). The time course of the averaged currents is shown in Fig. 1D. The effect of QX-314 on low-voltage-activated (LVA) I$_{Ca}$ was not tested as this is not expressed in dissociated lamprey motoneurons with reduced dendrites (El Manira and Bussières 1997). Thus I$_{Ca}$ is blocked by QX-314, but a substantial reduction occurred only at concentrations much higher than for fast inactivating I$_{Na}$ (Fig. 1D). One can assume that the intracellular concentration of QX-314 approximates that of the patch electrodes.

**QX-314 blocks action potentials in spinal neurons and axons**

To investigate the possible role of active inward conductances on membrane potential oscillations and action potentials in neurons participating in the locomotor network, intracellular sharp electrodes containing QX-314 were used to record from spinal neurons during fictive locomotion induced by N-methyl-d-aspartate (NMDA) in intact lamprey spinal cords. This is manifest in rhythmic, alternating left and right ventral root bursting and in synchronous membrane potential oscillations in active spinal neurons (Fig. 2A). In the spinal cord, sharp microelectrodes with a resistance of 22–66 MΩ were used because recording with patch electrodes cannot be accomplished in the adult lamprey spinal cord. The diffusion from these electrodes into the cell will be very much reduced as compared with patch electrodes used for the dissociated cells, discussed in the preceding text, therefore higher concentrations of QX-314 had to be used in the microelectrode. Concentrations of 10 mM had no effect on the action potential within 10 min, whereas at 30 mM QX-314 (in the pipette), the action potentials remained or could be evoked by current pulses throughout the period of recording indicating that at the latter concentration the intracellular level of QX-314 was not sufficient to block voltage-dependent Na$^+$ channels.
QX-314 reduces the amplitude of membrane potential oscillations

To investigate a possible contribution of increased inward conductance on membrane potential oscillations, we analyzed intracellular recordings from motoneurons and unidentified spinal neurons during fictive locomotion using electrodes containing QX-314 (see Methods). Figure 3A shows the motor pattern underlying the locomotor activity recorded in the ventral roots and the corresponding membrane potential oscillations in a motoneuron. The amplitude of oscillations was reduced in all neurons impaled with electrodes containing 30 mM QX-314 (Fig. 3B). This effect is illustrated in Fig. 3C by the superimposed traces of oscillations averaged from the initial recording and after 30 min for the motoneuron in Fig. 3A and B (see also Fig. 2, A and B). The time course of the reduction in amplitude for three motoneurons subjected to 30 mM QX-314 as compared with three control cells is shown in Fig. 4 (see legend). Action potentials could not be evoked after approximately 5 min, and a clear reduction of the synaptically driven potentials occurred, progressing to a stable level of about 60–65% of initial values within 30 min, which generally reflects the time within which the action occurred in all neurons tested. Control neurons recorded without QX-314 or with 10 mM (n = 5) did not show a reduction in peak-to-peak amplitude over time, and in some cases, it increased in amplitude (Fig. 4, discussed in the following text). Cells impaled with 10 mM QX-314 electrodes were therefore included as controls.

The mean reduction at 15 and 30 min compared with initial oscillation amplitude is shown in Fig. 5 for motoneurons and unidentified spinal neurons. Amplitudes were significantly reduced in 8/9 motoneurons (Fig. 5A, P < 0.05, mean reduction = 22 ± 11%, n = 8) and in 6/7 spinal neurons (Fig. 5B; P < 0.005, mean reduction = 37 ± 11%, n = 6) at 15 min. After 30 min, there was a significant reduction in 8/8 motoneurons (P < 0.05, mean reduction = 25 ± 10%) and 4/4 spinal neurons (P < 0.005, mean reduction = 53 ± 14%; see figure legend for further explanation). The greater reduction in unidentified neurons is likely due to the mixed group of cells and also that current injection of QX-314 was sometimes used as opposed to passive diffusion to decrease the latency of the action potentials.

FIG. 2. QX-314 blocks action potentials in spinal neurons and reticulospinal axons. A: intracellular recording from a motoneuron (MN; top) and a ventral root recording (VR; bottom) during NMDA-induced fictive locomotion. The membrane potential oscillations in the control were obtained immediately after penetration with an electrode containing 100 mM QX-314. They have depolarizing plateaus showing bursts of action potentials (clipped in amplitude for illustration). B: intracellular recording from the same neuron as in A 7 min later when the action potentials have been abolished. C: action potential responses to current pulse stimulation in a reticulospinal axon recorded intracellularly directly after impalement with an electrode containing 100 mM QX-314. D: intracellular record from the same axon as in C after approximately 2 min showing the decline and eventual block of action potentials.

FIG. 3. QX-314 reduces membrane potential oscillations in motoneurons. A: ventral root recording (bottom) and simultaneous contralateral intracellular recording (top) from a spinal motoneuron during fictive locomotion. In this motoneuron, action potentials ceased within 1 min after impalement and are therefore not present in the initial recording. B: a clear amplitude reduction occurs within 30 min after impalement with 30 mM QX-314 in the microelectrode. C: superimposed averages of 5 oscillations (osc.) from the initial recording and after 30 min in the motoneuron shown in A and B, illustrating the amplitude reduction.
A statistically significant reduction (see METHODS) of motoneurons was compared between initial controls and after 30 min recording with QX-314 electrodes. It showed a statistically significant reduction ($P < 0.005$, mean $= 61 \pm 9\%$, $n = 8$). This is less than the mean amplitude after 30 min using QX-314 electrodes and indicates a change in the shape (see Fig. 3C).

There were no consistent changes in the average level of membrane potential after QX-314 application; some cells were somewhat depolarized or hyperpolarized ($\pm 2$ mV). In all cases, there was a reduction in amplitude of the oscillations with QX-314 concentrations equal to or more than 30 mM. This applied also if the membrane potential was restored to the original value or shifted positively or negatively by current injection.

**Effect of QX-314 on glutamate-induced current**

Because membrane potential oscillations in lamprey motoneurons and interneurons are induced by AMPA and NMDA receptors (Dale 1986), it is important to determine if there are some effects of QX-314 on the postsynaptic responses of glutamate receptors. We therefore applied L-glutamate, while recording from dissociated neurons from the lamprey spinal cord (see METHODS) with and without QX-314 in the recording pipette. As in intact neurons, dissociated lamprey spinal neurons have been shown to have NMDA and AMPA receptors (Krieger et al. 2000; A. El Manira, personal communications). The responses of brief pulses ($2.5$ s) of $100 \mu M$ L-glutamate varied in amplitude from cell to cell. However, stable L-glutamate-induced inward currents could be obtained for more than 10 min while recording from both control neurons and from neurons dialyzed with QX-314 as illustrated in Fig. 6, A–D. The mean amplitude of L-glutamate-induced current in control neurons fell about 10% of the initial value 10 min after whole cell access was gained (Fig. 6E). Similar results were obtained in the neurons dialyzed with different concentrations of QX-314 (Fig. 6E). The changes in amplitude of L-glutamate-induced current after 10 min compared with initial values in the neurons dialyzed with QX-314 were not significantly different at any concentration from those in control neurons ($P > 0.05$). Thus we can infer that QX-314 will not affect the postsynaptic glutamate receptors in intact spinal neurons during fictive locomotion. Further, as QX-314 did not significantly reduce the L-glutamate response, which must be largely from receptors on the soma in the dissociated neurons, the results support the notion that its action on depolarizing synaptically driven potentials in the isolated spinal cord occurs out in the dendrites.

**No evidence for a persistent Na$^+$ current in dissociated neurons**

A persistent, nonactivating Na$^+$ current $I_{NaP}$ has been shown to amplify EPSPs in cortical neurons (Lipowsky et al. 1996; Stafstrom et al. 1982, 1985). It causes an inward conductance at potentials subthreshold for the fast-inactivating Na$^+$ channels. Dissociated spinal neurons were tested for the presence of $I_{NaP}$ by applying voltage steps from −70 to 10 mV. Both fast inactivating $I_{Na}$ and HVA $I_{Ca}$ were present at voltage steps above −40 mV when $I_{Na}$ was blocked (Fig. 7A). When $I_{Ca}$ was blocked with Cd$^{2+}$ in the bathing solution (Fig. 7A), no $I_{NaP}$ was observed during the voltage steps as illustrated in Fig. 7B with steps from −60 to −46 mV ($V_h = −70$ mV), the last step being just below threshold for the fast

![Figure 4](image4.png) Comparison of the time course of amplitude reduction in membrane potential oscillations compared with initial values following impalement in 3 motoneurons with 10 mM QX-314 and 3 with 30 mM QX-314 in the recording electrode. A clear trend of reduced oscillation amplitudes is illustrated for 30 mM QX-314 while with 10 mM, the oscillations remain at or above initial values.

![Figure 5](image5.png) Mean amplitudes of membrane potential oscillations at different times following impalement with QX-314-filled microelectrodes. A: a significant reduction in amplitude occurred in 8/9 motoneurons ($P < 0.05$, mean reduction $= 22 \pm 11\%$, $n = 8$) in 15 min and in 8/8 motoneurons recorded for 30 min ($P < 0.05$, mean reduction $= 25 \pm 10\%$). One neuron had a reduction at 30 min but not at 15 min. Another neuron, which showed effects at 15 min, was lost within 30 min. B: in spinal neurons 6/7 cells showed a significant decrease in 15 min ($P < 0.005$, mean reduction $= 37 \pm 11\%$, $n = 6$) and 4/4 after 30 min ($P < 0.005$, mean reduction $= 53 \pm 14\%$). *$P < 0.05$; **$P < 0.005$. J Neurophysiol • VOL. 87 • JUNE 2002 • www.jn.org
QX-314 had no effect on glutamate-induced postsynaptic currents in dissociated spinal neurons. A: whole-cell glutamate-induced currents in a control neuron 30 s following rupture. B: glutamate-induced currents in a neuron dialyzed with 10 mM QX-314 30 s after rupture. C: currents induced after recording 10 min in the same cell as A. D: currents in the same cell as B after 10 min of recording. In both neurons, 100 μM glutamate was perfused during the time indicated by the labeled bars. Recordings were made in the presence of 10 μM glycine in Mg²⁺-free extracellular solution. The holding potential was −60 mV. E: the amplitude of glutamate-induced current after 10 min compared with those after 30 s during recordings in experiments as described in A–D. Changes in current responses in neurons dialyzed with 3 different concentrations of QX-314 were similar to those of control neurons recorded without QX-314. (P > 0.05 for all concentrations compared with controls, mean = 90 ± 11, 88 ± 9, 92 ± 13, and 91 ± 18% for controls, 200 μM, 1 mM, and 10 mM QX-314, respectively).

**Discussion**

**QX-314-effects on I_{Na} and I_{Ca}**

In dissociated pyramidal neurons of hippocampus, Talbot and Sayer (1996) showed that in addition to sodium channels Ca²⁺ channels also were blocked by QX-314. They report that with 10 mM QX-314 nearly 80% of the I_{Ca} was blocked, whereas at 1 mM it was reduced by 26%. With 1 mM QX-314, the Na⁺ current was still not entirely blocked. In lamprey spinal cord neurons, I_{Na} is reduced by 90% of the control within 3 min with 0.2 mM QX-314, whereas I_{Ca} is unchanged even at 0.5 mM QX-314 after 4 min. With 10 mM, however, I_{Ca} is still at 25% after 3 min. Thus using 50-fold higher QX-314 (10 vs. 0.2 mM), the same level of depression is not reached for I_{Ca} as for I_{Na} (Fig. 1).

**Effects of QX-314 on locomotion-related membrane potential oscillations**

QX-314 had significant effects (P < 0.005) on the amplitude of the membrane potential oscillations, which were depressed by 20–25% consistently, whereas no reduction was observed in the controls. The sharp microelectrodes (22–66 MΩ), as opposed to the patch electrodes, will markedly delay the diffusion into the recorded cell. With 10 mM QX-314 in the microelectrode, no effect on the Na⁺-dependent action potentials was observed nor on the membrane potential oscillations, whereas at 30 mM or higher concentrations, there was a clear depression of the oscillations. The depressing effects of QX-314 occurred at a concentration in the electrode close to that where there is no effect at all, thus at a concentration that may preferentially block Na⁺ channels. Moreover, the likelihood that a Ca²⁺ channel blockade in the soma causes the effect is low because the oscillations occur at subthreshold membrane potentials (−55 to −65 mV) when HVA Ca²⁺ channels are not activated (El Manira and Bussières 1997). Although LVA Ca²⁺ channels are not present in dissociated lamprey motoneurons with reduced dendrites (D. Hess and A. El Manira, unpublished observations), they are present in a proportion of intact lamprey spinal neurons (Matsushima et al. 1993). In cat spinal motoneurons, QX-314 has been shown to have a profound effect on voltage-activated persistent inward currents, possibly due to mixed Na⁺ and Ca²⁺ currents (Lee and Heckman 1999). Further, dendritic L-type channels participate in NMDA-induced oscillations in the turtle spinal cord (Guertin and Houngsda 1998) and low thresholds for activation of L-type channels in the dendrites of spinal motoneurons have been reported in mouse (Carlin et al. 2000). In lamprey, however, previous studies on membrane potential oscillations syn-
apically driven from fictive locomotion in the lamprey showed no change in amplitude after applying the L-type Ca\(^{2+}\) channel blocker nimodipine (Büschges et al. 2000), suggesting it is unlikely that L-type channels contribute to the observed effect. Because depression of the oscillations occurred within several minutes, even with the lowest effective concentrations in the electrode, it seems unlikely that the primary effect was from blockade of Ca\(^{2+}\) channels by diffusion of QX-314 into the dendrite. bis-(o-aminophenoxy)-N,N,N′,N′-tetracetic acid injection, which blocks the slow, \(K_{Ca}\)-induced afterhyperpolarization by chelating Ca\(^{2+}\), does not appear to reduce the amplitude of the motoneuronal oscillations (Cangiano et al. 2000). This finding suggests that Ca\(^{2+}\)-activated processes (e.g., \(K_{Ca}\)) do not affect the amplitude of the membrane potential oscillations. QX-314 has also been reported to have effects on the hyperpolarization-activated inward current due to \(I_h\) (Perkins and Wong 1995) in hippocampal neurons. \(I_h\) does not occur in lamprey dissociated motoneurons (D. Hess and A. El Manira, unpublished observations) and thus probably does not account for the QX-314 effects observed here. QX-314 has also been shown to block GIRK channels (Andrade 1991; Buchan et al. 2000). There is no evidence that these channels are activated during fictive locomotion in lamprey. However, if they were active during fictive locomotion, a blockade of GIRK channels would have produced a consistent depolarization of the cells, an effect that was not observed. Thus it appears very unlikely that a blockade of GIRK channels should account for the observed reduction in amplitude of the locomotor oscillation. We also ascertained that there is no effect on ion channels activated by \(\gamma\)-glutamate (Fig. 6).

The results suggest that EPSPs produced in dendrites to a certain degree activate some type(s) of Na\(^{+}\) channels, which boost the amplitude of the depolarization, corresponding to 20–25% of the oscillation amplitude in motoneurons. When considering all of the preceding text, the most likely possibility is that the effects produced by QX-314 in lamprey motoneurons, regarding the amplitude reduction of the locomotor oscillations, is due to an effect on voltage-dependent Na\(^{+}\) channels. In dissociated lamprey neurons there is no trace of a persistent Na\(^{+}\) current (Fig. 7). The dendritic membrane is, however, much smaller than in intact neurons, and thus persistent Na\(^{+}\) channels present on dendrites may contribute to shaping the membrane potential oscillations. The effects observed in the intact spinal cord are thus presumably due to a blockade of voltage-dependent, inactivating Na\(^{+}\) channels.

The EPSPs in motoneurons during fictive locomotion are due to the activation of excitatory glutamatergic interneurons activating both NMDA and AMPA receptors (Buchanan and Grillner 1987). These EPSPs thus appear to be boosted by inward currents blocked by QX-314. It has been reported that EPSPs in neocortical pyramidial neurons have larger potentials than their algebraic sum (‘supralinear summation’) and that this is due to a QX-314-sensitive inward current, likely including a regenerative Na\(^{+}\)and/or persistent Na\(^{+}\) conductance (Nettleton and Spain 2000). Potentials recorded in the soma of the lamprey neurons are a summation of active conductances and of EPSPs and inhibitory postsynaptic potentials. Out in the dendrite, inactivating Na\(^{+}\) channels that could de-inactivate between individual EPSPs may enhance even large EPSPs. Further, even at depolarized potentials near -50 mV, a substantial proportion of inactivating Na\(^{+}\) channels are in the de-inactivated state (see Hille 1992). Depending largely on channel density, they could thus contribute to an inward conductance that potentiates membrane depolarization. In a previous report, indirect evidence has suggested that the reticulospinal EPSP produced in motoneurons is boosted by TTX-sensitive Na\(^{+}\) channels (Buchanan et al. 1992). This view of boosting of EPSPs, in the present case activated by other spinal neurons, is thus further supported by our findings.

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NOTES

**Note added in proof**

Taddese and Bean (Neuron 33: 587–600, 1992) demonstrate that a subthreshold Na\(^{+}\) current from rapidly inactivating Na\(^{+}\) channels produce a “persistent Na\(^{+}\) current-like” response, similar to the above results.

REFERENCES


