Paradoxical Effect of QX-314 on Persistent Inward Currents and Bistable Behavior in Spinal Motoneurons In Vivo

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Lee, R. H. and C. J. Heckman. Paradoxical effect of QX-314 on persistent inward currents and bistable behavior in spinal motoneurons in vivo. J. Neurophysiol. 82: 2518–2527, 1999. Spinal motoneurons can exhibit bistable behavior, which consists of stable self-sustained firing that is initiated by a brief excitatory input and terminated by brief inhibitory input. This bistable behavior is generated by a persistent inward current (I_{PIC}). In cat motoneurons with low input conductances and slow axonal conduction velocities, I_{PIC} exhibits little decay with time and thus self-sustained firing is long-lasting. In contrast, in cells that have high input conductances and fast conduction velocities, I_{PIC} decays with time, and these cells cannot maintain long duration self-sustained firing. An alternative way to measure bistable behavior is to assess plateau potentials after the action potential has been blocked by intracellular injection of QX-314 to block sodium (Na\(^{+}\)) currents. However, QX-314 also blocks calcium (Ca\(^{2+}\)) currents and, because \(I_{PIC}\) may be generated by a mixture of Ca\(^{2+}\) and Na\(^{+}\) currents, a reduction in amplitude of \(I_{PIC}\) was expected. We therefore systematically compared the properties of \(I_{PIC}\) in a sample of cells with QX-314 to a control sample of cells without QX-314, which was obtained in a previous study. Single-electrode voltage-clamp techniques were applied in spinal motoneurons in the decerebrate cat preparation following administration of a standardized dose of the noradrenergic \(\alpha\)1 agonist methoxamine. In this sample, the average value of \(I_{PIC}\) was only about half that in the control sample. However, the reduction of \(I_{PIC}\) was much greater in cells with slow as compared with fast conduction velocities. Because a substantial portion of \(I_{PIC}\) originates in dendritic regions and because conduction velocity covaries with the extent of the dendritic tree, this result suggests that QX-314 may fail to diffuse very far into the dendrites of the largest motoneurons. The analysis of the decay of \(I_{PIC}\) and plateau potentials in cells with QX-314 also produced an unexpected result: QX-314 virtually eliminated time-dependent decay in both \(I_{PIC}\) and plateau potentials. Consequently, \(I_{PIC}\) became equally persistent in high and low input conductance cells. Therefore the decay in \(I_{PIC}\) in high input conductance cells in the absence of QX-314 is not due to an intrinsic tendency of the underlying inward current to decay. Instead it is possible that the decay may result from activation of a slow outward current. Overall, these results show that QX-314 has a profound effect on \(I_{PIC}\) and thus plateau potentials obtained using QX-314 do not accurately reflect the properties of \(I_{PIC}\) in normal cells without QX-314.

INTRODUCTION

Many types of motoneurons have been shown to exhibit bistable behavior, in which tonic self-sustained firing can be toggled on and off by brief excitatory and inhibitory inputs (e.g., Bennett et al. 1998; Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988; Hsiao et al. 1998; Lee and Heckman 1998b; Rekling and Feldman 1997; Svirskis and Hounsgaard 1998; Zhang and Harris-Warrick 1995; Zhang et al. 1995). In most motoneurons, neuromodulators, such as the monoamines serotonin and norepinephrine, are required for expression of bistable behavior (Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988; Hsiao et al. 1998; Lee and Heckman 1999b). It has also been shown that, at least in spinal motoneurons in the cat and the turtle, much of the persistent inward current that generates bistable behavior originates in the dendrites (Bennett et al. 1998; Hounsgaard and Kiehn 1993; Lee and Heckman 1996).

One striking characteristic of spinal motoneurons in the cat is the existence of systematic differences in their bistable behaviors. Motoneurons with low input conductances and slow axonal conduction velocities have the capacity to be fully bistable, in that they can generate long periods of self-sustained firing (Lee and Heckman 1998a,b). This strong bistability is possible because the total persistent inward current (\(I_{PIC}\)) in low input conductance cells has a large amplitude and exhibits little or no decay with time. Motoneurons with high input conductances and fast conduction velocities possess an equally large amplitude \(I_{PIC}\), but it tends to slowly decay over the course of a few seconds. Consequently, high input conductance motoneurons are only partially bistable, often generating <1 s of self-sustained firing.

However, the duration of \(I_{PIC}\) does not precisely match the duration of self-sustained firing (Lee and Heckman 1998a). In the high input conductance cells, self-sustained firing decays more rapidly than does \(I_{PIC}\). Further, we have recently shown that the amplitude of \(I_{PIC}\) is a key factor affecting the duration of self-sustained firing in low input conductance cells (Lee and Heckman 1999b). When the amplitude of \(I_{PIC}\) is decreased, self-sustained firing fails within 1–2 s even though \(I_{PIC}\) remains highly persistent. These differences between the persistence of \(I_{PIC}\) and the duration of self-sustained firing may be partly due to the deactivating effects of the afterhyperpolarization (AHP) following each action potential. This suggests that if action potentials were eliminated by intracellular injection of the lidocaine derivative QX-314 to block sodium (Na\(^{+}\)) currents (Connors and Prince 1982; Narahashi et al. 1972), then the resulting plateau potentials should have properties very similar to those of \(I_{PIC}\).

However, QX-314 is now known to not block Na\(^{+}\) currents but to also reduce calcium (Ca\(^{2+}\)) currents (Talbot and Sayer 1996). Because \(I_{PIC}\) may be generated by a combination of persistent Ca\(^{2+}\) and Na\(^{+}\) currents (Hounsgaard and Kiehn...
creases the amplitude of distilled water. We have shown that methoxamine substantially in-

were approved by the local animal care committee at Northwestern 

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experiments, was 5

before recording from any cells. The standard dose, given in all 

for calculating axonal conduction velocity (see Data analysis).

other eight experiments provided the primary data for this paper on the 
effects of QX-314. In all of these eight primary experiments, we 
used the decerebrate cat preparation and administered the noradren-
ergic α1 agonist methoxamine via a modified intrathecal method 

before recording from any cells. The standard dose, given in all 
experiments, was 5 μmol of methoxamine dissolved in 100 μl of 
distilled water. We have shown that methoxamine substantially in-

creases the amplitude of $I_{\text{PIC}}$ in motoneurons in our decerebrate 

preparation (Lee and Heckman 1999b). The increased amplitude of $I_{\text{PIC}}$ 
greatly enhanced the probability that motoneurons would exhibit 

bistable behavior. All details of the surgical preparation, decerebra-

tion, and administration of methoxamine are given in our previously 
published studies (Lee and Heckman 1998a,b, 1999b). All procedures 
were approved by the local animal care committee at Northwestern 

University.

To assess the effects of QX-314 on $I_{\text{PIC}}$, we compared the 
properties of $I_{\text{PIC}}$ in the sample of cells recorded in this study using 
electrodes containing QX-314 (referred to as the “QX-314 sample”) to 
the properties obtained in a sample of cells without QX-314 (the 
control sample). The control sample consisted primarily of data from 
27 cells, which were obtained in a previous study in the decerebrate 
preparation with the same dose of methoxamine as in this study (Lee 
and Heckman 1998a). However, this previous data set only had seven 
cells in which the rate of decay of $I_{\text{PIC}}$ was measured (see Data analysis). Because measurements of decay of $I_{\text{PIC}}$ were a particularly 
important component of this study, data on decay for 4 more cells 
without QX-314 from 2 additional experiments were added to the 
control sample, to give a total of 31 cells. For the QX-314 sample, 
electrodes were filled with a solution consisting of 50–100 mM of the 
bromide salt of QX-314 and 3 M KCl. This sample consisted of 18 
cells from 6 experiments. We have found the effects of methoxamine 
to be remarkably consistent from experiment to experiment (Lee and 
Heckman 1998a,b), so the results of this study are unlikely to be due 
to interanimal variations.

Experimental protocols

Protocols were similar for both the QX-314 sample and the control 
sample. Data collection began with identification of the cell as a 

motoneuron by antidromic activation from either the medial gastro-
cnemius (MG) or lateral gastrocnemius/soleus (LGS) nerves. A series 
of 20 antidromic spikes were averaged for conduction time measure-

ments. We then injected a series of steady currents, 5–10 s in duration, 
to induce rhythmic firing and allow QX-314 to block the Na$^+$ spikes. 
Typically, spike generation for rhythmic firing failed after ~1–2 min. 
In some cells, a single small (~10 mV) spike continued to occur at the 
onset of each current step, and additional applications of current did 
not further reduce its amplitude. Thus our criterion for sufficient block 
of action potentials was the total failure of rhythmic firing.

The single-electrode voltage-clamp technique was then used to 
measure $I_{\text{PIC}}$ (see Lee and Heckman 1998a for details and limitations).

As in our previous work (Lee and Heckman 1998a), the primary 
characteristics of $I_{\text{PIC}}$ were assessed from the pattern of currents 
evoked by a slow triangular voltage command (usually 40 mV in 

amplitude applied at a rate of 8 mV/s for both ascending and descend-
ing phases). This triangular voltage command was applied ~1–3 min 
after rhythmic firing was eliminated. The persistence of $I_{\text{PIC}}$ was then 
measured from voltage-clamp steps with a long duration (10 s). In 
some cells, we also measured the plateau potentials generated by $I_{\text{PIC}}$.

The plateau potentials were evoked either by a triangular shaped 
injected current (typically 30 nA in amplitude with rates of rise and 
fall of 6 nA/s) or by a brief period of synaptic input. For this, a 1.5-s 
period of monosynaptic input was generated by low-amplitude, high-
frequency vibration of the Achilles tendon, which activates muscle 
spindle Ia afferents (Matthews and Stein 1969).

Data analysis

PROPERTIES OF $I_{\text{PIC}}$. Data analysis techniques for $I_{\text{PIC}}$ in the QX-314 

sample were identical to those for the control sample (see Lee 
and Heckman 1998a). Figure 1 illustrates the standard parameters we 

assess for $I_{\text{PIC}}$, which are taken from the I-V function generated by the 

triangular voltage command. These parameters are the onset and the 
initial peak on the ascending phase and the sustained peak and offset

FIG. 1. A current-voltage (I-V) function in a motoneuron with QX-314. A: 
a triangular-shaped voltage command was applied to the cell, which revealed a 
net persistent inward current, $I_{\text{PIC}}$. This cell has a low input conductance, 
~0.43 μS. B: I-V relationship for the data shown in A. The labeled points on 
the function indicate the main parameters we measured to characterize $I_{\text{PIC}}$. 

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on the descending phase. Leak conductance was subtracted to define the amplitudes of the initial and sustained peaks (see Lee and Heckman 1998a for leak subtraction details). However, onsets and offsets were measured without leak subtraction because we wished these parameters to be functionally relevant in terms of the firing behavior of the cell (Lee and Heckman 1998a,b). Input conductance was measured from the slope of the subthreshold region of the I-V function in a 5- to 10-mV range ending at least 5 mV below I_{PEP} onset.

**PERSISTENCE OF I_{PEP} AND PLATEAU POTENTIALS.** The persistence of I_{PEP} was assessed during a long-duration (10 s) voltage step. The baseline for the step was set ~10 mV below the onset of I_{PEP}, and the step amplitude was adjusted so that it reached the voltage level at the initial peak of I_{PEP} on the cell’s I-V function. Persistence of I_{PEP} was calculated by expressing the amplitude of I_{PEP} in the final second of the 10-s step as a percentage of the maximum amplitude in the first second (an example is shown in Fig. 9A in RESULTS). In the QX-314 sample, we measured the persistence of plateau potentials evoked by our standard 1.5-s period of monosynaptic Ia input (an example is shown in Fig. 9B in RESULTS). If necessary, a baseline current was applied to allow the depolarization produced by this monosynaptic input to exceed the voltage for plateau activation. The persistence of the plateau potential was calculated similarly to that of I_{PEP}. The average plateau potential amplitude in a 1-s time window starting 9 s after the end of the Ia input was expressed as a percentage of its average amplitude in the first second after the Ia input.

**CONDUCTION TIME MEASUREMENTS.** Conduction time was measured as the difference between time of stimulation in the muscle nerve and time of onset of the antidromic spike, measured as the average of 20 trials. In some cells, spike amplitude began to decline during the series of 20 spikes that went into the average, even though resting potential remained stable. Presumably this was due to QX-314. However, QX-314 did not influence conduction time. In six cells, we compared the conduction time measured from the initial antidromic spike, which was >70 mV in amplitude, to that measured after QX-314 had eliminated both the somatic-dendritic and initial segment components of the spike, leaving only the M-spike [the M-spike typically has amplitude of ~5–7 mV and is assumed to result from passive propagation of the axonal spike through the initial segment and soma (Eccles et al. 1957)]. In each cell, the times of onset of the full spike and the M-spike were the same, and hence conduction time was unchanged.

**CALCULATION OF CONDUCTION VELOCITY.** Interanimal variations in size have a significant effect on conduction times (Emonet-Denand et al. 1988). Measurements of conduction distances and calculation of the resulting conduction velocities are presumed to account for interanimal variations. Despite this normalization, pooling conduction velocity from more than one experiment can increase the variance in the sample of conduction velocities, resulting in a degradation in the relations between conduction velocity and other motor-unit properties (Emonet-Denand et al. 1988). In the present study, we have used an alternative normalization method for the effect of interanimal variations in size on conduction velocity and performed a series of control experiments to demonstrate its validity (see the following subsection). The alternative method was done as follows. After all intracellular data were collected, the electrode was positioned just outside the cell (the standard procedure to assess the resting membrane potential). The extracellular field due to antidromic stimulation was averaged (32 trial per average) and superimposed on the intracellular record of the first part of the spike (an average of 20 trials). Figure 2 shows three examples of this superimposition. The time of spike initiation was taken as the first digitized point in which the spike diverged from the waveform defined by the extracellular field (arrow labeled ‘S’ in each record; digitization rate: 100 KHz). The onset time of the extracellular field (arrow labeled ‘F’ in each case) was assumed to be generated by the initiation of the action potentials for nearby motoneurons with the shortest conduction times. Thus we normalized each cell’s conduction time by the onset time for the extracellular field. The actual conduction velocity was estimated by assuming the motoneurons producing the earliest onset of the field had conduction velocities at the high end of the range for motoneurons. We chose a value of 110 m/s (cf. Zengel et al. 1985).

**CONTROL EXPERIMENTS FOR CONDUCTION VELOCITY CALCULATIONS.** To assess how well normalization by the field onset worked in reducing interanimal variations in conduction velocity estimates, we measured conduction time, field onset time, and conduction distance in a sample of 147 cells in 8 experiments (no QX-314 was used). Two of these experiments primarily consisted of posterior biceps-semitendinosus (PBSt) motoneurons, which have a substantially shorter conduction distance than MG or LGS motoneurons. All cells with antidromic action potentials >20 mV were accepted for this sample because, as noted above, we have found that conduction time is not affected by spike height (cells with good spike heights provided data for experimental protocols that were not part of the work present here). The average conduction velocity based on the assumption that field onset corresponded to 110 m/s was 93.5 ± 9.6 (SD) m/s. This was not significantly different from the average conduction velocity calculated from estimated conduction distance, which was 94.2 ± 10.4 m/s (t-test, P > 0.6). Note, however, the standard deviation for the field onset normalization was less than that for conduction distance normalization. This reduced standard deviation suggested that the field onset time might have more accurately reflected interanimal variations in size. To test this possibility further, the relationship between conduction velocity and rheobase was compared for the two normalization methods. For this analysis, we included only cells where spike height was >70 mV because rheobase is sensitive to impalement injury (Binder et al. 1996). Further, we only included data...
from the 3 experiments with at least 10 cells with rheobase measurements to allow comparison of variance both within and between experiments. In the resulting sample of 33 cells, the correlation between rheobase and conduction velocity estimated by conduction distance was \( r = 0.32 \). The correlation based on field onset was higher, at \( r = 0.51 \). The latter correlation fell within the range of the three within-experiment correlation coefficients, which were 0.44, 0.50, and 0.70. Thus use of the onset of the field to estimate conduction velocity is at least as good if not better than use of conduction distance when attempting to reduce the effects of interanimal variations in size. The field onset method was therefore used in the present study. However, it should be noted that we restricted our sampling of motoneurons to areas where large extracellular fields were evoked from the appropriate muscle nerves to assure that the field was generated by a reasonably large sample of cells. Also, it is essential to generate the field solely from the nerve that antidromically activates the nerve, as the field onsets for different nerves at a given recording locus can vary substantially.

**TABLE 1. Comparison of characteristics of \( I_{\text{PIC}} \) in cells with QX-314 to control cells**

<table>
<thead>
<tr>
<th>Characteristics of ( I_{\text{PIC}} )</th>
<th>QX-314 Cells</th>
<th>Control Cells*</th>
<th>( t )-Test, ( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial peak, nA</td>
<td>8.4 ± 7.9</td>
<td>20.3 ± 5.6</td>
<td>( P &lt; 0.0000001 )†</td>
</tr>
<tr>
<td>Sustained peak, nA</td>
<td>6.8 ± 5.0</td>
<td>11.2 ± 4.8</td>
<td>( P &lt; 0.004 )†</td>
</tr>
<tr>
<td>Initial–sustained peaks, nA</td>
<td>1.6 ± 4.1</td>
<td>9.1 ± 4.7</td>
<td>( P &lt; 0.0000001 )†</td>
</tr>
<tr>
<td>Offset voltage, mV</td>
<td>-46.5 ± 5.1</td>
<td>-46.7 ± 5.3</td>
<td>( P &gt; 0.9 )</td>
</tr>
<tr>
<td>Onset voltage, mV</td>
<td>-57.3 ± 8.4</td>
<td>-52.3 ± 8.0</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>Hysteresis (onset-offset)</td>
<td>10.9 ± 6.3</td>
<td>5.6 ± 3.8</td>
<td>( P &lt; 0.001 )†</td>
</tr>
</tbody>
</table>

Values in QX-314 Cells and Control Cells are means ± SD. Number of QX-314 cells is 18 and Control cells is 31. * Data primarily from Lee and Heckman (1998a). † Significant at \( P = 0.05/6 = 0.0083 \) (see METHODS).

**RESULTS**

The primary goal of this study was to assess the effects of QX-314 on \( I_{\text{PIC}} \) in spinal motoneurons. This goal was accomplished by comparing the characteristics of \( I_{\text{PIC}} \) in the sample of 18 cells in this study, which were obtained after intracellular injection of QX-314, to the characteristics of \( I_{\text{PIC}} \) in a control sample of cells \( [n = 31]; \) most of control data were obtained in our previously published study (Lee and Heckman 1998a). As noted in METHODS, all studies, both with and without QX-314, were carried out after administration of a standardized dose of the noradrenergic \( \alpha_1 \) agonist methoxamine. This was done to enhance the probability of encountering bistable behavior in our decerebrate preparation (Lee and Heckman 1999b).

**Intracellular QX-314 reduces the amplitude of \( I_{\text{PIC}} \)**

Because intracellular injection of QX-314 reduces both Na\(^+\) and Ca\(^{2+}\) currents (Talbot and Sayer 1996), we expected that intracellular injection QX-314 would tend to reduce the amplitude of \( I_{\text{PIC}} \) in spinal motoneurons. The histograms in Fig. 3 summarize the distribution of values for the initial peak of \( I_{\text{PIC}} \) in the two cell samples. On average, QX-314 reduced the initial peak amplitude of \( I_{\text{PIC}} \) to ~42% of its value in the control sample, and this reduction was statistically significant (see Table 1). The sustained peak of \( I_{\text{PIC}} \) in the sample of cells with QX-314 was similarly reduced, averaging ~62% of its value in the control sample (also significant; see Table 1). The average values for initial and sustained peaks in the QX-314 sample were not significantly different from each other (\( t \)-test; \( P > 0.4 \)). These results show that QX-314 markedly reduces \( I_{\text{PIC}} \). However, it is clear from Fig. 3 that QX-314 had a wide range of effects on the amplitude of \( I_{\text{PIC}} \) and that, at least in some cells, its amplitude fell well within the control range. Figure 4 shows an \( I-V \) function for a cell in which \( I_{\text{PIC}} \) retained a reasonably large amplitude (~16 nA) despite injection of QX-314. For comparison, the \( I-V \) function for the cell illustrated in Fig. 1 is also included in Fig. 4. This comparison suggests that QX-314 tends to reduce \( I_{\text{PIC}} \) to a greater extent in low than high input conductance cell (0.43 \( \mu \)S) with QX-314. This is the same \( I-V \) function shown in Fig. 1B.

**FIG. 3.** QX-314 markedly reduces the amplitude of the initial peak of \( I_{\text{PIC}} \). The frequency histogram in the top panel shows that most cells recorded with QX-314 had amplitudes of <10 nA. However, some cells had amplitudes that reached well into the range for the control cell sample. Control data without QX-314 primarily from Lee and Heckman (1998a).

**FIG. 4.** QX-314 tended to reduce \( I_{\text{PIC}} \) amplitude to a greater extent in low than high input conductance cells. Thick line: \( I-V \) function for a high input conductance (~1.45 \( \mu \)S) cell with QX-314. Thin line: \( I-V \) function for a low input conductance cell (0.43 \( \mu \)S) with QX-314. This is the same \( I-V \) function shown in Fig. 1B.
input conductance cells. In fact there was a correlation between $I_{\text{PIC}}$ amplitude and input conductance among the cells with QX-314 (for the initial peak, $r = 0.70, P < 0.001$; for the sustained peak, $r = 0.62, P < 0.01$). However, input conductance in part depends on cell size (Binder et al. 1996), so the possibility that the effects of QX-314 vary with cell size is considered in the next section.

**Intracellular QX-314 effects and motoneuron size**

A significant portion of $I_{\text{PIC}}$ originates in dendritic regions (Bennett et al. 1998; Hounsgaard and Kiehn 1993; Lee and Heckman 1996a), so one factor that might influence the effectiveness of QX-314 is how well it diffuses into the dendritic tree. Because high input conductance motoneurons have more extensive dendritic trees than low input conductance motoneurons (Binder et al. 1996), the lesser effect of QX-314 on $I_{\text{PIC}}$ in high input conductance cells may result from a failure of QX-314 to diffuse very far into the dendrites of these larger cells. To evaluate this possibility, we focused on the electrical property of motoneurons that is most closely related to their size, the conduction velocity of the axon. Conduction velocity is directly correlated with dendritic surface area (Burke et al. 1982; Kernels and Zwaagstra 1981) and is unaffected by QX-314 (see METHODS). If QX-314 is less effective in reducing $I_{\text{PIC}}$ in larger cells, then there should be a strong correlation between $I_{\text{PIC}}$ amplitude and conduction velocity in the QX-314 sample. Furthermore, the same correlation in the control sample should be weaker.

Figure 5 shows that, in the control cell sample, there was a relation between estimated conduction velocity (see METHODS) and the initial peak of $I_{\text{PIC}}$, but it was indeed very weak and was not significant (intercept: $-1.51$; slope: $0.22; r = 0.25; P > 0.05$). In contrast, in the QX-314 sample, there is a very strong but highly nonlinear relationship between the initial peak of $I_{\text{PIC}}$ and estimated conduction velocity (Fig. 5). $I_{\text{PIC}}$ is markedly reduced in cells with conduction velocities below ~100 m/s. Above this point, $I_{\text{PIC}}$ amplitude increases very steeply with conduction velocity. The relation between the sustained peak of $I_{\text{PIC}}$ and conduction velocity was similarly nonlinear (not shown). Log transformations of the initial and sustained peaks of $I_{\text{PIC}}$ resulted in correlation coefficients of $r = 0.78 (P < 0.001)$ and $r = 0.68 (P < 0.01)$, respectively. These results suggest that QX-314 may fail to diffuse far into the dendrites of the higher conduction velocity cells. An alternative explanation, which is considered in the DISCUSSION, is that the ionic currents that generated $I_{\text{PIC}}$ differ in cells with slow and fast conduction velocities.

**QX-314 alters several other aspects of the motoneuron $I-V$ function**

In addition to its strong impact on $I_{\text{PIC}}$ amplitude, QX-314 had several other subtle but interesting effects. The following sections consider alterations in the onset and offset of $I_{\text{PIC}}$ and also changes in the subthreshold region of the $I-V$ function.

**QX-314 linearizes the subthreshold region of the $I-V$ function.** Figure 6 compares the average ascending $I-V$ functions for the QX-314 sample to the average for the control sample. The reduction in the initial peak of $I_{\text{PIC}}$ due to QX-314 is clear, but this figure also reveals a difference in the subthreshold region of the $I-V$ function. The average subthreshold $I-V$ function in the QX-314 sample lacks the curvature, or rectification, of the average subthreshold function in the control sample. To quantify these differences in curvature, quadratic functions were fit to the region of each cell’s $I-V$ function below $I_{\text{PIC}}$ activation.

The term describing the curvature of the fitted function was significantly smaller for the QX-314 sample than for the control sample (QX-314: $-0.001 \text{nA/mV}^2$; control: $-0.018 \text{nA/mV}^2$; t-test, $P < 0.003$). This difference in curvature did not have major impact on average input conductance, which was calculated over a 5- to 10-mV range in the subthreshold region and was not significantly different in the two data sets (QX-314 data sample: $0.82 \pm 0.43 \mu\text{S}$; control data sample: $0.94 \pm 0.28 \mu\text{S}$; t-test, $P > 0.3$). The linearity of the subthreshold $I-V$ function in the presence of QX-314 was...
seen in both low and high input conductance cells, as is apparent from the examples in Fig. 4. At depolarized levels, the linearity is probably due to the suppression in amplitude of the Ca$^{2+}$ and Na$^+$ currents that generate $I_{\text{PIC}}$ (cf. Schwindt and Crill 1980). At hyperpolarized levels, the linearity may reflect suppression of an H-current, because previous studies have shown that QX-314 is an effective H-current blocker (Perkins and Wong 1995).

QX-314 DEPOLARIZES THE ONSET VOLTAGE FOR $I_{\text{PIC}}$ IN SOME CELLS. In the control population, the voltages for the onset of $I_{\text{PIC}}$ tended to occur at more depolarized levels in partially bistable cells than in fully bistable cells. As a consequence, onset correlated with input conductance in the control sample, as shown in Fig. 7A. In the QX-314 sample, the relation between onset voltage and input conductance was seriously degraded (Fig. 7A). However, the average voltage level for onset in the QX-314 sample was not significantly different from in the control sample (see Table 1). Most of the reduced slope for the onset-input conductance relationship with QX-314 appears to be due to a depolarizing shift in onset in many of the low input conductance cells. One possible reason for poor onset-input conductance relation may be that QX-314 has its greatest effect in the soma and proximal dendrites. Thus much of $I_{\text{PIC}}$ remaining after QX-314 injection would originate in the distal dendrites, where it would be electrically distant from a voltage clamp applied at the soma (see DISCUSSION).

Previous work has shown that the bromide ions introduced into the cell by the use of the bromide salt of QX-314 can produce a depolarizing shift in the $I-V$ function (Talbot and Sayer 1996). The chloride salt of QX-314 does not have this effect (Talbot and Sayer 1996). In the present study, the concentration of chloride in our electrodes was very much higher than bromide because our main filling solution was 3 M KCl, whereas the QX-314 concentration was only 50–100 mM. Consequently, the changes in the $I-V$ function in the present study are unlikely to be due to intracellular bromide.

QX-314 HYPERPOLARIZES THE OFFSET VOLTAGES FOR $I_{\text{PIC}}$ IN SOME CELLS. Like onset, the offset of $I_{\text{PIC}}$ tends to be more hyperpolarized in low input conductance cells. Figure 7B shows that the strong relation between offset voltage and input conductance seen in the control cell sample was still very evident in the QX-314 sample. Note however that, in the high input conductance cells, offset voltage appears to be shifted to a more hyperpolarized level in the QX-314 sample than in the control sample. As a result, average voltage level in the QX-314 sample was 7 mV lower than in the control sample (this difference did not quite reach our conservative level of statistical significance during multiple comparisons; see Table 1). This modest shift in offset voltage for high input conductance cells may reflect a change in the persistence of $I_{\text{PIC}}$ (see the final section of RESULTS).

QX-314 INCREASES THE HYSTERESIS IN $I_{\text{PIC}}$ ONSETS AND OFFSETS. Comparison of Fig. 7, A and B, reveals an important characteristic of the activation and deactivation of $I_{\text{PIC}}$: offset occurs at a substantially more hyperpolarized voltage level than onset (see also Figs. 1 and 4). The primary source of this hysteresis...
is probably that a major portion of $I_{PIC}$ originates in dendritic regions, which are likely under poor space clamp (Lee and Heckman 1998a). In addition, in the control cell sample, voltage hysteresis in $I_{PIC}$ was greatest in fully bistable cells. As a result, there was a strong inverse relation between hysteresis and input conductance. Figure 7C shows that QX-314 actually increased hysteresis by shifting this relationship upward without much altering its slope. This suggests that, even in the presence of QX-314, a substantial portion of $I_{PIC}$ still originates in dendritic regions. The tendency for increased hysteresis in the cells with QX-314 in low input conductance cells may occur because this agent is most effective in reducing $I_{PIC}$ near the soma, leaving the dendritic portion relatively unaffected (see DISCUSSION).

Plateau potentials in cells with QX-314

The application of QX-314 to bistable motoneurons reveals the existence of plateau potentials during current clamp. A triangular shaped injected current was applied in six cells to reveal the plateau potential generated by $I_{PIC}$. The time course of the injected current was slow (5 nA/s), giving overall rates of change of voltage that roughly matched those during the voltage-clamp protocols used to measure $I_{PIC}$. Figure 8A shows that the plateau potential evoked by the triangular current input had a sharp onset and also that its offset current was considerably below its onset current. This plateau behavior was seen in all six cells and is very similar to that seen in previous studies of motoneurons with QX-314 (Bennett et al. 1998; Brownstone et al. 1994). In Fig. 8B, the relations between current and voltage obtained in both current-clamp and voltage-clamp conditions for one cell (same as in 8A) are superimposed. The resulting functions are very similar, with the exception that, during current clamp, the onset of the plateau produces a sharp jump in voltage. This is expected from the instability generated by the negative slope conductance evident in the same region during voltage clamp. The offset of the plateau was then more gradual than during voltage clamp, which presumably reflects the lesser degree of control over dendritic regions during current clamp as compared with voltage clamp. Similar results were obtained for all six cells. It was noteworthy that even though QX-314 dramatically reduces $I_{PIC}$, it still provided sufficient current to generate a substantial plateau potential ($\sim 10$ mV in the case of Fig. 8A). Overall, the plateau potential behaviors observed in this study were very similar to the behaviors seen in previous studies (Bennett et al. 1998; Brownstone et al. 1994).

Intracellular QX-314 eliminated the slow decay of $I_{PIC}$ in high input conductance cells

In the control cell sample, $I_{PIC}$ exhibited a much greater tendency for decay with time in high input conductance cells than in low input conductance cells (Lee and Heckman 1998a).
However, Fig. 9A shows a high input conductance cell with QX-314 in which there is no detectable decay in $I_{\text{PIC}}$. Figure 9B shows a nondecaying plateau potential in another high-input conductance cell with QX-314. Figure 10 compares the persistence of $I_{\text{PIC}}$ for 11 cells with QX-314 compared with that seen in the 11 cells from the control population, with each data set plotted as a function of input conductance. In the QX-314 sample, persistence in 3 of the 11 cells was assessed from the plateau potential, whereas, in the other 8 cells, persistence was measured from $I_{\text{PIC}}$ (in 3 of these 8 cells, plateau potential persistence was found to be nearly identical to that of $I_{\text{PIC}}$). In the control data set, persistence declines precipitously with input conductance. In contrast, $I_{\text{PIC}}$ was highly resistant to decay in all of the cells with QX-314, regardless of input conductance.

An additional way of estimating the persistence of $I_{\text{PIC}}$, using the $I-V$ function, strongly supported the conclusion that QX-314 made $I_{\text{PIC}}$ highly resistant to decay. The triangular voltage command used to generate the $I-V$ function was slow, so that $\sim 3-4$ s usually elapsed between the initial and sustained peaks of $I_{\text{PIC}}$ (see Fig. 1). Thus the closer the amplitude of the sustained peak to the initial peak, the greater the persistence of $I_{\text{PIC}}$. Table 1 shows that the sustained peak was reduced by $\sim 9$ nA compared with the initial peak in the control data set, whereas this reduction was much smaller in the cells with QX-314. $\sim 1.5$ nA. As noted above, this meant that the initial and sustained peaks in the QX-314 data set were not significantly different from each other. In five of the cells in the QX-314 sample, the sustained peak was actually slightly larger than the initial peak (as illustrated by the $I-V$ function for the cell in Fig. 1). The lack of decay of $I_{\text{PIC}}$ in the cells with QX-314 indicates that a major portion of the inward current that generates $I_{\text{PIC}}$ is highly persistent even in high input conductance motoneurons. Thus the tendency for decay of $I_{\text{PIC}}$ in high input conductance motoneurons without QX-314 may be due to activation of slow outward currents (see Discussion).

**DISCUSSION**

The effect of QX-314 on $I_{\text{PIC}}$ in spinal motoneurons in the cat was paradoxical in the sense that QX-314 greatly reduced the amplitude of $I_{\text{PIC}}$ but nonetheless increased its persistence. However, these effects of QX-314 varied in a systematic fashion between high and low input conductance motoneurons. The largest reduction in amplitude of $I_{\text{PIC}}$ with QX-314 was seen in low input conductance, slow conduction velocity motoneurons, which probably correspond to the fully bistable cells in the control sample. QX-314 did not noticeably increase persistence of $I_{\text{PIC}}$ in these low input conductance cells because fully bistable cells already exhibit little or no time-dependent decay in $I_{\text{PIC}}$. In high input conductance, fast conduction velocity motoneurons, which probably correspond to the partially bistable cells in the control cell sample, QX-314 had a lesser impact on the amplitude of $I_{\text{PIC}}$ but entirely eliminated its tendency to decay.

These results show that, as in other cells (e.g., Perkins and Wong 1995; Talbot and Sayer 1996), QX-314 does much more than block action potentials in motoneurons. Thus measurements of plateau potentials in motoneurons with QX-314 do not provide an accurate reflection of the characteristics of bistable behavior or the properties of $I_{\text{PIC}}$ in cells without QX-314. In some types of studies, the suppression of $I_{\text{PIC}}$ along with the spike may in fact be desirable. $I_{\text{PIC}}$ provides very potent amplification of synaptic inputs (Lee and Heckman 1996), which might tend to obscure the precise pattern of synaptic inputs generated by different sources onto motoneurons. Thus intracellular QX-314 might aid in investigating the functional anatomy of synaptic inputs onto motoneurons. On the other hand, it is likely that suppression of $I_{\text{PIC}}$ and other currents by QX-314 would dramatically alter synaptic integration within the motoneuron. This alteration precludes conclusions about normal synaptic integration based on cells with QX-314, but comparisons of synaptic integration between cells with and without QX-314 might provide useful insights about this fundamental issue.

**Relation between the amplitude of $I_{\text{PIC}}$ and conduction velocity**

One possible explanation for the strong relationship between $I_{\text{PIC}}$ amplitude and conduction velocity (Fig. 5) is that the ionic composition of $I_{\text{PIC}}$ may differ in fully and partially bistable cells. Because QX-314 is more effective in blocking Na$^+$ than Ca$^{2+}$ currents, the reason that $I_{\text{PIC}}$ was smallest in low input conductance cells may be that a larger proportion of the total current in fully bistable cells is due to Na$^+$ rather than Ca$^{2+}$ channels. Our results do not exclude this possibility, but one key aspect of the data suggests that a different ionic composition is not the whole explanation. In fast conduction velocity cells, the amplitude of $I_{\text{PIC}}$ was only slightly reduced (see the high conduction velocity region of Fig. 5). Based on measurements in hippocampal cells in a slice preparation (Talbot and Sayer 1996), the concentration of QX-314 that blocks Na$^+$ spikes should reduce Ca$^{2+}$ currents to $\sim 20\%$ of their control.

![Graph showing lack of decay in $I_{\text{PIC}}$ in both low and high input conductance cells](http://jn.physiology.org/)
amplitude. If a Ca-mediated current contributes to $I_{\text{PC}}$, it too would be drastically reduced by such a sharp drop in Ca$^{2+}$ entry. Thus QX-314 did not reduce the amplitude of $I_{\text{PC}}$ in high input conductance motoneurons to the extent expected from its actions on both Na$^+$ and Ca$^{2+}$ currents in other types of neurons.

For this reason, we suggested an alternative explanation in results, namely the failure of QX-314 to diffuse far enough into the dendrites of the fast conduction velocity cells to suppress the dendritic component of $I_{\text{PC}}$. Although the rate of diffusion of QX-314 into the narrow branches of dendritic trees is not known, it is clear that motoneurons with fast conduction velocities tend to have more extensive dendritic trees than motoneurons with slow conduction velocities (Burke et al. 1982; Kernell and Zwagstra 1981). Furthermore, it is clear that a substantial portion of $I_{\text{PC}}$ originates in dendritic regions (Bennett et al. 1998; Hounsgaard and Kiehn 1993; Lee and Heckman 1996). In motoneurons with conduction velocities of $\sim 100$ m/s or slower, QX-314 did in fact reduce the initial peak of $I_{\text{PC}}$ to the level expected on the basis of its effect on Ca$^{2+}$ currents (Talbot and Sayer 1996), i.e., $\sim 20\%$ of the control amplitude (see Fig. 5). In contrast, in some cells with conduction velocities above 100 m/s, the amplitude of $I_{\text{PC}}$ was well within the range of control values. This lack of effect is consistent with a failure of diffusion of QX-314 into dendritic regions of the large, fast conduction velocity cells. Note that QX-314 was effective in blocking rhythmic firing in all cells, including the fastest conduction velocity neurons. Thus it is unlikely that the lesser reduction of $I_{\text{PC}}$ in the fastest conduction velocity cells was due to a failure to attain sufficient entry of QX-314 from the electrode into the soma and initial segment.

One prediction of slow diffusion of QX-314 is that the amplitude of $I_{\text{PC}}$ should decline with time in the fast conduction velocity cells. This prediction was difficult to test in our in vivo preparation because $I_{\text{PC}}$ seems to be very sensitive to recording quality and often tends to decline with time even in the control cells without QX-314. Therefore all comparisons were based on data in a similar time window, 1–3 min post spike failure. Higher concentrations of QX-314 in the electrodes may have increased the rate of diffusion into the dendrites, but we found that concentrations above 100 mM tended to degrade transient behavior of our electrodes and prevent good discontinuous voltage clamp.

A differential impact of QX-314 on the somatic versus dendritic components of $I_{\text{PC}}$ may also account for the increased onset-offset hysteresis seen in low input conductance cells with QX-314 (Fig. 7C). Computer simulations with a simple motoneuron model consisting of a somatic compartment coupled to a single dendritic compartment showed that increasing the electrical isolation between the two compartments increased onset-offset hysteresis (Lee and Heckman 1999a). Increasing the proportion of $I_{\text{PC}}$ in the dendrites versus the soma has a similar effect (unpublished observations). Thus a differential effect of QX-314 on the soma versus dendritic components of $I_{\text{PC}}$ should also increase hysteresis. However, this explanation may not apply to high input conductance cells. It was argued above that the nonlinear relationship between conduction velocity and $I_{\text{PC}}$ meant that QX-314 did not much affect the dendritic component of $I_{\text{PC}}$ in high input conductance cells. In these cells, the increased hysteresis with QX-314 may largely be due to the hyperpolarization of the offset of $I_{\text{PC}}$. This hyperpolarization is probably due to the increased persistence of $I_{\text{PC}}$ in high input conductance cells (see the following section).

**Persistence**

The most surprising result in this study was the marked enhancement of the persistence of $I_{\text{PC}}$ in the high input conductance cells with QX-314. This enhanced persistence provides an important insight into why $I_{\text{PC}}$ tends to decay in these cells in the absence of QX-314. $I_{\text{PC}}$ decay could be due to slow inactivation of one or more channels producing $I_{\text{PC}}$ or to slow development of an outward current to counterbalance $I_{\text{PC}}$. The elimination of decay by QX-314 shows that at least part of $I_{\text{PC}}$ in high input conductance cells does not undergo inactivation over the course of 10 s. Furthermore, because QX-314 was less effective in suppressing the amplitude of $I_{\text{PC}}$ in high input conductance motoneurons, the proportion of $I_{\text{PC}}$ that does not inactivate forms the majority of the total current. Therefore QX-314 probably does not alter decay by simply removing an inactivating component of $I_{\text{PC}}$. It seems reasonable to suppose that the decay of $I_{\text{PC}}$ in partially bistable cells is due to slow activation of an outward current instead of inactivation of $I_{\text{PC}}$.

How is it that QX-314 can have such a large impact on persistence in the cell in which it had the least effect on amplitude? As for many of the actions of QX-314, this paradox probably arises from a differential impact on somatic versus dendritic regions of the cell. The strong effect on persistence suggests that QX-314 is somehow reducing an outward current that is in or near the soma. The lack of effect on $I_{\text{PC}}$ amplitude, as noted above, likely occurs for the converse reason: most of $I_{\text{PC}}$ is generated in dendritic regions where the impact of QX-314 is limited by slow diffusion.

The present results do not reveal which outward current might produce the slow decay in high input conductance cells, but one factor that is likely to be important is that $I_{\text{PC}}$ onset and offset occur in a more depolarized range in these cells (see Fig. 7, A and B). In fact, in high input conductance, partially bistable cells, $I_{\text{PC}}$ is not activated until several millivolts above spike threshold (Lee and Heckman 1998b). Thus one possibility is that the onset of $I_{\text{PC}}$ is accompanied by some degree of activation of the delayed rectifier K$^{+}$ current. However, it is not clear how activation of the delayed rectifier could account for the slow time course of decay. Typically, the time constant for decay of $I_{\text{PC}}$ in high input conductance cells is on the order of several seconds, whereas the slowest time constants for activation of the delayed rectifier are on the order of milliseconds.

An alternative possibility is that the more depolarized level results in a greater influx of Ca$^{2+}$ in the soma, which then slowly increases intracellular Ca$^{2+}$ concentration. The increased Ca$^{2+}$ concentration could then slowly activate a Ca-mediated K$^{+}$ current, such as that which generates the spike AHP. By limiting Ca$^{2+}$ entry, QX-314 could prevent excessive buildup of intracellular Ca$^{2+}$ and reduce activation of the Ca-mediated K$^{+}$ current. Because QX-314 may be ineffective in reaching dendritic regions, the crucial area for reducing Ca$^{2+}$ entry and buildup of Ca$^{2+}$ concentration may be at or near the soma. The possibility that QX-314 acts directly on the
K+ channels cannot be excluded. QX-314 has been shown to suppress the large-conductance, Ca-mediated K+ channel in patch-clamp recordings in excised patches from hippocampal neurons (Oda et al. 1992). However, this suppression may only occur at very high concentrations of QX-314 because this K+ conductance was not affected by intracellular injection in hippocampal cells (Connors and Prince 1982).

Finally, it should be noted that reduction in net amplitude of IPSC is not necessarily enough to prevent its decay. We have recently completed a study of IPSC in the decerebrate preparation without application of methoxamine (Lee and Heckman 1999b). In that study, IPSC average amplitude was ~11 nA, which is only slightly larger than the 8 nA seen in the QX-314 sample in the present study in the decerebrate with methoxamine. Despite the reduction in amplitude of IPSC in the decerebrate without methoxamine, the tendency for IPSC to slowly decay in high input conductance cells was still clearly apparent. We suspect this is because the main outward current that provides this decay in high input conductance cells is located at or near the soma, where it is strongly affected by QX-314 in the present study.

Overall, the enhanced persistence of IPSC in partially bistable cells in the presence of QX-314 is an important result because it suggests that IPSC in partially bistable cells is essentially similar to IPSC in fully bistable cells. This suggests that the ionic composition of IPSC is similar in both types of motoneurons. There remains, however, the important issue of understanding why IPSC is activated at a more depolarized level in high input conductance motoneurons than low input conductance ones. One possibility is that differences in electrical structures of the dendrites in low and high input conductance cells affect the apparent voltage threshold as seen from the soma. However, considerable further work examining the types and distributions of voltage-sensitive channels on motoneuron dendrites is required before this possibility can be evaluated.

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