Characterization of Na\textsuperscript{+}-Activated K\textsuperscript{+} Currents in Larval Lamprey Spinal Cord Neurons

Dietmar Hess, E. Nanou, and Abdeljabbar El Manira
Nobel Institute for Neurophysiology, Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Submitted 18 July 2006; accepted in final form 15 February 2007

Hess D, Nanou E, El Manira A. Characterization of Na\textsuperscript{+}-activated K\textsuperscript{+} currents in larval lamprey spinal cord neurons. J Neurophysiol 97: 3484–3493, 2007. First published February 28, 2007; doi:10.1152/jn.00742.2006. Potassium channels play an important role in controlling neuronal firing and synaptic interactions. Na\textsuperscript{+}-activated K\textsuperscript{+} (K\textsubscript{Na}) channels have been shown to exist in neurons in different regions of the CNS, but their physiological function has been difficult to assess. In this study, we have examined if neurons in the spinal cord possess K\textsubscript{Na} currents. We used whole cell recordings from isolated spinal cord neurons in lamprey. These neurons display two different K\textsubscript{Na} currents. The first was transient and activated by the Na\textsuperscript{+} influx during the action potentials, and it was abolished when Na\textsuperscript{+} channels were blocked by tetrodotoxin. The second K\textsubscript{Na} current was sustained and persisted in tetrodotoxin. Both K\textsubscript{Na} currents were abolished when Na\textsuperscript{+} was substituted with choline or N-methyl-D-glucamine, indicating that they are indeed dependent on Na\textsuperscript{+} influx into neurons. When Na\textsuperscript{+} was substituted with Li\textsuperscript{+}, the amplitude of the inward current was unchanged, whereas the transient K\textsubscript{Na} current was reduced but not abolished. This suggests that the transient K\textsubscript{Na} current is partially activated by Li\textsuperscript{+}. These two K\textsubscript{Na} currents have different roles in controlling the action potential waveform. The transient K\textsubscript{Na} appears to act as a negative feedback mechanism sensing the Na\textsuperscript{+} influx underlying the action potential and may thus be critical for setting the amplitude and duration of the action potential. The sustained K\textsubscript{Na} current has a slow kinetic of activation and may underlie the slow Ca\textsuperscript{2+}-independent afterhyperpolarization mediated by repetitive firing in lamprey spinal cord neurons.

INTRODUCTION

Potassium channels are critical for determining the shape of the action potentials, the neuronal firing pattern and the strength of synaptic transmission (Augustine 1990; D’Incamps et al. 2004; Geiger and Jonas 2000; Meir et al. 1999; Rooper and Pongs 1996; Sabatini and Regehr 1999). Various types of K\textsuperscript{+} channels have been characterized using biophysical, pharmacological, and molecular analyses; these include channels activated by voltage changes and intracellular Ca\textsuperscript{2+} (Coetzee et al. 1999; Song 2002). Evidence is accumulating that K\textsuperscript{+} channels activated by intracellular Na\textsuperscript{+} exist in neurons, but their function is largely unclear (Bhattacharjee and Kaczmarek 2005; Dryer 1994). Sodium-activated K\textsuperscript{+} (K\textsubscript{Na}) channels were originally described in heart myocytes (Kameyama et al. 1984) and were subsequently found in neurons in invertebrates (Hartung 1985) and vertebrates (Dale 1993; Dryer 1991; Dryer et al. 1989; Haiman et al. 1992; Koh et al. 1994; Safronov et al. 1996). They can be activated by Na\textsuperscript{+} influx via voltage-gated channels or through leak channels (Bhattacharjee and Kaczmarek 2005; Dryer 1994; Zhou et al. 2004). In addition, there is evidence that Na\textsuperscript{+} influx following single action potentials can activate K\textsubscript{Na} channels (Dryer 1994; Hartung 1985; Liu and Stan Leung 2004).

Two genes encoding for K\textsubscript{Na} channels have recently been identified, slick (Slo2.1) and slack (Slo2.2) (Bhattacharjee et al. 2003; Yuan et al. 2003). The distribution of these channels in the brain studied by immunohistochemistry corresponds to the regions where neurons possessing K\textsubscript{Na} channels have been characterized (Bhattacharjee et al. 2002, 2005). The physiological function of the native K\textsubscript{Na} channels in cellular and synaptic processing has been difficult to undertake because of the lack of specific blockers. However, there is evidence suggesting that K\textsubscript{Na} channels play a role in regulating the firing frequency of neurons (Sanchez-Vives et al. 2000), intrinsic bursting in cortical neurons (Francescetti et al. 2003), and the timing of spindle waves in thalamus (Kim and McCormick 1998). In addition these channels are the target of modulation by G-protein-coupled receptors and intracellular signaling molecules (Santi et al. 2005).

Neurons in the spinal cord of the lamprey display a slow afterhyperpolarization that is important for spike frequency adaptation that has been thought to be exclusively mediated by activation of K\textsubscript{Ca} channels (Grillner 2003; Wallen et al. 1989). Recently, however, it has been shown that blockade of voltage-gated Ca\textsuperscript{2+} channels or chelating intracellular Ca\textsuperscript{2+} only reduced the amplitude of the slow AHP (Cangiano et al. 2002). A residual component of the AHP insensitive to Ca\textsuperscript{2+} was present and has been suggested to be mediated by K\textsubscript{Na} channels (Cangiano et al. 2002; Wallen et al. 2005). However, there is no direct evidence showing the existence of K\textsubscript{Na} current in lamprey spinal cord neurons. In this study, we have used patch-clamp recordings from lamprey spinal cord neurons to determine if they possess K\textsuperscript{+} currents activated by Na\textsuperscript{+} influx. Our results show that spinal cord neurons display two types of K\textsubscript{Na} currents: a transient current activated by the Na\textsuperscript{+} influx during the action potential that appears to be important for initiating the repolarization of the action potential and a second component consisting of a sustained K\textsubscript{Na} current most likely activated by Na\textsuperscript{+} influx through leak channels and which may play a role in mediating the Ca\textsuperscript{2+} insensitive AHP induced by high-frequency firing (Wallen et al. 2005).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

Cell dissociation

Larval lampreys (Petromyzon marinus) were used in all experiments. The spinal cord was dissociated in Leibovitz’s L-15 culture medium (Sigma, St. Louis, MO) supplemented with penicillin-streptomycin (2 μg/ml; Sigma), and the osmolarity was adjusted to 270 mosm. After treatment with collagenase (1 mg/ml; 30 min; Sigma) and protease (2 mg/ml, 45 min; Sigma), the tissue was subsequently washed with the culture medium and triturated through a sterilized pipette. The dissociated cells were distributed in petri dishes and incubated at 10°C for 2–5 days (El Manira and Bussieres 1997).

Electrophysiology

All spinal neurons were recorded using a patch-clamp amplifier (Axopatch 200A, Axon Instruments, Foster City, CA). When investigating very fast currents, like Na+, currents, a high quality of the clamping condition is required. To guarantee the best space-clamp condition possible, only small neurons with a diameter <10–15 μm were chosen. The neurons were either mono- or multipolar corresponding mainly to moto- and interneurons. The mechanosensory dorsal cells could be easily identified by their round and large cell bodies. Because these neurons are not part of the locomotor network, they were not included in this study. The series resistance range was compensated for electronically by 75–85%. Linear leak and residual capacity currents were subtracted on-line with the use of a P/4 subtraction protocol. The liquid junction potential was calculated to range between 3 and 5 mV and was not corrected. The neurons were clamped at a holding potential of −60 mV, and response currents were evoked by depolarizing voltage steps of 100 ms with 5 ms interstimulus intervals. Current and voltage signals were sampled at 10 or 100 kHz.

The control solution contained (in mM) 124 NaCl, 2 KCl, 1.2 MgCl2, 2 CaCl2, 10 glucose, and 10 HEPES with pH adjusted to 7.6 with NaOH. In some experiments, extracellular Na+ was replaced with Li+, choline, or N-methyl-d-glucamine (NMDG). In experiments regarding the biophysical properties of Na+ and Li+ passing the voltage-gated Na+ channel, an extracellular solution containing TEA (20 mM), CsCl2 (100 μM), Ni2+ (50 μM) was used. For whole cell recordings, the pipettes were filled with a solution containing (in mM) 102 KCH3SO3, 1.2 MgCl2, 10 glucose, and 10 HEPES, pH 7.6 adjusted with KOH. To study Na+ and Li+ current activation, KCH3SO3 was replaced with CsCH3SO3 to block K+ currents.

Analysis

Current peak corresponds to the maximum current reached. Time to peak was measured as the time between the onset of the stimulus and the peak of the current. To obtain the Na+ and the different K+ current activation and inactivation curves, values of the chord conductance (G) were calculated from the respective peak or sustained currents assuming ohmic behavior. The Na+ and K+ equilibrium potentials were determined using the Nernst equation and calculation of the linear part of the I-V plot. The analysis was performed using Axon Instruments software, PlotIt (Scientific Programming Enterprises, Haslett, MI) or Origin (Microcal Software, Northampton, MA). Unless otherwise stated, the results are expressed as means ± SE. Means were compared using Student’s t-test or one-way ANOVA (Graphpad).

RESULTS

TTX blocks a transient sodium-dependent potassium current

To determine if lamprey spinal cord neurons possess Na+ -dependent K+ currents, whole cell patch-clamp recordings were performed from neurons in culture. Ca2+ currents were blocked by Cd2+ (50–100 μM) and Ni+ (200 μM). Application of voltage steps to −10 mV from a holding potential of −60 mV activated the transient Na+ inward current (INa) that was always followed by a transient and a sustained outward K+ current (Fig. 1A). Application of TTX (600 nM) blocked the Na+ current and also abolished the transient K+ current (Fig. 1, A and B). Subtraction of the current induced in TTX from that induced in control revealed the currents blocked by TTX that consisted of an inward Na+ current and an outward Na+-dependent K+ (KNa) current (Fig. 1, C and D). The amplitude of the Na+ current was 8.12 ± 0.52 nA (n = 36), and its peak was reached 0.43 ± 0.01 ms after the onset of the voltage step. The amplitude of the transient KNa current was 3.24 ± 0.25 nA (n = 36) and reached its peak 1.0 ± 0.02 ms after the onset of the voltage step. The application of TTX had little effect on the sustained outward K+ current the amplitude of which was slightly increased from 1.73 ± 0.13 to 1.81 ± 0.13 nA (105 ± 0.78%; n = 33; P > 0.05; Fig. 1, A and B).

In the neurons examined, the series resistance was always adequately compensated and the voltage command settled within <400 μs, and the peak Na+ current occurred at around 500–700 μs after the onset of the voltage step (Fig. 2, A and B), indicating that the neurons were adequately clamped. In these neurons, the inward Na+ current was always followed by a transient KNa current, suggesting that this current is not merely an artifact of inadequate voltage-clamp conditions. To further determine if the neurons were voltage clamped appropriately, we examined the effect of TTX on the kinetics of the Na+ current. Voltage steps to −10 mV were applied from a holding potential of −60 mV to activate Na+ and transient KNa currents. Application of TTX gradually decreased the amplitude of the Na+ current (Fig. 1, B) and the transient KNa currents (Fig. 2, A and B). The time to peak of the inward Na+ current did not show any shift as its amplitude gradually decreased (Fig. 2, A and B). In addition, there was a clear correlation between the TTX-induced
decrease of the inward Na\(^+\) current and that of the transient \(K_{Na}\) (Fig. 2C). The amplitude of the sustained \(K^+\) current was not significantly changed in the presence of TTX (Fig. 2C). These results suggest that the TTX-sensitive transient outward current is not due to incomplete voltage clamp but is mediated by \(K_{Na}\) channels activated by Na\(^+\) influx underlying action potentials.

\(K_{Na}\) currents in spinal cord neurons

To examine if the sustained \(K^+\) current is mediated by activation of \(K_{Na}\) channels, we tested the effect of replacement of extracellular Na\(^+\) with equimolar amount of lithium, choline, or NMDG on the amplitude of this current. Li\(^+\) is known to enter voltage-gated Na\(^+\) channels but does not usually activate Na\(^+\)-dependent targets such as \(K_{Na}\) channels (Bischoff et al. 1998; Dryer et al. 1989). Replacing Na\(^+\) with Li\(^+\) decreased the amplitude of the transient outward current providing further support for the existence of a transient \(K_{Na}\) current that is partially activated by Li\(^+\). The amplitude of the transient \(K_{Na}\) was 4.23 \pm 0.25 nA in control and decreased to 3.32 \pm 0.21 nA in Li\(^+\) (\(P < 0.001\); \(n = 9\); Fig. 3, A and B), whereas its time to peak increased from 0.94 \pm 0.02 ms in control to 1.08 \pm 0.03 ms in Li\(^+\) (\(P < 0.001\); \(n = 9\); Fig. 3, A and B). The amplitude of the inward current was not significantly changed in Li\(^+\) (\(P > 0.05\); Fig. 3B). TTX (600 nM) blocked both the inward current mediated by Li\(^+\) and the resulting transient \(K^+\) current (Fig. 3C). In the presence of TTX, replacing Na\(^+\) with Li\(^+\) decreased the amplitude of the sustained \(K^+\) current from 1.48 \pm 0.14 to 1.04 \pm 0.12 nA (by 30.71 \pm 2.28%; \(P < 0.03\); \(n = 9\); Fig. 3C). These results suggest that a component of the sustained current is mediated by activation of \(K_{Na}\) channels that is blocked when Na\(^+\) is replaced with Li\(^+\). It thus appears that lamprey spinal cord neurons possess two types of \(K_{Na}\) channels. One is transient, activated by Na\(^+\) influx through the voltage-gated channels underlying the action potential and is partially activated by Li\(^+\). The second is sustained and is blocked when Na\(^+\) is replaced with Li\(^+\).

We compared the activation and inactivation curves of Na\(^+\) and Li\(^+\) inward current to determine if the change in the kinetics of the inward current can be due to a shift in the activation of the Na\(^+\) channels when Li\(^+\) is used as charge carrier. In these experiments, both Ca\(^{2+}\) and K\(^+\) currents were blocked by Cd\(^{2+}\) and intracellular Cs\(^+\), respectively, leaving only the inward Na\(^+\) or Li\(^+\) current (Fig. 3E). Li\(^+\) shifted the activation curve of the inward current by +3.43 mV (1/2 maximum activation: Na\(^+\) = -21.4 mV vs. Li\(^+\) = -17.97 mV; Fig. 3D; \(n = 16\)). There was no change in the inactivation curve between Na\(^+\) and Li\(^+\) (Fig. 3D).
Pharmacological isolation of the transient $K_{Na}$ current

In addition to the two types of $K_{Na}$ currents, lamprey spinal cord neurons also possess a high-voltage-activated $K^+$ current with a transient ($K_t$) and a sustained component (Hess and El Manira 2001). To address the functional role of the transient $K_{Na}$ current and its contribution to the action potential waveform, it was necessary to isolate this current from the voltage-activated $K_t$ (Fig. 4A). We previously showed that catechol at low concentration (≤100 µM) blocked the voltage-activated $K_t$ current (Hess and El Manira 2001). At high concentrations, catechol (200–500 µM; $n = 7$) also blocked the transient $K_{Na}$ current (Fig. 4Bi). To be able to compare the contribution of the $K_{Na}$ current and that of the $K_t$ to the waveform of the action potential, these currents need to be separated. To this end, voltage steps to −10 mV were applied from a holding potential of −60 mV, and the effect of catechol and TTX (600 nM) was first tested separately on the elicited current, and after their respective washout, they were added together (Fig. 4, A and Bi). By subtracting the currents elicited in the different conditions (Fig. 4B), it was possible to separate the individual currents. The current blocked by TTX (I-TTX), with its $Na^+$ and transient $K_{Na}$ components, was isolated by subtracting the current elicited in TTX from that of control (black trace in Fig. 4Bi). The voltage-gated $Na^+$ current (I-Na) corresponded to the current blocked by TTX in the presence of catechol (cyan trace in Fig. 4Bi). The transient $K_{Na}$ current (I-$K_{Na}$) was isolated by subtracting I-Na from the $I_{TTX}$ (Fig. 4Bi). The sustained high-voltage-activated $K^+$ current (I-$K_t$) corresponded the current persisting in the presence of catechol and TTX, whereas the transient high-voltage-activated $K^+$ current (I-$K_t$) represented the current blocked by catechol in the presence of TTX (green trace in Fig. 4Bi).

Voltage activation range of the $K_{Na}$ and $K_t$

The voltage range at which the $K_{Na}$ and the $K_t$ are activated was determined using a protocol with voltage steps from −50 to +40 mV with +10-mV increments from a holding potential of −60 mV. These experiments were performed in the presence of $Cd^{2+}$ (100 µM) and Ni$^2+$ (50 µM) to block all Ca$^{2+}$ currents. Application of catechol (200–500 µM) blocked the transient $K_{Na}$ at lower voltage steps and blocked both the $K_{Na}$ and the $K_t$ at high-voltage steps (Fig. 5, A and Bi; green traces). TTX alone blocked the inward $Na^+$ current and the transient $K_{Na}$ (Fig. 5, A and Bi; magenta traces). Co-application of TTX and catechol blocked the total current at low voltage steps (Fig. 5A; blue trace) and blocked all transient currents at high-voltage steps leaving only the voltage-activated sustained $K^+$ current (Fig. 5B, blue trace). The contribution of the transient $K_{Na}$ and voltage-activated $K_t$ current to the total outward current was plotted as a function of the test potential (Fig. 5E). It was clear that the two currents were activated within different voltage windows. The transient $K_{Na}$ started activating at lower voltage steps compared with the voltage-gated $K_t$. The peak $K_{Na}$ current was reached at −10 mV, and its contribution to the total $K^+$ current decays with increased voltage steps (Fig. 5, C–E). In contrast, the voltage-dependent $K_t$ started activating at high-voltage steps with the half-maximum activation of −1.0 ± 1.0 mV (Hess and El Manira 2001). The amplitude of this current increased with increasing voltage steps (Fig. 5, C–E). Thus these two currents display different biophysical properties, suggesting that they may play complementary roles in controlling the waveform of the action potential.

Contribution of the transient $K_{Na}$ current to the action potential waveform

To determine the contribution of the transient $K_{Na}$ current during action potentials, we have used the waveform of an action potential, corresponding to an original recording, as the voltage command (Fig. 6). The spike waveform started from a resting membrane potential of −60 mV with the peak at 46.3 mV reached after 0.7 ms. The spike width measured at the half-maximal amplitude was 0.96 ms. The different currents were isolated by applying catechol (200 µM) and TTX (600 nM) first separately and then in combination to separate the different currents (see preceding text, Fig. 8A). In these experiments $Cd^{2+}$ (100 µM) and Ni$^2+$ (50 µM) were added later to isolate the Ca$^{2+}$ current. The different currents activated by the voltage waveform of the action potential were separated and corresponded to $I_{Na}$, $I_{KNa}$, $I_{Kt}$, $I_{Ca}$, and $I_{Ki}$ (Fig. 6A). The peak amplitude and time to peak in relation to the onset of the stimulus of all currents were calculated (Fig. 6B; $n = 21$). The $I_{Na}$ was activated first 0.85 ± 0.01 ms after the start of the stimulus waveform and reached the peak amplitude of 5.36 ± 0.29 nA after 1.80 ± 0.01 ms (Fig. 6, A–C). The transient $K_{Na}$ current was activated 1.05 ± 0.01 ms after the start of the stimulus that correspond to the time when the $I_{Na}$ reached the peak amplitude. The $K_{Na}$ peak amplitude was 4.48 ± 0.41 nA and was reached after 1.33 ± 0.01 ms, which corresponded to the peak of the action potential waveform, and it decayed completely after 2.25 ± 0.09 ms (Fig. 6, A–C). The kinetics and activation properties of the transient $K_{Na}$ current suggest that this current contributes to setting the spike peak by
limiting the amplitude of the depolarization by a counter current and thereby initiating the repolarization of the action potential.

The high-voltage-activated \( K^+ \) current started activating after 1.07 ± 0.01 ms from the onset of the stimulus and its peak amplitude was 7.19 ± 0.43 nA, which is significantly higher than that of \( K_{Na} \) and was reached after 1.54 ± 0.01 ms that was always later than the \( K_{Na} \) current (Fig. 6, A–C). The decay of \( K_i \) was slower than that of the transient \( K_{Na} \) as it was not completely deactivated at the end of the stimulus waveform (Fig. 6, A and C). Comparing the area underlying the transient \( K^+ \) currents showed that the \( K_i \) was much bigger than \( K_{Na} \) current (Fig. 6D). These results suggest that there are two transient \( K^+ \) currents serving complementary roles in determining the action potential waveform. The \( K_{Na} \) current seems to contribute to the early repolarization of the action potential, whereas the \( K_i \) current appears to determine the time course of the repolarization and the fast afterhyperpolarization and as a consequence the spike width.

The voltage-activated \( K_i \) current started activating around the peak of the action potential waveform (1.18 ± 0.01 ms after the onset of the stimulus; Fig. 6A) and reached its peak of 1.76 ± 0.2 nA after 1.79 ± 0.01 ms, which corresponds to the repolarization phase of the action potential (Fig. 6, C and D). The last current activated by the action potential waveform was the \( I_{Ks} \) which started 1.49 ± 0.02 ms after the onset of the stimulus and showed the smallest peak amplitude of 0.58 ± 0.04 nA reached after 1.95 ± 0.02 ms (Fig. 6, A–D).

Activity-dependent changes in the \( K^+ \) currents

To reveal activity-dependent changes in the amplitude of the different currents, we stimulated the neurons with a voltage command composed of 10 action potential waveforms and applied the different blockers to isolate \( I_{Na}, I_{KNa}, I_{Ks} \), and \( I_K \) (see preceding text). Frequencies of 10, 50, and 100 Hz were used. We compared the amplitude of the different current between the 1st and the 10th stimulus waveform (Fig. 7A). There was a large change in the amplitude of the different current when the stimulus waveforms were applied at a frequency of 100 Hz. The amplitude of the inward \( I_{Na} \) was reduced to 88.7 ± 3.1% of control (\( n = 14 \)), and the amplitude of the transient \( K_{Na} \) current decreased to 83.9 ± 7.1% of control between the first and the last stimulus (Fig. 7A). In the same neurons, the amplitude of the \( K_i \) current was also reduced but to a lesser extent, to 92.7 ± 3.4% of control. By contrast, the \( K_k \) amplitude increased to 111.7 ± 8.0% of control. The activity-dependent decrease in the \( K_{Na} \) current amplitude was less pronounced at lower than at higher frequencies [reduced to 83.9 ± 7.1% at 100 Hz (\( n = 14 \)), to 90.4 ± 6.2% at 50 Hz (\( n = 5 \)), and to 93.5 ± 2.2% at 10 Hz (\( n = 11 \));

FIG. 6. Activation of different currents by action potential waveform. A: action potential waveform (dotted line) was used to activate different voltage- and Na⁺-activated currents. Na⁺ current, transient \( K_{Na} \) (I-KNa), transient voltage-activated \( K^+ \) current (I-Ks), sustained \( K^+ \) current (I-Ki), and Ca²⁺ current (I-Ca²⁺) were isolated and their activation in relation to the action potential waveform was studied. B: peak amplitude of the different currents plotted as a function of the onset of the action potential waveform stimulus. C: onset and offset of the different currents plotted as a function of the action potentials waveform. D: area of each of the currents showing the total charge they contributed during an action potential waveform.

FIG. 5. Contribution of the transient \( K_{Na} \) and voltage-activated \( K^+ \) currents to the total current at different membrane potentials. A: inward Na⁺ and outward currents elicited by a test voltage step to −10 mV in control (black trace). Application of TTX (magenta trace) blocked the inward Na⁺ and the transient \( K_{Na} \) current. Catechol alone blocked the transient \( K_{Na} \) and voltage-activated \( K^+ \) current, without affecting the inward Na⁺ current (green trace). The combined application of TTX and catechol (blue trace) blocked both Na⁺ and transient \( K^+ \) current. B: application of a test voltage step to +30 mV elicited a smaller inward Na⁺ current and a large outward \( K^+ \) current. TTX (magenta trace) blocked the inward Na⁺ and transient \( K_{Na} \) current, which contributed only a small fraction to the total outward current. Catechol blocked the transient \( K_{Na} \) and voltage-activated \( K^+ \) currents. Application of TTX in the presence of catechol blocked the inward Na⁺ current. C: current traces showing the current blocked by TTX (I-TTX, black trace), transient \( K_{Na} \) current (I-KNa, red trace), and transient voltage-activated \( K^+ \) current (I-Ks, green trace) elicited by a test voltage step to −10 mV. D: current blocked by TTX (I-TTX, black trace), transient \( K_{Na} \) current (I-KNa, red trace), and transient voltage-activated \( K^+ \) current (I-Ks, green trace) elicited by a test voltage step to −10 mV. E: plot of the amplitude of the transient \( K_{Na} \) current and transient voltage-activated \( K^+ \) current as a ratio of total current at different membrane potentials.
Choline and NMDG were also used to replace extracellular Na⁺ to determine the proportion of the sustained K⁺ current that is mediated by activation of K<sub>Na</sub> channels. Choline and NMDG had similar effects, they completely abolished the Na⁺ current and the transient K<sub>Na</sub> current (Fig. 8, A–D). They also reduced the amplitude of the sustained K⁺ current in a similar proportion to Li⁺. Choline decreased the sustained K⁺ current from 1.48 ± 0.16 to 1.05 ± 0.15 nA (by 31.56 ± 3.09%; n = 13; Fig. 8, A, B, and E), and NMDG decreased it from 1.52 ± 0.17 to 1.08 ± 0.13 nA (by 29.17 ± 1.68%; n = 6; Fig. 8, C, D, and E). The remaining sustained K⁺ current is likely to be mediated by activation of voltage-activated channels. Application of TTX in the presence of choline or NMDG had no further effect on the amplitude of the K⁺ current (Fig. 8, A and C). To determine if the Na⁺-sensitive sustained current is indeed mediated by K⁺, the reversal potential of the tail current was estimated by varying the holding membrane potential after the test pulse. The Na⁺-sensitive tail current was isolated by subtracting the current induced in the presence of Na⁺ from that induced when Na⁺ was replaced with choline (n = 5) or NMDG (n = 5). There was a linear relationship between the amplitude of the Na⁺-sensitive tail current and the holding membrane potential with the current reversing at around −105 (Fig. 8F). This is close the K⁺ reversal potential of −94 mV calculated using the Nernst equation. It thus seems that the Na⁺-sensitive sustained current is mediated by K⁺.

Decrease in the amplitude of the sustained current is not due to change in the intracellular Ca<sup>2+</sup> concentration of pH

Removing external Na⁺ would affect the Na⁺-Ca<sup>2+</sup> exchanger leading to increased intracellular Ca<sup>2+</sup> levels that could decrease K⁺ currents other than K<sub>Na</sub>. To test if the decrease of the sustained K⁺ current by choline or NMDG was not the consequence of elevated intracellular Ca<sup>2+</sup>, neurons were dialyzed with the Ca<sup>2+</sup> chelator bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid (BAPTA, 10 mM). Neurons were held at −60 mV, and test voltage steps to −10 mV were applied in the presence of TTX, Cd²⁺, and Ni²⁺ to block Na⁺ and Ca²⁺ currents. In the presence of BAPTA, replacement of Na⁺ with choline and NMDG reduced the amplitude of the sustained K⁺ current in a reversible manner (Fig. 9, A and C). In total, choline reduced the sustained K⁺ current from 1.0 ± 0.19 to 0.80 ± 0.14 nA in BAPTA (n = 6) and from 0.50 ± 0.075 to 0.34 ± 0.052 nA (n = 5) in control (Fig. 9B). NMDG decreased the sustained K⁺ current from 1.1 ± 0.095 to 0.87 ± 0.10 nA (n = 5) in BAPTA and from 0.75 ± 0.18 to 0.60 ± 0.18 (n = 5) in control (Fig. 9D). These results suggest the decrease of the sustained K⁺ current by removal of Na⁺ is not mediated by an effect on Na⁺-Ca²⁺ exchanger.

Removing Na⁺ would also affect the Na⁺-H⁺ exchanger, resulting in changes in intracellular pH that could decrease the sustained K⁺ currents. To rule out this possibility, neurons were recorded using an intracellular solution containing 100 mM HEPES, and the effect of substituting Na⁺ with NMDG was tested on the sustained K⁺ current (see Dale 1993). In neurons recorded with high HEPES solution, NMDG was still able to reduce the amplitude of the sustained K⁺ current from 1.45 ± 0.12 to 1.10 ± 0.10 nA (n = 4, data not shown), suggesting that the effect of removing Na⁺ on the sustained current is not due to change in the intracellular pH.

Contribution of K<sub>Na</sub> current to the total sustained current

The effect of choline and NMDG was also tested on the amplitude of the sustained K⁺ current elicited by voltage steps with increased amplitude. The neurons were held at −60 mV and successive voltage steps were applied to −50 mV with 10-mV increments up to +50 mV. The amplitude of the K⁺ current was measured before and after Na⁺ substitution with choline (n = 6) or NMDG (n = 8). Both substitutions de-
creased the amplitude of the $K^+$ current at all test voltage steps (Fig. 10, A and C). The $K_{Na}$ current was isolated by subtracting the current elicited in choline or NMDG from control. The amplitude of the $K_{Na}$ current blocked by choline (Fig. 10B) or NMDG (Fig. 10D) increased in response to increased amplitude of voltage steps. In addition, the $K_{Na}$ current showed a tendency to decline at voltage step above +30 mV (Fig. 10, B and D). These results suggest that the $K_{Na}$ channels in addition to being sensitive to intracellular Na$^+$ are also gated in a voltage-dependent manner.

**FIG. 8.** Evidence for the presence of a sustained $K_{Na}$ current. A: recording from a spinal cord neuron with its membrane potential held at −60 mV and application of a test pulse to −10 mV elicited an inward Na$^+$ current followed by a transient and sustained $K^+$ currents. Application of TTX (red trace) blocked the inward Na$^+$ and the transient $K^+$ current. Substitution of Na$^+$ with choline after washout of TTX (green trace) reduced the amplitude of the sustained $K^+$ current and abolished the inward Na$^+$ and transient $K^+$ current. The effect of choline in the presence of TTX on the sustained $K^+$ current (cyan trace) was similar to that of choline alone (green trace). B: current traces with enlarged time scale showing the effect of TTX and choline on the inward and outward currents. C: in control, application of a test voltage step induced Na$^+$ and $K^+$ currents. TTX blocked the inward Na$^+$ and the transient $K^+$ current (red trace). Substitution of Na$^+$ with N-methyl-d-glucamine (NMDG) abolished the Na$^+$ and the transient $K^+$ currents, whereas it reduced the amplitude of the sustained $K^+$ current (green trace). Application of NMDG and TTX together had no further effect on the amplitude of the sustained $K^+$ current (cyan trace). D: current traces with enlarged time scale in control, in TTX and in NMDG. E: graph showing the percentage of the sustained $K^+$ current inhibited by Li$^+$, choline and NMDG. F: plot of the amplitude of the Na$^+$-sensitive tail current at different membrane potentials that reversed at around −105 mV.

**FIG. 9.** Blockade of the sustained $K^+$ current by replacement of Na$^+$ does not depend on intracellular Ca$^{2+}$. A: recording from a spinal cord neuron with an intracellular solution containing bis-(o-aminophenxy)-N,N',N'-tetraacetic acid (BAPTA). The sustained $K^+$ current elicited by a voltage step to −10 mV from a holding potential of −60 mV was reduced when Na$^+$ was replaced with choline. B: graph showing the percent decrease in the amplitude of the sustained $K^+$ current in neurons recorded with control solution and those recorded with a solution containing BAPTA. C: replacement of Na$^+$ with NMDG also reduced the amplitude of the sustained $K^+$ current in a neuron recorded with an intracellular solution containing BAPTA. D: change in the amplitude of the sustained $K^+$ current in control neurons and in neurons recorded with an intracellular solution containing BAPTA.

**FIG. 10.** Voltage activation of sustained $K_{Na}$ current. A: amplitude of the sustained $K^+$ current elicited at different test potentials in control and in choline. B: sustained $K_{Na}$ current was isolated by subtracting the current induced in choline from control and plotted as a function of voltage. C: plot showing the amplitude of the sustained $K^+$ current at different test potentials in control and in NMDG. D: graph of the amplitude of the sustained $K_{Na}$ current at different membrane potentials. The $K_{Na}$ current was isolated by subtracting the current elicited in NMDG from that in control.
Leak current permeable to Na⁺ in spinal cord neurons

The transient $K_{Na}$ current is linked to the Na⁺ influx through voltage-gated Na⁺ channels underlying the action potential. It is unlikely that they contribute to activation of the sustained $K_{Na}$ current because it was unaffected by TTX and was blocked only when the total extracellular Na⁺ was replaced by Li⁺, choline, or NMDG. In lamprey spinal cord neurons, there is no persistent TTX-insensitive Na⁺ current because all voltage-activated currents were blocked by TTX when applied in the presence of Ca²⁺ and K⁺ channel blockers (see El Manira and Bussieres 1997). One possible source of Na⁺ is through Na⁺ influx via voltage-activated Na⁺ channels. This current is linked to the Na⁺ influx because replacement of extracellular Na⁺ with Li⁺, NMDG and choline blocked the transient $K_{Na}$ and reduced the amplitude of the sustained Na⁺ current in the same proportion as Li⁺.

To test if lamprey spinal cord neurons possess leak channels permeable to Na⁺, the holding current and membrane conductance of neurons were monitored in control and when Na⁺ was replaced with NMDG or choline (Fig. 11). NMDG decreased the holding current, which resulted in an outward current with the mean amplitude of 232.8 ± 65.7 pA ($n = 10$; Fig. 11, A and C). The outward current was associated with a decrease in membrane conductance from 10.5 ± 6.9 nS in control to 5.8 ± 3.9 nS ($n = 10$) in NMDG (Fig. 11A). Similarly, substituting Na⁺ with choline also induced an outward current with a mean amplitude of 203.62 ± 52.14 pA ($P < 0.001$; $n = 10$; Fig. 11, B and C). This was associated with a decrease in the membrane conductance from 18.3 ± 16.17 nS ($n = 10$) to 7.6 ± 8.2 nS ($P < 0.001$; $n = 10$; Fig. 11B). These results indicate that spinal cord neurons possess a Na⁺-mediated leak conductance, which may activate a sustained $K_{Na}$ current.

**DISCUSSION**

$K_{Na}$ current in spinal cord neurons

In the present study, we present results showing that lamprey spinal cord neurons possess K⁺ channels activated by intracellular Na⁺. Several lines of evidence support the existence of $K_{Na}$ current in the spinal cord of the lamprey and that it consists of two components; one transient and the other sustained. The transient $K_{Na}$ current appears to be activated by Na⁺ influx through TTX-sensitive Na⁺ channels that underlying the action potential because this was completely blocked by TTX, whereas the sustained $K_{Na}$ current was not affected. The transient current does not appear to be the result of the lack of space clamp because the neurons examined were small with very short processes, their series resistance was compensated, and TTX gradually reduced the amplitude of Na⁺ current without shifting its kinetics.

The amplitude of the transient $K_{Na}$ current was significantly reduced by ~30% when Na⁺ was substituted with Li⁺, indicating that these channels are effectively activated by Li⁺ influx via voltage-activated Na⁺ channels. A similar current has been described in crayfish motoneurons (Hartung 1985). In contrast, the sustained $K_{Na}$ current was insensitive to Li⁺ because replacement of extracellular Na⁺ with Li⁺, NMDG and choline blocked the transient $K_{Na}$ and reduced the amplitude of the sustained K⁺ current in the same proportion as Li⁺.

The effect of replacing Na⁺ on the sustained current did not appear to be a result of an action on Na⁺-Ca²⁺ exchanger or Na⁺-H⁺ exchanger because the sustained $K_{Na}$ current was blocked by removing Na⁺ in neurons recorded with an intracellular solution containing BAPTA or high HEPES concentration.

The sustained current in lamprey spinal cord neurons is insensitive to TTX, and its amplitude was reduced when Na⁺ was replaced with Li⁺, NMDG, or choline. The kinetics of this current is similar to that of the delayed rectifier and represents ~30% of the total outward sustained current. Lamprey spinal cord neurons do not display persistent TTX-insensitive Na⁺ current that could provide a source for Na⁺ during voltage command used in this study. The sustained $K_{Na}$ current can be activated by the basal Na⁺ levels under the membrane. One way of maintaining the steady-state Na⁺ levels necessary to activate the sustained $K_{Na}$ is through Na⁺ influx via leak channels. This is supported by the fact that substitution of Na⁺ with NMDG or choline produced an outward current associated with a decrease in membrane conductance. A sustained $K_{Na}$ current has been described in different preparations (Bhattacharjee and Kaczmarek 2005; Dryer 1994). In Xenopus embryo spinal, sustained $K_{Na}$ current has been suggested to be activated by baseline Na⁺ levels that are controlled by Na⁺ entry via leak channels (Dale 1993). The sustained $K_{Na}$ current has been shown to display a marked voltage dependency (Bhattacharjee and Kaczmarek 2005; Dale 1993; Dryer 1994).

In lamprey spinal cord neurons, the sustained $K_{Na}$ current appears also to be voltage-dependent because its amplitude increases in response to depolarizing voltage steps.

**Na⁺ concentration required for activation of $K_{Na}$ channels**

Previous studies have shown that $K_{Na}$ channels have relatively low sensitivity to Na⁺, being activated only when the intracellular Na⁺ concentration is >10 mM (Bhattacharjee and Kaczmarek 2005; Dryer 1994). For this reason, it was first suggested that they play a role only under pathological conditions (see Dryer 1994). Studies of the dynamics of intracellular Na⁺ have shown that Na⁺ concentration can reach ≤100 mM
in the dendrites after repetitive stimulation (Rose 2002; Rose and Konnerth 2001). There is also evidence that Na\(^+\) and K\(_{Na}\) channels are closely localized (Koh et al. 1994). This allows an increase in Na\(^+\) concentration during a single action potential to result in activation of K\(_{Na}\) channels that is sufficient to contribute to the control of the amplitude and duration of the action potential.

Lamprey spinal cord neurons possess leak channels permeable to Na\(^+\) similar to those shown in Xenopus embryo spinal neurons (Dale 1993). The Na\(^+\) influx via leak channels alone is able to maintain the steady-state levels of Na\(^+\) necessary to activate the sustained K\(_{Na}\) current. However, this may not be the only source of Na\(^+\) involved in activating sustained K\(_{Na}\) current. During repetitive firing of spinal cord neurons, Na\(^+\) entry through voltage-activated channels can lead to sufficient elevations of intracellular Na\(^+\) concentration to activate K\(_{Na}\) channels. Indeed it has been reported that lamprey spinal cord neurons display a Ca\(^{2+}\)-independent slow afterhyperpolarization during repetitive firing that is abolished by removing Na\(^+\) (Cangiano et al. 2002; Wallen et al. 2005).

**Comparison of lamprey K\(_{Na}\) channels with cloned genes encoding for K\(_{Na}\) currents**

Two genes (Slick and Slack) encoding for K\(_{Na}\) currents have recently been cloned and their pharmacology profile is being characterized as well as their distribution in the CNS (Bhattacharjee et al. 2002, 2003, 2005; Yuan et al. 2003). These channels have different kinetics and are modulated differently by G-protein-coupled receptors and intracellular messengers (Bhattacharjee et al. 2003; Santi et al. 2006). Slick channels activate rapidly in response to depolarization, whereas Slack channels are slowly activating (Bhattacharjee et al. 2002, 2003, 2005; Yuan et al. 2003). The two lamprey K\(_{Na}\) currents also display different kinetics with the transient current displaying faster activation compared with the sustained current. Although the molecular identity of lamprey K\(_{Na}\) channels is not yet known; the two types may be encoded by different genes.

**Functional role of K\(_{Na}\) channels**

The physiological role of K\(_{Na}\) channels have been difficult to characterize because of the lack of specific blockers. However, there are now several studies showing that K\(_{Na}\) channels contribute to the regulation neuronal activity. In other preparations, K\(_{Na}\) channels have been shown to regulate the action potential waveform (Dale 1993; Haimann et al. 1990; Hartung 1985), to produce adaptation of firing rate, and to contribute to setting the resting membrane potential.

Our present results suggest that the two types of K\(_{Na}\) currents described in lamprey spinal cord neurons may play different roles. The transient current is closely associated with the Na\(^+\) influx during the action potentials and contributes to the early repolarization of the spikes. The transient K\(_{Na}\) current may serve as a negative feedback mechanism sensing the increase in Na\(^+\) concentration during single action potentials and may thus be essential in setting the spike amplitude as well as duration. Under our experimental conditions, the sustained K\(_{Na}\) current seems to be activated by Na\(^+\) influx via leak channels but not by Na\(^+\) influx during a single action potential. However, Na\(^+\) accumulation during repetitive firing may reach sufficient levels to activate the sustained K\(_{Na}\) current and underlie the Ca\(^{2+}\)-independent slow afterhyperpolarization (Cangiano et al. 2002; Wallen et al. 2005).

In the present study, we have used isolated spinal cord neurons that may undergo some changes in the composition of the ionic currents in culture. However, there is evidence showing that K\(_{Na}\) channels exist in neurons recorded from adult lamprey spinal cord in vitro and contribute to the Ca\(^{2+}\)-independent slow afterhyperpolarization and thus regulate their firing activity (Cangiano et al. 2002; Wallen et al. 2005). The characterization of these K\(_{Na}\) currents in lamprey spinal cord neurons represents a first step toward understanding their function and modulation. The use of the lamprey spinal cord with its characterized network architecture and output will help gaining further insights into the significance of K\(_{Na}\) channels in a network controlling locomotor function.

**ACKNOWLEDGMENTS**

We thank Drs. Sten Grillner and Russell Hill for comments on the manuscript.

**GRANTS**

This work was supported by Swedish Research Council Project 11562 and Karolinska Institutet funds. D. Hess received a fellowship from the Deutsche Forschungsgemeinschaft, Germany.

**REFERENCES**


Bhattacharjee A, Kaczmarek LK. For K\(^+\) channels, Na\(^+\) is the new Ca\(^{2+}\). Trends Neurosci 28: 422–428, 2005.


