Intrinsic Properties Shape the Firing Pattern of Ventral Horn Interneurons From the Spinal Cord of the Adult Turtle

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Smith, Morten and Jean-François Perrier. Intrinsic properties shape the firing pattern of ventral horn interneurons from the spinal cord of the adult turtle. J Neurophysiol 96: 2670–2677, 2006. First published August 9, 2006; doi:10.1152/jn.00609.2006. Interneurons in the ventral horn of the spinal cord play a central role in motor control. In adult vertebrates, their intrinsic properties are poorly described because of the lack of in vitro preparations from the spinal cord of mature mammals. Taking advantage of the high resistance to anoxia in the adult turtle, we used a slice preparation from the spinal cord. We used the whole cell blind patch-clamp technique to record from ventral horn interneurons. We characterized their firing patterns in response to depolarizing current pulses and found that all the interneurons fired repetitively. They displayed bursting, adapting, delayed, accelerating, or oscillating firing patterns. By combining electrophysiological and pharmacological tests, we showed that interneurons expressed slow inward rectification, plateau potential, voltage-sensitive transient outward rectification, and low-threshold spikes. These results demonstrate a diversity of intrinsic properties that may enable a rich repertoire of activity patterns in the network of ventral horn interneurons.

INTRODUCTION

Interneurons located in the ventral horn of the spinal cord are fundamental components of motor circuits. They are involved in the coordination of synergist and antagonist muscles (for review, see Baldissera et al. 1981); they contribute to the left–right coordination of the body (Butt and Kiehn 2003; Butt et al. 2002; Jankowska and Noga 1990; Stein et al. 1995; Stokke et al. 2002); and are active during rhythmic movements such as locomotion (Butt and Kiehn 2003; Kjerulf and Kiehn 1996; Pratt and Jordan 1987), swimming (Grillner 2003; Sillar and Roberts 1993), or scratching (Berkowitz 2005).

Three fundamentally different approaches have been used to categorize spinal neurons. The traditional way is to determine the nature of primary afferents contacting neurons (Baldissera et al. 1981; Jankowska 1992). A more recent approach consists in identifying functional subclasses of neurons according to the homeodomain transcription factors they express (Jessel 2000; Lanuzza et al. 2004; Wilson et al. 2005). A third method is based on the determination of intrinsic properties expressed by individual spinal neurons (Hounsgaard and Kjerulf 1992; Morisset and Nagy 1999; Murase and Randic 1983; Russo and Hounsgaard 1996a,b; Yoshimura and Jessell 1989). This approach is physiologically relevant because, together with synaptic inputs, currents mediated by voltage-gated ion channels determine the precise timing of action potentials in individual neurons (Llinas 1988). Moreover, the behavior of small neuronal networks is dictated by the intrinsic properties of individual cells in combination with the properties of synaptic connections and the pattern of interconnections between nerve cells (Arshavsky 2003; Marder and Calabrese 1996; Stein et al. 1997).

Only a few studies performed in preparations from embryonic or neonatal animals have investigated the intrinsic properties from ventral horn interneurons (Butt and Kiehn 2003; Butt et al. 2002; Szucs et al. 2003; Theiss and Heckman 2005; Wilson et al. 2005). However, the motor repertoire of neonates is quite poor compared with that from adult animals. The increase in complexity of motor behaviors that occurs throughout development is correlated with dramatic changes in the nature and variety of ion channels expressed in neuronal membranes (Furlan et al. 2005; Gao and Ziskind-Conhaim 1998; Huang et al. 2006; Jiang et al. 1999; Martin-Carballo and Greer 2000; Perrier and Hounsgaard 2000; Song et al. 2006; Spitzer and Ribera 1998; Vinay et al. 2000). For this reason, the contribution of intrinsic properties to the function of mature spinal motor network ought to be established in adult vertebrates. In the absence of a slice preparation from an adult mammal in which ventral horn interneurons remain viable these studies have not yet been performed.

Here we have used the spinal cord of the adult turtle because of its high resistance to anoxia. Using the whole cell blind patch-clamp technique, we examined the intrinsic properties of ventral horn interneurons. We show that ventral horn interneurons display a broad variety of discharge patterns produced by different intrinsic properties.

METHODS

Slice preparation

Experiments were performed in vitro on transverse slices (1.5 mm thick) from the spinal cord lumbar enlargement (D8–S2) from the adult turtle (Chrysemys scripta elegans). The turtles were anesthetized by intraperitoneal injection of 100 mg sodium pentobarbitone and killed by decapitation. The surgical procedures complied with Danish legislation and were approved by the controlling body under The Ministry of Justice. Experiments were performed at room temperature (20–22°C) in a solution containing (in mM): 120 NaCl, 5 KCl, 15 NaHCO3, 2 MgCl2, 3 CaCl2, and 20 glucose, saturated with 98% O2 and 2% CO2 to obtain pH 7.6.

Electrophysiological recordings

Whole cell blind patch-clamp recordings of ventral horn interneurons were performed with borosilicate pipettes filled with Mg-glu-
conate (1.53 mM), MgCl₂ (3.7 mM), CaCl₂ (300 mM), HEPES (5 mM), Na₂-HEPES (5 mM), NE prim (2 mM), K₂CH₂SO₄ (127 mM), and biocytin (10 mM). The pipette resistance was typically 5–10 MΩ when measured in the bath. Current-clamp recordings were performed either with an Axoclamp 2A, an Axoclamp 2B, or a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Data were collected and analyzed by means of pCLAMP software (Molecular Devices), sampled at 10–20 kHz with a 12- or 16-bit A/D converter (Digidata 1200 or Digidata 1322A; Molecular Devices), and stored on a hard disk for later analysis. Membrane potential values were not corrected for liquid junction potential.

Staining procedure

Cells were injected with biocytin (10 mM, Sigma) using 500-μs depolarizing pulses of current at 1 Hz (10–100 pA). Slices were immersed in 4% paraformaldehyde overnight and rinsed three times in PBS. Sections (50–100 μm) were made with a vibratome or a cryostat. Slices, incubated with streptavidin conjugated with Alexa 488 (Molecular Probes) together with Triton-X (0.3%) and 0.2% fish gelatin, were then mounted on objective glass with antifade medium (Prolong gold or Prolong antifade Kit (Molecular Probes). Neurons were visualized with a confocal microscope (Leica TCS SP2). The location of soma was normalized to the position of the central canal when measured in the bath. Current-clamp recordings were performed when the latency was >267 ms (mean 187 ± 57 m; Fig. 1A).}

Drugs

Fast synaptic inputs were eliminated by a mixture of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 25 μM; Tocris), d-2-amino-5-phosphonopentanoic acid (d-AP5, 50 μM; Tocris), (+)-bicuculline (20 μM; Tocris), and strychnine (10 μM) added to the extracellular medium. Other drugs used were ZD7288 (100 μM; Sigma), nifedipine (10 μM; Sigma), 4-aminopyridine (4-AP; 4–5 mM; Merck, Darmstadt, Germany), and tetrodotoxin (TTX; 1 μM; Alomone Labs, Jerusalem, Israel).

Data quantification

The input resistance of recorded cells was estimated as the voltage change induced by small hyperpolarizing current pulses (−10 to −100 pA) applied from −60 to −65 mV. The rheobase was calculated as the amount of current necessary to generate one action potential. For cells firing at rest (n = 66), a negative bias current was injected to hold the membrane potential at −70 mV. The rheobase was then estimated from this value.

Firing patterns were characterized during 2-s depolarizing current pulses of twice the rheobase value. A firing pattern index (FPI) was defined as the difference between the mean frequency of action potentials occurring during the last 500 ms (ML) and the first 500 ms (MF) of the current pulse divided by the mean frequency during the whole pulse (MW)

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FPI = \frac{ML - MF}{MW}
\]

The firing was categorized according to the FPI value, as burst (FPI < −0.3), adaptation (−0.3 < FPI < −0.1), regular (−0.1 < FPI < 0.1), or acceleration (FPI > 0.1). The latency of the first action potential was systematically measured. The firing was considered to be delayed when the latency was >200 ms.

Data were analyzed statistically by using two populations (paired or independent when appropriate) t-test (OriginPro 7.5; OriginLab, Northampton, MA). Significance was accepted when P < 0.05. Data are presented as means ± SD.

RESULTS

Our study is based on the recording of 207 ventral horn interneurons. They had an input resistance ranging from 100 to 3,300 MΩ (mean = 560 ± 489 MΩ; n = 187; Fig. 1A). Neurons that were not firing at rest had a mean membrane potential of −58.4 ± 5.9 mV; n = 97). The size of cell bodies recovered from recorded stained neurons (n = 42) ranged from 11.5 to 51 μm (long diameter; mean: 23.1 ± 7.7 μm; Fig. 1D) or from 7.8 to 29 μm (short diameter; mean: 12.9 ± 3.8 μm; Fig. 1C). These values are significantly lower than the mean diameters of turtle motoneurons reported in other studies (40 ± 9.7 μm; P < 0.01; McDonagh et al. 2002). The somas were located in the intermediate zone or in the ventral horn, dorsally to the motor columns (Fig. 1B). The cells from which we recorded were located 22–404 μm (mean 145 ± 95 μm) from the surface of the slice. Each of them had between 1 and 5 primary dendrites (mean 2.6 ± 1.1) with a length ranging from 37 to 737 μm (mean: 167 ± 162 μm). All the interneurons had dendrites projecting in the transversal plan (n = 42); 16 of these neurons had one or more dendrites projecting in the rostrocaudal direction.

Firing patterns

We characterized the firing pattern of ventral horn interneurons by calculating two indices. First, we calculated the slope of a linear regression of the instantaneous frequency of action potentials during 2-s depolarizing current pulses at twice the rheobase value (55 of the 207 neurons were tested). For 19 cells (35%) the slope value was below −1, indicating a degree of adaptation of the firing frequency. For 21 neurons (38%), the
frequency of action potentials did not significantly change because the slope ranged from −1 to 1. For the last 15 cells (27%), the slope was >1, demonstrating an increasing firing frequency.

To refine the categorization of firing patterns, we determined a firing pattern index (FPI) from the response to 2-s depolarizing current pulses at twice the rheobase value (see METHODS). Of the 207 interneurons, 56 were tested in this way. Of the 56 cells tested, nine (16%) had a bursting firing pattern (Fig. 2A) with a spike frequency decay of >30% (FPI < −0.3); five of these cells were firing at rest. The others had a resting potential of −59.7 ± 3.3 mV. Twelve cells (21%) had an FPI value between −0.3 and −0.1 and were therefore categorized as adaptation (Fig. 2B). Nine cells (16%) had a firing frequency within ±10% of the mean value (0.1 < FPI < 0.1). Such a firing pattern was considered regular (Fig. 2C). All other interneurons tested (n = 26; 47%) displayed incremental firing because they responded with an increased spike frequency of >10% (Fig. 2D; FPI > 0.1). The distribution of the different categories of FPI index is summarized in Fig. 2E.

The latency of the first action potential generated by current-pulse injections was systematically measured, ranging from 4 to 565 ms. For eight of the 56 interneurons, the latency was >200 ms and the firing was categorized as delayed (14%, Fig. 2F). Finally, four cells (of the 207 of our sample) responded with an oscillating membrane potential (Fig. 2G1). This firing pattern was still present when fast synaptic transmission was blocked (n = 4/4; see METHODS) and was therefore probably not driven by the surrounding neural network. Moreover, the frequency of the bursts of action potentials was voltage sensitive, increasing when the cell was depolarized and decreasing when it was hyperpolarized (Fig. 2G2; P < 0.05 for three of the four cells; independent t-test; the fourth cell was not recorded long enough to perform statistical tests). These results demonstrate that ventral horn interneurons display a rich repertoire of firing patterns. We next examined the molecular basis of these cellular behaviors.

Plateau properties

The most common firing pattern observed during depolarizing current pulses was an acceleration of the firing frequency (Fig. 2D; n = 76 of 166, i.e., 46%; this number includes cells that were not tested at twice the rheobase value). Action potentials generated during 2-s depolarizing current pulses were either followed by an afterhyperpolarization (n = 22/76; Fig. 3A), or by and afterdepolarization that could be sufficient to trigger action potentials (Fig. 3B; n = 54/76). Depolarizing pulses could also trigger a bistable firing pattern (Fig. 3C; n = 32). Figure 3C1 illustrates an interneuron that was kept silent with a negative bias current. A depolarizing current pulse to 0 pA generated action potentials with an accelerating frequency and triggered firing that continued for seconds after the current pulse was turned off. The bistable firing could be terminated with a negative current pulse (Fig. 3C2). This example demonstrates that plateau potentials contributed to the resting membrane potential of some interneurons (n = 12). Extracellular addition of nifedipine (10 μM) blocked the acceleration in spike generation during the current pulse as well as the after-discharge (Fig. 3D; n = 6/6), suggesting that both were mediated by L-type calcium channels.

Voltage-sensitive transient outward rectification

We observed delayed firing in 14% of the interneurons tested at twice the rheobase (Fig. 2F). In the presence of blockers for fast synaptic transmission (see METHODS), we studied the voltage sensitivity of the delay. We found that depolarizing current pulses after a hyperpolarizing current pulse generated a voltage-sensitive transient hyperpolarization that postponed the occurrence of the first action potential (n = 36 of 163 neurons tested; i.e., 22%; Fig. 4, A and C). In a few instances, however, when the depolarizing current pulse reached a sufficient level, an action potential was generated before the transient hyperpolarization (n = 8/36; Fig. 4F). This could be a result of the interaction between a postinhibitory rebound (see following text) and the transient hyperpolarization. The amplitude of the negative transient induced by depolarizations was sensitive to the amplitude of the preceding negative current pulse (Fig. 4, B and C; n = 19/19). The negative transient disappeared in the presence of 4-aminopyridine (4–5 mM; Fig. 4E; n = 10/10). These results demonstrate that a voltage-sensitive transient outward rectifying conductance delays the firing in a sizeable fraction of interneurons.
Postinhibitory rebound

Sixteen percent of the interneurons responded to depolarizing pulses of twice the rheobase value by a bursting firing pattern (Fig. 2A). We tested whether this pattern was mediated by a postinhibitory rebound (PIR). We recorded the response of interneurons to hyperpolarizing current pulses of increasing amplitudes. For the vast majority of the cells from which we recorded (n = 156 of 181; i.e., 86%), current pulses induced a depolarizing sag with an amplitude that increased linearly with the level of hyperpolarization (Fig. 5A and B; threshold for the sag $-71.9 \pm 6.4$ mV). In most cases, when the current pulse was turned off, the sag was followed by a PIR (n = 133 of 151 cells tested, i.e., 88%) that could reach the threshold for action potentials (Fig. 5A). The threshold for the PIR, estimated with an activation protocol similar to that used for characterizing the transient outward rectification (Fig. 4A), ranged from $-53$ to $-85$ mV (mean value: $-70.2 \pm 7.5$ mV), which was not significantly different from the threshold for the depolarizing sag ($P > 0.05$; paired t-test). The threshold for the PIR overlapped with the resting membrane potential (ranging from $-44$ to $-75$ mV), indicating that, at least in some instances, PIRs were evoked by depolarizing current pulses applied from resting membrane potential. In support of this hypothesis, we found that a hyperpolarizing current pulse, applied from a positive level to 0 pA, evoked a depolarizing sag (Fig. 5C and D; n = 10 of 21 cells tested). Both the sag and the PIR were sensitive to ZD7288 (100 μM; n = 17; Fig. 5, E and F), suggesting that they were mediated by a hyperpolarization-activated inward cationic current ($I_h$).

In a smaller fraction of interneurons, hyperpolarizing pulses were followed by a different type of PIR (13 of 151 cells tested; i.e., 8.6%). It appeared in an all-or-none manner and had a constant amplitude in response to depolarizing current pulses (Fig. 6, A, C, and E), in contrast to the PIR mediated by $I_h$ (Fig. 5A). It also disappeared in an all-or-none manner when the amplitude of the current pulse decreased (n = 5; Fig. 6, B, D, and E). Moreover, its threshold estimated with an activation protocol (Fig. 6C), was significantly higher than the threshold for $I_h$ (mean value: $-58.2 \pm 4.5$ mV; n = 13; independent

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**FIG. 3.** A fraction of ventral horn interneurons express plateau potentials. A: response of an interneuron to a depolarizing current pulse. Frequency of action potentials increased during the current injection. Response to the current pulse was followed by an afterhyperpolarization of $-4$ mV. Cell recorded in the presence of CNQX (25 μM), AP5 (50 μM), bicuculline (20 μM), and strychnine (10 μM). B: response of another interneuron with an increase in firing frequency during the depolarization. Response to the current pulse was followed by an afterdepolarization that was sufficient to trigger action potentials. C1 and C2: in another interneuron, a depolarizing current pulse generated a bistable firing (C1) that could be interrupted by a negative current pulse (C2). D1: response of another interneuron to a depolarizing current pulse. D2: addition of nifedipine (10 μM) inhibited both the increase in firing frequency and the afterdischarge. Cell recorded in the presence of CNQX (25 μM), AP5 (50 μM), bicuculline (20 μM), and strychnine (10 μM).

**FIG. 4.** Voltage-sensitive transient hyperpolarizations delay action potentials. A: depolarizing current pulse applied from a negative level induces a voltage-sensitive transient hyperpolarization that delays the occurrence of action potentials. B: amplitude of the transient hyperpolarization is sensitive to the voltage preceding the depolarization. C: amplitude of the transient hyperpolarization as a function of the depolarizing current pulse (dotted line in A; activation) and of the preceding holding current (dotted line in B; inactivation). Insert: how the amplitude of the hyperpolarization was measured. D: transient hyperpolarization evoked by a depolarizing current pulse. E: hyperpolarization disappeared in the presence of 4-aminopyridine (5 mM). F: response of another interneuron to a depolarizing current pulse. Note the action potential preceding the transient hyperpolarization. Recordings from D–F made in the presence of CNQX (25 μM), AP5 (50 μM), bicuculline (20 μM), and strychnine (10 μM). A–B, D–E, and F are from 3 different interneurons.
It persisted in the presence of TTX (Fig. 6, C and D; n = 2). Taken together, these results demonstrate that this second type of PIR is attributed to a low-threshold spike (LTS). However, the infrequency of this type of PIR prevented us from determining its ionic basis.

Combinations of intrinsic properties

We showed above that different intrinsic properties (plateau potential, transient outward rectification, slow inward rectification, and low-threshold spikes) control the firing pattern of ventral horn interneurons. These properties were usually not expressed alone but in different combinations. To estimate the occurrence of these combinations, we systematically tested the presence of the four intrinsic properties (n = 135). We first estimated the incidence of cells expressing two properties together. With the exception of LTS and transient outward rectification that were never expressed together, we found all the possible pairs of properties (results summarized in Table 1). We also looked for neurons expressing three properties together and found 14 interneurons out of 135 (10%) in which the transient outward rectification, plateau properties, and slow inward rectification were coexpressed and three cells (2%) that coexpressed LTS, plateau properties, and slow inward rectification. Because LTS and transient outward rectification were never recorded in the same neuron, none of the interneurons expressed all four properties.

DISCUSSION

In the present study we demonstrated that ventral horn interneurons from the adult spinal cord display a rich variety of firing patterns. We showed that these electrical behaviors are mainly controlled by plateau potentials, transient outward rectification, slow inward rectification, and low-threshold spikes.

Sample of ventral horn interneurons

Previous work investigated the firing patterns of ventral horn interneurons from the adult turtle (Berkowitz 2005; Berkowitz et al. 2006; Houngsaard and Kjarulf 1992). In these studies, interneurons were recorded by means of sharp electrodes. This technique allows the recording of the biggest interneurons. By comparison, whole cell patch-clamp recording gives access to much smaller cells. In support of this assertion, we found that the mean input resistance of the

FIG. 5. Slow inward rectification generates postinhibitory rebounds. A: response of an interneuron to hyperpolarizing current pulses. Note the depolarizing sag (black square) and the postinhibitory rebound (PIR, star) that could trigger an action potential. Cell recorded in the presence of CNQX (25 μM), AP5 (50 μM), bicuculline (20 μM), and strychnine (10 μM). B: amplitude of the sag and PIR as a function of current. C: hyperpolarizing current pulse applied from +10 to 0 pA generated a depolarizing sag of +3 mV, demonstrating that the slow inward rectifying current contributes to the resting membrane potential. D: average response of the neuron illustrated in C (21 sweeps). E and F: addition of ZD7288 (100 μM) inhibited the sag and the PIR. Cell recorded in the presence of CNQX (25 μM), AP5 (50 μM), bicuculline (20 μM), and strychnine (10 μM). A, C, and E are from 3 different interneurons.

FIG. 6. Low-threshold spikes (LTSs) generate PIRs. A: depolarizing current pulse applied from a negative level induces a voltage-sensitive burst of action potentials. B: burst of spikes is sensitive to the voltage preceding the depolarization. Cell recorded in the presence of CNQX (25 μM), AP5 (50 μM), bicuculline (20 μM), and strychnine (10 μM). C: in another interneuron, in the presence of tetrodotoxin (TTX, 1 μM) a depolarizing current pulse generates a voltage-sensitive LTS. E: amplitude of the LTS as a function of the depolarizing current pulse (dotted line in C; activation) and of the preceding holding current (dotted line in D; inactivation).
interneurons from our sample was higher than that reported by Hounsgaard and Kjærulff (560 vs. 130 MΩ). However, we cannot rule out that the high-input resistance of the cells in our sample also arises from the absence of shunting with patch recording technique, washout of leak conductance, or amputation of dendritic trees in the slices. To compare more directly the sizes of cells recorded with sharp and patch electrodes, we measured the diameter of the 12 ventral horn interneurons recorded with sharp electrodes and illustrated in Berkowitz (2005) and Berkowitz et al. (2006). Their diameter was significantly larger (long: 30.6 ± 10.8 μm; short: 16.9 ± 5.5) compared with the interneurons from our sample (independent t-test; P < 0.01 both for the long and the short diameter). These differences strongly suggest that our sample of interneurons contains cell types that have not been recorded before.

The broad diversity of firing patterns is specific to adult interneurons

Few studies have investigated the nature of intrinsic properties present in interneurons from the ventral horn of neonatal or embryonic animals (Butt and Kiehn 2003; Butt et al. 2002; Szucs et al. 2003; Theiss and Heckman 2005; Wilson et al. 2005). These studies reported firing patterns including single-spike firing (Szucs et al. 2003; Theiss and Heckman 2005), repetitive firing (Szucs et al. 2003; Theiss and Heckman 2005), and bursting firing (Theiss and Heckman 2005; Wilson et al. 2005). In our study, all the interneurons were able to fire repetitively. This observation suggests that single-spike firing is a transient behavior occurring only during development, as is the case for motoneurons (Gao and Ziskind-Conhaim 1998; Vinay et al. 2000). In addition to repetitive and bursting firing, we found that ventral horn interneurons from the adult turtle have accelerating, delayed, and oscillatory firing patterns (Fig. 2). To the best of our knowledge, none of these latter behaviors was previously reported in neonatal or embryonic spinal interneurons. In the absence of intrinsic properties, interneurons would fire at a constant rate as is the case in the majority of ventral horn neurons from the spinal cord of neonatal rats (Szucs et al. 2003; Theiss and Heckman 2005). Here we found that only 16% on the ventral horn interneurons displayed such a regular firing pattern. These differences indicate that intrinsic properties from ventral horn interneurons are altered during development.

### Table 1. Combination of two intrinsic properties in ventral horn interneurons

<table>
<thead>
<tr>
<th>Intrinsic Property</th>
<th>Plateau Potential</th>
<th>Voltage-Sensitive Transient Outward Rectification</th>
<th>Slow Inward Rectification</th>
<th>Low-Threshold Spike</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plateau potential</td>
<td>50–100</td>
<td>14–28</td>
<td>44–87</td>
<td>3–6</td>
</tr>
<tr>
<td>Voltage-sensitive transient outward rectification</td>
<td>14–63</td>
<td>22–100</td>
<td>16–70</td>
<td>0–0</td>
</tr>
<tr>
<td>Slow inward rectification</td>
<td>44–51</td>
<td>16–18</td>
<td>86–100</td>
<td>7–8</td>
</tr>
<tr>
<td>Low-threshold spike</td>
<td>3–36</td>
<td>0–0</td>
<td>7–82</td>
<td>8–100</td>
</tr>
</tbody>
</table>

The first number represents the absolute percentage of neurons expressing the two intrinsic properties and the second number is the relative percentage. For example, the second cell of the third line (14–63) means that 14% of the interneurons expressed both a voltage sensitive outward rectification and a plateau potential, and that among the neurons with a voltage sensitive outward rectification, 63% had plateau properties.

Intrinsic properties determine the firing patterns of interneurons

The vast majority of interneurons (86%) expressed a slow inward rectification property. It was characterized by a slowly activating depolarizing sag appearing on hyperpolarization and a postinhibitory rebound (PIR) on return to initial membrane potential. Because both were blocked by ZD7288, they were probably mediated by an I_h current (Robinson and Siegelbaum 2003). I_h was active at resting membrane potential in 48% of neurons. For these neurons, a depolarizing pulse applied from resting membrane potential induced an extra depolarization resulting from the relaxation of I_h (Fig. 5, C and D). Because the PIR can trigger action potentials, I_h may contribute to the adaptation of spike frequency during depolarizing current pulses. Other mechanisms such as the increase of the afterhyperpolarization arising from calcium accumulation (Yarom et al. 1985), the activation of an M-current (Alaburda et al. 2002a), or the slow inactivation of the fast, inactivating Na⁺ conductance (Miles et al. 2005) may also contribute to the adaptation of action potential frequency. We ascribed the accelerating firing pattern observed in 47% of the interneurons in response to depolarizing current pulses at twice the rheobase value (Fig. 2D) to a plateau potential mediated by L-type calcium channels. Our assumption is based on the fact that the acceleration of the spike frequency as well as the afterdepolarization present after the current pulse injection disappeared in the presence of nifedipine. This result is in agreement with previous observations made in ventral horn interneurons (Hounsgaard and Kjærulff 1992), deep dorsal interneurons (Russo and Hounsgaard 1996a), or motoneurons (Hounsgaard and Mintz 1988; Simon et al. 2003). However, we cannot rule out that other conductances such as a persistent sodium current (Li and Bennett 2003) or a calcium-activated nonsselective cationic current (Morisset and Nagy 1999; but see Perrier and Hounsgaard 1999) contributed to plateau properties.

The voltage-sensitive transient outward rectification recorded in 22% of the interneurons generates a transient hyperpolarization that delays the occurrence of action potentials generated by depolarizing current pulses (Fig. 4). It was activated by depolarization after hyperpolarization, had a threshold close to that for action potentials, and was sensitive to 4-aminopyridine. Taken together, these results suggest that the transient outward rectification was mediated by a low-voltage-activated A-current (Rogawski 1985). However, we cannot rule out the contribution of a D-current (Storm 1988). In some instances, a delayed firing was not present when tested with a
depolarizing current pulse from −70 mV, but was detected when the depolarization was preceded by a transient hyperpolarization. This explains why we observed a delayed firing in only 14% of the interneurons and a transient outward rectification in 22% of them.

We found that burst firing arising from a postinhibitory rebound can be mediated either by slow inward rectification or by a low-threshold spike (LTS). The LTS was induced by depolarization after hyperpolarization in an all-or-none manner. It had a low threshold (−58 mV) and was insensitive to TTX. Although we did not demonstrate it, these results are compatible with a spike mediated by a T-type calcium current, as the one recorded in deep dorsal horn neurons of the turtle (Russo and Hounsgaard 1996b) or in ventral horn interneurons of the neonatal mouse (Wilson et al. 2005).

We recorded four interneurons with oscillatory behaviors. Two arguments suggest that they were mediated by intrinsic properties. First, the oscillations persisted when fast synaptic transmission was blocked; and second, the frequency of oscillations was voltage sensitive. Oscillations of the membrane potential promoted by metabotropic modulation were previously recorded in deep dorsal horn interneurons of the neonatal rat (Derjean et al. 2003). Here we show that oscillations also occur in ventral horn interneurons, even in the absence of pharmacological activation.

Functional considerations

We recorded plateau potentials and bistable firing in interneurons at resting membrane potential and in the absence of metabotropic modulation. This observation contrasts with that of spinal motoneurons, in which plateau potentials are not expressed in the absence of neuromodulation (Alaburda et al. 2002b; Perrier et al. 2002). This suggests that plateau potentials from ventral horn interneurons may participate in tonic firing in interneurons and thereby contribute to the background activity of the spinal network.

A major function of the spinal motor network is to generate rhythmic activity such as locomotion (Graham-Brown 1911). It is not known whether the activity of the spinal network depends on intrinsic properties of interneurons, as in networks from invertebrates (Arshavsky 2003), or whether it is an emergent property originating from synaptic interactions between neurons in the network. Recent works have provided evidence for (Paton et al. 2006; Pena et al. 2004) and against (Alaburda et al. 2005; Del Negro et al. 2002) the contribution of intrinsic properties to network activity. An alternative possibility would be that rhythmic activity results from a combination of these two mechanisms. In the present study, we found that a small fraction of ventral horn interneurons displayed intrinsic oscillation properties. Despite lack of evidence, it is tempting to link this observation to the global activity generated by the spinal network during rhythmic activities.

GRANTS

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