Depolarization-Induced Facilitation of a Plateau-Generating Current in Ventrall Horn Neurons in the Turtle Spinal Cord

GYTIS SVIRSKIS AND JØRN HOUNSGAARD
Laboratory of Neurophysiology, Biomedical Research Institute, Kaunas Medical Academy, 3000 Kaunas, Lithuania; and Department of Medical Physiology, The Panum Institute, Copenhagen University, DK-2200 Copenhagen N, Denmark

Svirskis, Gytis and Jørn Hounsgaard. Depolarization-induced facilitation of a plateau-generating current in ventral horn neurons in the turtle spinal cord. J. Neurophysiol. 78: 1740–1742, 1997. Plasticity at the neuronal level commonly involves use-dependent changes in strength of particular synaptic pathways or regulation of postsynaptic properties by modulatory transmitters. Here we analyze a novel form of short-term plasticity mediated by use-dependent facilitation of postsynaptic responsiveness. Using current- and voltage-clamp recordings, we found that all spinal ventral horn neurons able to generate plateau potentials showed depolarization-induced facilitation of the underlying inward current. Facilitation was noticeable when the neurons were depolarized to more than −50 mV at intervals <4 s. When stimulation with fast triangular voltage ramps was used, the inward current activated at a less depolarized potential during the second ramp. The inward current and facilitation was eliminated by nifedipine, a selective antagonist of L-type calcium channels. Depolarization-induced facilitation of low-voltage-activated L-type calcium channels is suggested to be the underlying mechanism. It is noted that facilitation occurs on a time scale compatible with a role in phasic motor activity.

INTRODUCTION

Integration of synaptic potentials is influenced by passive membrane properties and voltage-sensitive channels. The integration can be transformed by a change in synaptic weights and/or modulation of postsynaptic channels. A simple form of postsynaptic modulation results from changes in channel state induced and maintained by a change in membrane potential. The depolarization-induced facilitation of calcium channels described in many cell types (Dolphin 1996) is of particular interest in neurons as a possible mediator of a novel form of use-dependent postsynaptic plasticity.

Here we show that the contribution of plateau potentials to synaptic integration in motoneurons and ventral horn interneurons depends on depolarization-induced facilitation. We have found that all motoneurons and interneurons able to generate plateaus show depolarization-induced facilitation of the inward current underlying plateau potentials. Depolarization-induced facilitation of dihydropyridine-sensitive plateau potentials was previously shown to mediate the windup in dorsal horn neurons (Morisset and Nagy 1996; Russo and Hounsgaard 1994). Our analysis suggests that facilitation of L-type calcium channels is the underlying mechanism. The time scale of facilitation is much longer than the duration of synaptic potentials but is compatible with a role in phasic motor behavior. In the turtle, this is supported by the finding that the facilitation required for induction of the scratch reflex occurs at stimulus intervals <5 s (Currie and Stein 1988). Elsewhere it has been shown that a neuron with these properties can become a complex integration unit despite a modest electrotonic length (Baginskas et al. 1997).

METHODS

Transverse sections, 1–2 mm thick, of the lumbar spinal cord were obtained from turtles (Pseudemys scripta elegans) deeply anesthetized by mebumal (≈100 mg/kg) injected intraperitoneally (Hounsgaard et al. 1988). The bath medium contained (in mM) 120 NaCl, 5 KCl, 15 NaHCO3, 20 glucose, 2 MgCl2, and 3CaCl2. The liquid level was 0.2 mm above slice surface. Sharp and patch electrodes were pulled from borosilicate glass tubes with an outer diameter of 1.5 mm and an inner diameter of 0.86 mm. Sharp electrodes were filled with 1.5 M KCl and 0.5 M potassium acetate. Patch electrodes were filled with 125 mM potassium gluconate and 9 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid.

Voltage clamp was performed with an Axoclamp 2A amplifier in discontinuous single-electrode voltage-clamp mode. The gain ranged from 0.7 to 3 and the sample rate from 6 to 9 kHz. For voltage-ramp clamp, the recorded currents and potentials were low-pass filtered at 0.1 kHz and digitized at 0.8 kHz. All other recordings were low-pass filtered at 3 kHz and digitized at 6 kHz. To reduce noise during slow voltage-clamp ramps, four sweeps were averaged on a HIOKI digital oscilloscope. The data from the oscilloscope were transferred to an IBM-compatible computer. Recordings were obtained from motoneurons and interneurons in the ventral horn. Cell type and health was inferred from firing properties (Hounsgaard and Kjaerulff 1992; Hounsgaard et al. 1988).

Drugs used were tetrodotoxin (2 μM), cis-(±)-1-amino-1-cyclopentane-1,3-dicarboxylic acid (ACPD, 20 μM), muscarine (25 μM), atropine (1 μM), CoCl2 (2 mM), nifedipine (20 μM), tetraethylammonium (0.2–1 mM), and apamin (1 μM).

RESULTS

We tested for depolarization-induced facilitation in interneurons and motoneurons in the ventral horn. Neurons without plateau potentials did not show facilitation. Facilitation was present in all interneurons showing plateaus (Fig. 1A, n = 15 of 30, 6 sharp and 24 whole cell recordings) and in all motoneurons in which plateau properties were induced or enhanced by application of ACPD, muscarine, or tetroethylammonium and apamin (n = 40 of 46, 35 sharp and 11 whole cell recordings). As illustrated in Fig. 1B, the neurons displayed windup of the response to current pulses repeated at intervals <4 s. After elimination of sodium spikes with tetrodotoxin, the facilitation of the response persisted and could lead to full activation of the plateau potential (Fig. 1C). The properties of the inward current were further inves-
DEPOLARIZATION-INDUCED FACILITATION OF PLATEAU CURRENT

The increasing afterdepolarization indicates involvement of plateau potential. The simplest explanation for the observed phenomena is facilitation of low-voltage-activated L-type calcium channels.

Turtle motoneurons and interneurons have physically long dendrites (Hounsgaard and Kjaerulff 1992; Ruigrok et al. 1984). Distal dendrites of turtle motoneurons possess L-type calcium channels (Hounsgaard and Kiehn 1993). These distal channels could be persistently active in weakly clamped dendrites during a train of short depolarizations causing facilitated activation of the proximal L-type channels. However, the electrotonic length of dendrites in motoneurons and ventral interneurons is moderate, ~1 λ (G. Svirskis, A. Baginskas, J. Hounsgaard, and A. Gutman, unpublished data). Also, responses generated by distal dendritic currents are sensitive to changes in bias current at the

![Image](https://via.placeholder.com/150)

**FIG. 1.** A: in ventral horn interneurons, plateau potentials can be activated in normal solution. B: windup of the response to a repeated current pulse. The increasing afterdepolarization indicates involvement of plateau potential. C: after elimination of sodium spikes, facilitation was still present and could lead to the full activation of the plateau potential. All figures from the same cell.

tigated in voltage-clamp experiments. The response to slow triangular voltage ramps showed clockwise hysteresis, reflecting the slow kinetics of the inward current (Fig. 2A) (for details see G. Svirskis and J. Hounsgaard, unpublished data). We were unable to obtain stationary current voltage characteristics without hysteresis even with ramps lasting >10 s. This suggested that slow changes in inward current were activated during the voltage ramp depolarization. When the potential was stepped to a level of depolarization close to the threshold for plateau potentials, activation lasted several hundred milliseconds and was voltage sensitive (Fig. 2B). Consistent with the facilitation observed in current-clamp recordings, the inward current during a step depolarization to more than −50 mV increased when the step was repeated (Fig. 2C). Facilitation was also observed in response to triangular voltage ramps lasting <500 ms. The inward current was activated earlier and was larger during the second voltage ramp (Figs. 2D and 3A).

Plateau potentials in interneurons and motoneurons are due to a low-threshold L-type calcium current (Hounsgaard and Kiehn 1993; Hounsgaard and Kjaerulff 1992). The voltage-dependent inward current and facilitation was eliminated by Co²⁺, a nonselective blocker of calcium channels (n = 6), and by nifedipine, a selective blocker of L-type calcium channels (n = 12, Fig. 3, A and B).

**DISCUSSION**

We have shown that all ventral horn neurons able to generate plateau potentials show depolarization-induced facilitation of the response to repeated depolarizations. This facilitation is present in current clamp and voltage clamp. The simplest explanation for the observed phenomena is facilitation of low-voltage-activated L-type calcium channels.

Turtle motoneurons and interneurons have physically long dendrites (Hounsgaard and Kjaerulff 1992; Ruigrok et al. 1984). Distal dendrites of turtle motoneurons possess L-type calcium channels (Hounsgaard and Kiehn 1993). These distal channels could be persistently active in weakly clamped dendrites during a train of short depolarizations causing facilitated activation of the proximal L-type channels. However, the electrotonic length of dendrites in motoneurons and ventral interneurons is moderate, ~1 λ (G. Svirskis, A. Baginskas, J. Hounsgaard, and A. Gutman, unpublished data). Also, responses generated by distal dendritic currents are sensitive to changes in bias current at the

![Image](https://via.placeholder.com/150)

**FIG. 2.** A: during slow triangular voltage ramp, the slowly activating and deactivating inward current caused hysteresis in current-voltage relation. B: during a step depolarization, the slow activation of the inward current was voltage dependent. Leak current subtracted. C: inward current during a depolarizing step was facilitated when repeated. Leak current subtracted. D: inward current activated at less depolarized potentials and was enhanced during a 2nd ramp (see current-voltage plot). All records from the same interneuron in presence of tetrodotoxin (TTX), tetraethylammonium, and apamin. In A and D, the solid horizontal line indicates the 0 current value. Arrows: direction of the potential change.
soma (Hounsgaard and Kiehn 1993). Thus it is unlikely that dendritic bistability can account for the observed facilitation. However, hysteresis in response to very slow ramps may be due to slow development and spread of plateaus in dendrites if windup of the inward current is assumed (Baginskas et al. 1997).

The selective blocker of L-type calcium channels eliminated the inward current and facilitation. It seems possible, that L-type calcium channels in the cells investigated here and in dorsal horn neurons (Morisset and Nagy 1996; Russo and Hounsgaard 1994) show depolarization-induced facilitation. In turtle spinal neurons generating plateau potentials, the inward current develops slowly, over several hundred milliseconds, at moderate levels of depolarization (Figs. 1A and 2, A and B). This slow activation may reflect the slow change in gating responsible for the facilitation. Depolarization-induced facilitation of L-type calcium channels has been observed in skeletal muscle (Feldmeyer et al. 1992), hippocampal pyramidal cells (Kavalali and Plummer 1994), cerebellar granule cells (Parri and Lamsan 1996), and neostriatal neurons (Song and Surmeier 1996). The facilitation observed here stands out because of the low level of depolarization required and the long-lasting change in gating induced. Usually, conditioning depolarizations near 0 mV are required for induction of facilitation lasting at most a few hundred milliseconds (Dolphin 1996). The observed activation of L-type channels near resting membrane potential (Figs. 2A and C, and 3A) is not unique. In hippocampal pyramidal cells, L-type calcium channels activate at the same level of potentials (Avery and Johnston 1996).

The results obtained do not allow us to rule out a role for calcium-dependent currents (Greene et al. 1994; Sciancalepore and Constanti 1995) in depolarization-induced facilitation. However, plateau potentials were observed in turtle motoneurons when sodium was replaced with choline (Mintz 1987). If other calcium-dependent currents are involved, their sensitivity to calcium should be steep or they should be voltage dependent, because the response to the second potential ramp coincided with the previous response in the 15-mV range preceding activation of the inward current (Figs. 2C and 3A).

REFERENCES


