Ca\(^{2+}\)-Activated Nonselective Cationic Current (\(I_{\text{CAN}}\)) in Turtle Motoneurons

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Perrier, Jean-François and Jørn Hounsgaard. Ca\(^{2+}\)-activated nonselective cationic current (\(I_{\text{CAN}}\)) in turtle motoneurons. J. Neurophysiol. 82: 730–735, 1999. The presence of a calcium-activated nonspecific cationic (CAN) current in turtle motoneurons and its involvement in plateau potentials, bistability, and windup was investigated by intracellular recordings in a spinal cord slice preparation. In the presence of tetraethylammonium (TEA) and tetrodotoxin (TTX), calcium action potentials evoked by depolarizing current pulses were always followed by an afterdepolarization associated with a decrease in input resistance. The presence of the afterdepolarization depended on the calcium spike and not on membrane potential. Replacement of extracellular sodium by choline or N-methyl-D-glucamine (NMDG) reduced the afterdepolarization, confirming that it was mediated by a CAN current. Plateau potentials and windup were evoked in response to intracellular current pulses in the presence of agonist for different metabotropic receptors. Replacement of extracellular sodium by choline or NMDG did not abolish the generation of plateau potentials, bistability, or windup, showing that Na\(^{+}\) was not the principal charge carrier. It is concluded that plateau potentials, bistability and windup in turtle motoneurons do not depend on a CAN current even though its presence can be detected.

**Introduction**

In the spinal cord, plateau potentials have been described in motoneurons (Bennett et al. 1998a,b; Hounsgaard et al. 1988; Hounsgaard and Kiehn 1985; Hounsgaard and Mintz 1988; Lee and Heckman 1998a,b) and several subtypes of interneurons (Hounsgaard and Kjaerulff 1992; Morisset and Nagy 1996; Russo and Hounsgaard 1996). Currents responsible for plateau potentials are modulated by metabotropic receptors for glutamate, serotonin (5-HT), and acetylcholine (Delgado-Lezama et al. 1997; Svirskis and Hounsgaard 1998). Windup (i.e., the gradual increase of the response to repeated depolarizations) has also been described in several types of spinal cord neurons (Morisset and Nagy 1996; Russo and Hounsgaard 1994; Svirskis and Hounsgaard 1997). Both phenomena depend on Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels, which triggers the plateau potential (Hounsgaard and Mintz 1988) and windup of the plateau potential during repeated depolarizations (Russo and Hounsgaard 1994; Svirskis and Hounsgaard 1997). It is not known, however, whether the Ca\(^{2+}\) influx is the charge carrier for the plateau potential or merely the trigger for a noninactivating Ca\(^{2+}\)-activated conductance generating the current underlying the plateau potential. The nonselective calcium-activated cationic current (\(I_{\text{CAN}}\)) is a possible candidate because CAN channels, usually permeant for Na\(^{+}\), K\(^{+}\), and also Ca\(^{2+}\), do not undergo voltage- or Ca\(^{2+}\)-dependent inactivation and thus provide a potential mechanism for maintaining depolarization and Ca\(^{2+}\) entry in the cell (Partridge et al. 1994). Recent studies have shown an involvement of such a \(I_{\text{CAN}}\) in plateau potentials in several types of neurons. In the dorsal gastric motor neuron of the stomatogastric ganglion in the crab for example, \(I_{\text{CAN}}\) current plays an important role in generation and maintenance of plateau potentials (Zhang et al. 1995). In rostral ambiguous neurons in the brain stem of newborn mice, a \(I_{\text{CAN}}\) current is the main charge carrier for plateau potentials (Rekling and Feldman 1997). In rat deep dorsal horn neurons of the spinal cord, \(I_{\text{CAN}}\) represents a fraction of the plateau current, the rest being mediated by noninactivating calcium channels (Morisset and Nagy 1999). Moreover, \(I_{\text{CAN}}\)S are modulated by intracellular pathways compatible with metabotropic modulation of plateau potentials. For example, \(I_{\text{CAN}}\) currents in *Helix* burster neurons are modulated by cyclic AMP-dependent membrane phosphorylation (Partridge et al. 1990); in CA1 hippocampal neurons, \(I_{\text{CAN}}\)S are activated by metabotropic receptors for glutamate (Crepel et al. 1994). Finally, cumulative increase in the concentration of intracellular Ca\(^{2+}\) is a plausible mechanism for windup of a \(I_{\text{CAN}}\) in response to repeated depolarizations.

Several questions remain to be answered. 1) Is such a \(I_{\text{CAN}}\) expressed in turtle motoneurons? 2) Does it contribute significantly to plateau potentials? 3) Does metabotropic modulation, known to regulate generation of plateau potentials, act on \(I_{\text{CAN}}\) currents? 4) Is \(I_{\text{CAN}}\) current involved in windup?

In the present report we show that \(I_{\text{CAN}}\)S are expressed in turtle motoneurons. We also show that the contribution of \(I_{\text{CAN}}\) to plateau potentials, bistability, and windup is insignificant, and that modulatory pathways known to regulate the generation of plateau potentials do not affect \(I_{\text{CAN}}\).

**Methods**

Transverse slices (1.5–2 mm thick) were obtained from the lumbar enlargement of adult turtles (*Chrysemys picta*) anesthetized by intraperitoneal injection of 100 mg sodium pentobarbital and killed by decapitation. The surgical procedures comply with the Danish legislation and are 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) NaCl, 5 KCl, 15 NaHCO\(_3\), MgCl\(_2\), 3CaCl\(_2\), and 20 glucose saturated with 98% O\(_2\)-2% CO\(_2\) to obtain pH 7.6. Intracellular recordings in current-clamp mode were performed with an Axoclamp 2B amplifier (Axon Instruments). Pipettes were filled with a solution containing 1 M K-acetate. Motoneurons were selected for study if they had a stable membrane potential of more than −60 mV. The data were sampled at 16.6 kHz with a 12-bit A/D converter (DIGIDATA 2000 from Axon) and displayed by...
means of Axoscope software and stored on a hard disk for later analysis.

Low-sodium medium was prepared by substituting choline chloride or \(N\)-methyl-\(d\)-glucamine chloride (NMDG; Sigma) for sodium chloride. The pH of medium prepared with NMDG was carefully adjusted to 7.6 by addition of the necessary amount of HCl. This was necessary because medium added NMDG-HCl stock solution often had a pH 8–8.4 when saturated with carbogen. In four of four experiments, high pH reduced or abolished plateau potentials. In normal medium an increase in pH above 8.0, produced by adding HEPES sodium salt, greatly increased the threshold for plateau potentials \((n = 2)\). In low sodium ringer a high pH value had the same effect \((n = 2)\).

Plateau potentials were facilitated directly with an agonist for L-type \(Ca^{2+}\) channels (BayK8644; 2 \(\mu\)M; Sigma) or indirectly by activation of group I metabotropic glutamate receptors with cis-(\(\pm\))-1-aminocyclopentane-1,3-dicarboxylic acid (cis-ACPD; 40 \(\mu\)M; Tocris), muscarine receptors (Muscarine; 20 \(\mu\)M; Sigma), or serotonin receptors (5-HT; 10 \(\mu\)M; Sigma), all known to promote the generation of plateau potentials (Delgado-Lezama et al. 1997; Hounsgaard and Mintz 1988; Svirskis and Hounsgaard 1998). \(Ca^{2+}\) spikes and plateau potentials were facilitated by addition of tetraethylammonium (TEA; 10 mM) to the medium (Hounsgaard and Mintz 1988).

Other drugs that were used (all from Sigma) are as follows: tetrodotoxin (TTX; 2 \(\mu\)M); Nifedipine (10 \(\mu\)M); cobalt; HEPES sodium salt \((N\text{-}[2\text{-hydroxyethyl}]\text{piperazine-N'-[2-ethanesulfonic acid]})\). Data are presented as means \(\pm\) SE.

**RESULTS**

**I\(_{\text{CAN}}\) current in turtle motoneurons**

One way to test whether a CAN current \((I_{\text{CAN}})\) is present in motoneurons is to record the effect of a transient increase in the level of intracellular calcium. We have taken advantage of the ability of motoneurons to generate calcium action potentials in the presence of TEA (10 mM) and TTX (2 \(\mu\)M) (Hounsgaard and Kiehn 1993; Hounsgaard and Mintz 1988) to induce a transient calcium increase. Under these conditions, a \(Ca^{2+}\) spike was evoked by a depolarizing current pulse of sufficient amplitude (Fig. 1A). The involvement of calcium channels in this regenerative response was confirmed by its disappearance when 2 mM \(Co^{2+}\) was added to the medium \((n = 2)\). In all the cells recorded from, calcium spikes were followed by a slow
depolarizing potential, lasting >500 ms (arrow in Fig. 1B; n = 26). The existence of this afterdepolarization was strictly correlated to the presence of calcium spikes and was not dependent on voltage, because it was possible to induce it even when the cell was hyperpolarized by a strong negative bias current. As shown in Fig. 1C calcium spikes were always followed by a prolonged afterdepolarization independent of the membrane potential. This is consistent with mediation by a calcium-activated inward current. This afterdepolarization was not mediated by L-type Ca channels because it persisted in the presence of nifedipine (10 μM; n = 4; see Fig. 2).

Application of trains of low-amplitude, negative current pulses following calcium spikes showed that the afterdepolarization was associated with a decrease in input resistance by up to 60% and lasting up to 600 ms (n = 6), i.e., the same duration as the afterdepolarization. (Fig. 1D).

The amplitude of the afterdepolarization following calcium spikes increased with increasing levels of hyperpolarizing holding current (Fig. 2A1). The relation between the amplitude of the afterdepolarization and the holding potential was linear (Fig. 2A2), showing that the current mediating the afterdepolarization was not voltage sensitive. The afterdepolarization was reversible in response to depolarizing holding current (Fig. 2A). The reversal potential was \(-55 \pm 2.2\) mV (means ± SE; n = 11), suggesting that different ion species were involved. As shown in Fig. 2B the afterdepolarization was partly mediated by Na\(^+\) ions because its reversal potential was shifted to more hyperpolarized levels when extracellular NaCl was replaced by choline chloride or NMDG (n = 7).

Taken together these data showed that the afterdepolarization following calcium spikes in turtle motoneurons was due to activation of a CAN with an amplitude linearly related to the membrane potential.

**I\(_{\text{CAN}}\) current not necessary for plateau potentials**

If the I\(_{\text{CAN}}\) makes a significant contribution to plateau potentials, then their amplitude should be sensitive to the concentration of extracellular Na\(^+\). We tested this by subjecting motoneurons, facilitated to generate plateau potentials in normal medium, to low sodium medium. In all motoneurons, independent of the way in which plateau potentials were promoted, the generation of plateau potentials was uninhibited by low sodium medium (n = 16 of 16; 7 with choline medium and 9 with NMDG medium). In particular the generation of plateau potentials was not shifted to more hyperpolarized membrane potentials. These findings are illustrated in Fig. 3. In normal medium the train of spikes evoked by a depolarizing current

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**FIG. 2.** Afterdepolarization involves inward Na\(^+\) current. For all recordings: top trace: membrane potential; bottom trace: current. All records in presence of TEA (10 mM) and TTX (2 μM) and Nifedipine (10 μM). A1: afterdepolarization following Ca\(^{2+}\) spikes inverted by injecting depolarizing bias current. A2: plot of the difference in potential before and after the calcium spike (V2-V1) as a function of the holding potential (V1). B1: low-sodium Ringer affects the afterdepolarization. Top trace: control recording. Bottom trace: replacement of Na\(^+\) by N-methyl-D-glucamine (NMDG). B2: in low-sodium Ringer, the reversal potential of the afterdepolarization is more negative. Plot of the difference in potential before and after the calcium spike (V2-V1) as a function of the holding potential (V1). ■, control; ○, low-sodium ringer.
pulse did not outlast the duration of the stimulus (Fig. 3A1). In the presence of 40 μM ACPD, the same stimulus evoked a sustained discharge only terminated by a hyperpolarizing current (Fig. 3A2). This bistable response pattern was maintained when sodium chloride was replaced with NMDG (Fig. 3A3). Similar results were obtained when plateau potentials were promoted by serotonin (Fig. 3B; n = 3), by muscarine (n = 3), or BAY K (n = 2; not illustrated). These experiments show that I_{CAN} current is not a major contributor to plateau potentials in turtle motoneurons.

**I_{CAN} current not necessary for windup**

We finally tested whether windup of plateau potentials was preserved in low-Na\(^+\) medium. With the same protocol as used previously (Russo and Hounsgaard 1994; Svirskis and Hounsgaard 1997), we found that the plateau potential, generated by a depolarizing current of near threshold intensity, increased in amplitude and decreased in latency with each of the first few pulses in a stimulus train (n = 8). Facilitation was present in all motoneurons in low-sodium medium whether plateau properties were induced by cis-ACPD (n = 2; Fig. 4A), 5-HT (n = 3; Fig. 4B), or muscarine (n = 3; not illustrated). Therefore we conclude that I_{CAN} is not necessary for windup.

**DISCUSSION**

Our experiments show that I_{CAN} is present in turtle motoneurons. Two experimental observations suggest that this current is activated by calcium. First, under our experimental conditions, the presence of this current was strictly correlated with the presence of calcium spikes. Second, when calcium influx was removed by addition of cobalt, the current was absent. The ions carrying I_{CAN} include Na\(^+\), because its removal shifted the reversal potential substantially. K\(^+\) ions probably also contribute because the reversal potential for the CAN-mediated afterdepolarization is near −55 mV in normal medium and because the reversal shifts toward the equilibrium potential for potassium when NaCl is replaced by NMDG chloride. In agreement, at the resting membrane potential the CAN-induced afterdepolarization was converted to an afterhyperpolarization in NMDG medium.

Our experiments also show that removal of a depolarizing component of I_{CAN} (i.e., the Na\(^+\) inward current) does not affect the ability of motoneurons to generate plateau potentials and windup. These results demonstrate that Na\(^+\) is not the main charge carrier for plateau potentials or windup in turtle motoneurons. The increased conductance during plateau potentials is incompatible with decreased Cl\(^-\) or K\(^+\) currents. Therefore our results support the hypothesis that plateau po-
I CAN is outward and therefore hyperpolarizes the cell and thus 50 mV) (Svirskis and Hounsgaard 1997). Above the presence of 5-HT (10 \mu M)-ACPD (40 \mu M) sodium ringer. A counteracts plateau potentials. Finally the absence of voltage current is around 55 mV, which is below the threshold for CAN amplitude (Partridge et al. 1994) (see also Fig. 3). The linear relationship between the holding potential and $I_{\text{CAN}}$ amplitude (Partridge et al. 1994) (see Fig. 2) also allows us to exclude its involvement in the nonlinear termination of plateau potentials. However, our data do not exclude the possibility that a sustained $I_{\text{CAN}}$ may assist in holding the cell near the threshold for action potentials.

Our results are at odds with recent findings suggesting that CAN currents mediate plateau potentials in rostral ambiguous neurons in the brain stem of newborn mice (Rekling and Feldman 1997) or in rat deep dorsal horn neurons of the spinal cord (Morisset and Nagy 1999) and do not support involvement of a TTX-sensitive sodium channel as in trigeminal motoneurons (Hsiao et al. 1998). These differences undoubtedly reflect differences in the functional sets of ion channels in dendrites and cell bodies in different types of neurons. One obvious difference between Ca$^{2+}$- and Na$^{-}$-mediated plateau potentials is that Ca$^{2+}$ accumulation may serve as second messenger and also be potentially harmful.

FIG. 4. Windup persists in low-Na$^{+}$ medium. Windup induced in low-sodium ringer. A: windup of the response of a motoneuron in presence of cis-ACPD (40 \mu M). B: windup of the response of another motoneuron in presence of 5-HT (10 \mu M). In both experiments NaCl was replaced by NMDG.

This work was funded by The Danish Medical Research Council, The Lundbeck Foundation, and The NOVO-Nordisk Foundation. J.-F. Perrier is recipient of a Marie Curie Research Training Grant ERB4001GT970998.

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Received 9 February 1999; accepted in final form 9 April 1999.

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