NMDA-Induced Intrinsic Voltage Oscillations Depend on L-Type Calcium Channels in Spinal Motoneurons of Adult Turtles

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Guertin, P. A. and J. Hounsgaard. NMDA-induced intrinsic voltage oscillations depend on L-type calcium channels in spinal motoneurons of adult turtles. J. Neurophysiol. 80: 3380–3382, 1998. In a slice preparation from adult turtles, bath-applied N-methyl-D-aspartate (NMDA) induced rhythmic activity in spinal motoneurons. The underlying intrinsic oscillation in membrane potential was revealed in the presence of tetrodotoxin (TTX). NMDA-induced rhythmicity, in the presence or absence of TTX, was abolished or reduced by NMDA receptor antagonists and by three different classes of antagonists for L-type calcium channels. It is suggested that both NMDA receptor channels and L-type calcium channels contribute to NMDA-induced intrinsic oscillations in mature spinal motoneurons.

INTRODUCTION

Rhythmic activity, resembling the locomotor pattern generated in vivo, is induced in vitro preparations of the lumbar spinal cord by bath-applied N-methyl-D-aspartate (NMDA). Under these conditions, rhythmic firing in motoneurons is produced by sequential bouts of excitatory and inhibitory synaptic input from the premotor network assisted by intrinsic oscillatory membrane properties in motoneurons (Guertin and Hounsgaard 1998; Hochman et al. 1994; Sillar and Simmers 1994; Wallén and Grillner 1987). In lampreys, these intrinsic oscillatory properties are mediated by NMDA receptor channels and calcium-dependent potassium channels (Wallén and Grillner 1987) with only minor contribution from calcium channels (Matsumisha et al. 1993). Here we show that NMDA-induced oscillations in spinal motoneurons of adult turtles are blocked by three different classes of antagonists for L-type calcium channels. This finding indicates that ion channels contribute with different weight to rhythmicity in motorneurons from different species.

METHODS

Experiments were performed on 14 adult turtles (Pseudemys scripta). From deeply anesthetized animals (pentobarbitone, 100 mg kg⁻¹ ip), transverse slices (1.5 mm) were obtained from the hindlimb enlargement of the spinal cord and kept at room temperature (20–22°C) in a recording chamber perfused with oxygenated (98% O₂-2% CO₂) medium (in mM: 120 NaCl, 5 KCl, 15 NaHCO₃, 2 MgCl₂, 3 CaCl₂, and 20 glucose, to obtain a pH of 7.5). Activity from motoneurons was monitored intracellularly with 40–55 MΩ sharp microelectrodes filled with K-acetate (1.0 M). Intracellular activity was digitized (10 kHz), stored on a computer, and displayed on-line with Axoscope version 1.1, which was also used for analysis.

Drugs that were used are as follows: NMDA (20–40 µM, Tocris), 2-amino-5-phosphonopentanoic acid (AP5, 25–100 µM, Tocris), nifedipine (0.05–7 µM, Sigma), BAY K 8644 (1–5 µM, RBI), gallopamil (D600, 25–100 µM, RBI), verapamil (25–100 µM, RBI), diltiazem (25–100 µM, RBI), tetrodotoxin (TTX, 1–2 µM, Sigma).

RESULTS

Rhythmic activity induced in turtle motoneurons by NMDA (Guertin and Hounsgaard 1998) is blocked by the competitive antagonist AP5. In the presence of AP5, the NMDA-induced rhythmic firing (Fig. 1A) was replaced by tonic firing if the membrane potential was maintained above threshold for action potentials with a depolarizing bias current (Fig. 1Ba), while a steady membrane potential was observed with subthreshold bias currents (Fig. 1, Bb and Bc). The rhythmic activity induced by NMDA was also abolished by application of L-type calcium channel antagonists (Table 1). Figure 1 shows that rhythmicity restored after washing out AP5 (Fig. 1C) was again abolished by application of 7 µM nifedipine, an antagonist specific for the dihydropyridine binding site on L channels. Rhythmicity was replaced by tonic firing at suprathreshold potentials (bias current of +1.1 nA; Fig. 1Da) and by a stable resting potential at more hyperpolarized levels (Fig. 1, Db and Dc). The effect of nifedipine was reversible (Fig. 1E). This suppressive effect was detectable with concentrations of nifedipine as low as 50 nM. Note that in absence of TTX in the bath, the antagonist effects in Fig. 1 may include presynaptic actions.

In slice preparations, rhythmic activity induced by NMDA is generated by intrinsic membrane properties resistant to TTX (Fig. 2). The intrinsic oscillations induced by NMDA in the presence of TTX were abolished by nifedipine (n = 8; Fig. 2, Ba and Bb). This suggests that the effect of nifedipine was produced postsynaptically.

NMDA-induced oscillations were also reduced by antagonists, selective for the benzothiazepine and the phenylalkylamine sites on L-type calcium channels (Fig. 3 and Table 1). Figure 3A shows an example where 100 µM D600, a phenylalkylamine, applied for 10 min, reduced the NMDA-induced rhythmicity to irregular fluctuations of low ampli-
TABLE 1. Suppressive effects evoked by antagonists of NMDA receptors or L channels on NMDA-induced rhythmicity in mature lumbar motoneurons

<table>
<thead>
<tr>
<th></th>
<th>AP5</th>
<th>Nifedipine</th>
<th>D600</th>
<th>Verapamil/Diltiazem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abolish</td>
<td>13</td>
<td>13</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Suppress only</td>
<td>2</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>No effect</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

Abolishing or suppressing effects evoked by AP5, nifedipine, and verapamil + diltiazem were seen in the presence of tetrodotoxin in 4, 8, and 4 cases, respectively. ‘Suppress only’ means that the rhythm is irregular and the amplitude is reduced as in Fig. 3A, NMDA, N-methyl-D-aspartate; AP5, 2-amino-5-phosphonopentanoic acid; D600, gallopamil.

DISCUSSION

The results show that intrinsic oscillations induced by NMDA in mature lumbar motoneurons are abolished or reduced by antagonists of L-type calcium channels. As in other preparations, the oscillations are also blocked by NMDA receptor antagonists.

NMDA-induced oscillations were blocked by three different classes of antagonists with distinct binding sites on the α1 subunit of the L-type calcium channel (Hockerman et al. 1997). This makes cross reaction with the NMDA receptor channel complex (Filloux et al. 1994) and other nonspecific actions (Diochot et al. 1995) unlikely explanations for the present findings. Furthermore, the higher effectiveness of...
The small irregular voltage oscillations after adding 100 μM NMDA in other systems: convulsions (Palmer et al. 1993; channel blocker nifedipine attenuates slow excitatory amino acid neurotransmission in lampreys (M. Wikström, A. Buschges, A. El Manira, and S. Grillner, personal communication)).

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REFERENCES


Dihydropyridines over the other antagonists in the present experiments is in agreement with their respective affinities and sites of action on L-type calcium channels (Hockerman et al. 1997).

The fact that activation of plateau potentials mediated by L-type channels do not lead to oscillations (Hounsgaard and Kiehn 1989; Hounsgaard and Mintz 1988) raises the possibility that oscillations in turtle motoneurons may involve mechanisms beyond the cell membrane. For example, calcium entry per se might trigger calcium-induced-calcium-release, which could phasically regulate the properties of L channels and other channels via second messengers (Chavis et al. 1996; Delgado-Lezama et al. 1997). These questions need further attention.

L-type calcium channels contribute to properties induced by NMDA in other systems: convulsions (Palmer et al. 1993; Weiss et al. 1990), LTP (Huber et al. 1995) and nitric oxide production (Rodriguez-Alvarez et al. 1997). Our results reveal another layer of complexity to the mechanisms of intrinsic voltage oscillations in motoneurons. This is underlined by recent experiments showing that L-type calcium channels contribute to serotonin-induced oscillations in guinea pig trigeminal motoneurons (Hsiao et al. 1998) and to other aspects of NMDA-induced oscillations in the spinal cord of lampreys (M. Wikström, A. Buschges, A. El Manira, and S. Grillner, personal communication).