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Research Report

Pudendal nerve stimulation, interneurons post-discharge and delayed depolarization in hind limb motoneurons of the female cat

E.J. Muñoz-Martínez, Rodolfo Delgado-Lezama*

Departamento de Fisiología, Biofísica y Neurociencias, CINVESTAV del IPN, A.P. 14 740, C.P. 07000, México D. F., México

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The present experiments were done in the spinal female cat. In a prior work in the decerebrate female cat, stimulation of the sensory pudendal nerve (SPN) induced a depolarizing wave (LD) in hind limb motoneurons that outlasted the stimulus by up to 6 s. LD triggered self-sustained motoneuron firing (bistability). An intrinsic potential underlies bistable firing, which, in the cat, depends on two main factors; first, the integrity of pathways descending from the brain stem to the spinal cord and, second, the membrane potential of the motoneuron just before the stimulus; at high resting potential, excitatory short-lasting inputs induce transient but no sustained firing. Thus, no bistability occurs in the spinal cat or in hyperpolarized motoneurons of the decerebrate cat. LD might be an intrinsic potential that could also be absent in the spinal cat, or an extrinsic (synaptic) potential induced by spinal interneurons. In the latter case, the interneurons generating LD should show post-discharge as prolonged as LD. LD was produced in spinal cats and its amplitude did not change or increase slightly during hyperpolarizing pulses, which suggests that LD might be a synaptic response. Interneurons showing post-discharge to train of stimulation to SPN were located 100–200 μm above the pools of hind limb motoneurons. Some post-discharges were as prolonged as LD. We conclude that LD might be a synaptic response to local interneurons.

1. Introduction

In hind limb motoneurons of the decerebrate female cat, stimulation of the sensory pudendal nerve (SPN) with train of shocks induces a depolarizing wave (LD) that outlasts the train from 0.2 to 6 s and may reach the firing threshold. When the threshold is reached, LD may trigger self-sustained firing depending upon the resting membrane potential just before the SPN stimulation. This is a case of the cell property known as bistable behavior (bistability) of the motoneuron, that is, no firing or self-sustained firing (Hounsgaard et al., 1984, 1988a,b; Crone et al., 1988; Lee and Heckman, 1998a,b; Hounsgaard and Khien, 1989, 1993; Perrier and Hounsgaard, 2000; Paroschy and Shefchyk, 2000; Cueva-Rolón et al., 2002; Raya et al., 2004). Human motoneurons might also show this behavior (Collins et al., 2001). A plateau potential underlies bistable firing, which depends on two conditioning factors. First, the resting (or holding) potential before excitation must be 3 mV or less

* *Corresponding author*. Fax: +52 5061 3800x5713.
E-mail address: jmunoz@fisio.cinvestav.mx (E.J. Muñoz-Martínez).

Abbreviations: LD, late depolarization in hind limb motoneurons; SPN, sensory pudendal nerve; SPNint, sensory pudendal nerve interneurons; MG, medial gastrocnemius; LG-Sol, lateral gastrocnemius plus soleus; Tib, tibial; DP, deep peroneal

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below the firing threshold (Hounsgaard et al., 1984; Cueva-Rolón et al., 2002). At larger potential difference, LD only elicits transient firing (Cueva-Rolón et al., 2002). Second, bistable firing also depends on the integrity of serotonergic axons descending from the brain stem. Thus, spinal cats do not show bistable firing (Hounsgaard et al., 1988a). We wondered whether LD also might be an intrinsic potential with the same conditioning factors as the plateau potential of bistability or a synaptic potential induced by local interneurons. If this were the case, the interneurons should show post-discharge as prolonged as LD.

Hind limb motoneurons of spinal cats show LD, which does not change or increase slightly by hyperpolarizing the cell membrane. Thus, LD does not depend on descending pathways or membrane potential. This suggests that LD is a synaptic potential. Interneurons responding to SPN stimulation with post-discharge of similar duration as LD were found near hind limb motoneurons.

2. Results

2.1. *LD in spinal cats*

Intracellular recordings were taken from 22 motoneurons in the L7 segment of the spinal cord. These neurons did not show background firing and had stable membrane potential (≥60 mV) through the observation. Nineteen motoneurons responded to nerve stimulation: MG (*N*=12), Tib (*N*=4), or SP (*N*=3). Three motoneurons only responded to stimulation of the ventral root (unidentified motoneurons). Single shock to SPN evoked either IPSP-EPSP (*N*=14) or EPSP-IPSP sequence (*N*=3). EPSPs were <3 mV in amplitude in the former sequence but 4–6 mV in the latter. Two motoneurons only showed a single, larger EPSP that reached threshold (not shown; see Cueva-Rolón et al., 2002).

As in decerebrate cats, a train of shocks (100 Hz, 0.1–0.5 s) to SPN elicited in spinal cats a depolarizing wave (LD) outlasting the train by 0.2–4 s (see Cueva-Rolón et al., 2002). Thus, LD does not depend on the integrity of descending pathways.

In the cells showing IPSP-EPSP sequence evoked by single shocks, IPSPs preceded LD. During train of stimulation at 10 Hz, series of IPSP-EPSP were seen (*N*=5, see Fig. 1C). Both, IPSP and EPSP increased in amplitude rather randomly during the train. Note in Fig. 1C that LD rose from the last EPSP. It appears that during the train, IPSP prevented that LD rise. After LD, the membrane potential increased with respect to control.

In the cases of IPSP-EPSP sequence, LD showed two peaks (Fig. 1A). The first LD peak occurred during the train of stimulation and later LD declined, but it rose again and reached the major peak after the stimulus. When the response to either single shock or a train started with EPSP, potential oscillations followed but hyperpolarization prevailed during the train, and LD rose after a long delay (Fig. 1B).

In nine motoneurons, LD produced single or repetitive firing lasting less than 2 s (Figs. 1B, C). The amplitude and duration of subthreshold LDs varied in different motoneurons of the same cat, but the differences showed no relation with the innervated muscle.

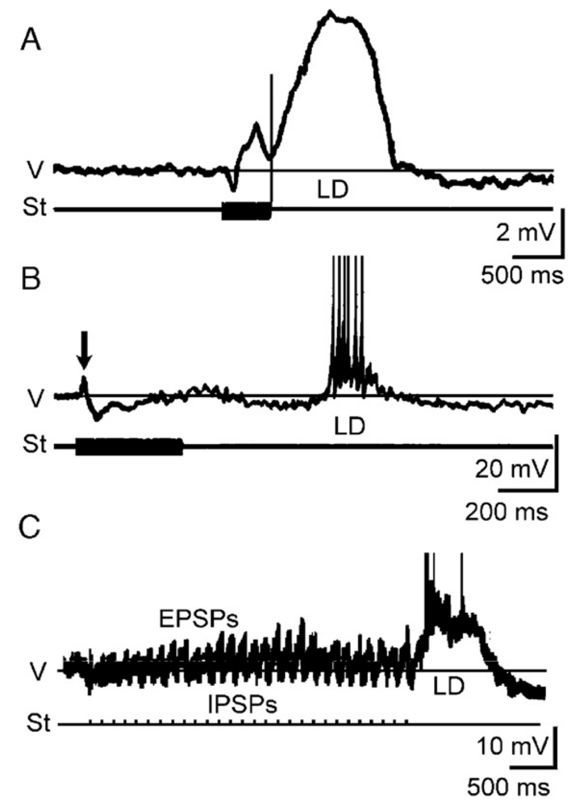

Fig. 1 – Late depolarization (LD) induced in motoneurons of spinal cats by stimulation the sensory pudendal nerve (SPN) with a train of shocks at 100 Hz. In all panels, the upper trace is the motoneuron membrane potential (V) and the lower one is the stimulus artifact (St); tracings V show reference line. In panel A (MG motoneuron), the first event is an IPSP followed by l depolarization that peaks and declines during the train (100 Hz, 500 ms). Coinciding with the train offset (vertical line), LD rises In panel B (MG motoneuron), the first event is an EPSP followed by IPSPs for most of the stimulating period. Small EPSPs as well as IPSPs follow the stimulus. In panel C (LG-Sol motoneuron), SPN train at 10 Hz elicited a repetitive IPSP-EPSP sequence. Synaptic potentials grow during the train. IPSP coincide with the raising of the precedent EPS, and LD rises from the last EPSP. In the three cases (A–C), the membrane potential increases after LD.

2.2. *LD during hyperpolarizing pulses*

Cathodic current pulses were injected to motoneurons prior to evoking subthreshold LDs. The LD amplitude did not change significantly (*N*=5) or increased (*N*=3) during cathodic pulses with amplitude greater than the IPSP reversal potential (Figs. 2A, B). Therefore, it appears that LD is not an intrinsic potential but a synaptic one.

2.3. *LD amplitude vs. number of stimulating pulses*

LD amplitude depends on the number of train pulses to SPN. This relation was studied in four LDs that did not reach threshold (Figs. 2A, B). As shown in Fig. 2, LD amplitude