In Vivo Patch-Clamp Analysis of IPSCs Evoked in Rat Substantia Gelatinosa Neurons by Cutaneous Mechanical Stimulation

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INTRODUCTION

Noxious information is carried through fine myelinated Aδ and unmyelinated C fibers to the superficial laminae (Light and Perl 1979; Yoshimura and Jessell 1989), particularly substantia gelatinosa (SG) lamina II of the spinal dorsal horn where the information is modulated. Such a modulation is expected to partly occur through the activation of inhibitory transmission. To address this issue, we have developed a patch-clamp technique applicable to SG neurons in an in vivo rat preparation (Furue et al. 1999). The aim of the present work was to know whether IPSCs are evoked in SG neurons in response to cutaneous mechanical stimulation and if so to reveal what kinds of neurotransmitters are involved in the IPSCs.

METHODS

The methods used for the current experiment were similar to those in our preceding study (Furue et al. 1999). Briefly, male Sprague-Dawley rats (7–10 wk old) were anesthetized with urethane (1.2–1.5 g/kg ip) and artificially ventilated; if a withdrawal reflex appeared, a supplemental dose of urethan was given during surgery and data collection. Bilateral pneumothorax was made to reduce a respiratory movement of thorax. A lumbar laminectomy was performed at the level of L3 or L5, and then the animal was placed in a stereotaxic apparatus. After removing the dura and cutting arachnoid membrane to make a window large enough to let a patch electrode, the surface of spinal cord was irrigated with 95% O2-5% CO2 –equilibrated Krebs solution (in mM): 117 NaCl, 3.6 KCl, 2.5 CaCl2, 1.2 MgCl2, 1.2 NaH2PO4, 11 glucose, and 25 NaHCO3 at 38.0 ± 0.5°C. A whole cell voltage-clamp technique was applied to SG neurons with an electrode that had a tip resistance of 10–15 MΩ and was filled with a solution having the following composition (in mM): 110 Cs2SO4, 0.5 CaCl2, 2 MgCl2, 5 EGTA, 5 HEPES, 5 ATP-Mg, and 5 tetraethylammonium; pH 7.2. Data were digitized with an A/D converter, stored, and analyzed with a personal computer using pCLAMP6 and Axograph3.5 data acquisition program (Axon Instruments, Foster City, CA). The recorded neurons were identified as being in the SG based either on their morphological features revealed by an intrasomatic injection of biocytin or on the depth of the neurons from the surface of the spinal cord. The mechanical stimuli used were pinching of skin folds with a toothed forceps and brushing the surface of skin or the hairs in the ipsilateral hind limb. Drugs used were bicuculline and strychnine (Sigma, St. Louis, MO); they were given by superfu-

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RESULTS

An animal preparation was maintained in a stable state over 10 h, and patch-clamp recordings of up to 3 h were obtained from 31 SG neurons having input resistances of 447 ± 26 MΩ (mean ± SE, n = 25). All of the neurons examined exhibited spontaneous excitatory postsynaptic currents (EPSCs) at a holding potential (V_H) of −70 mV, as reported by Furue et al. (1999), and when measured at 0 mV where spontaneous EPSCs were invisible, spontaneous IPSCs could be recorded in all of these cells (see Fig. 2, A and B).

IPSCs evoked by cutaneous mechanical stimulation

In the majority (80%) of the SG neurons examined (n = 15), a brush produced a barrage of IPSCs, which persisted during the stimulus (left panel of Fig. 1; see also Fig. 3A). They had an average amplitude and frequency, respectively, of 87.9 ± 12.3 pA and 18.3 ± 2.6 Hz (n = 12), values being significantly larger than those of spontaneous IPSCs (27.2 ± 3.6 pA and 6.6 ± 0.5 Hz, respectively; n = 12; P < 0.05). These IPSCs subsided within 1 s after the stimuli were terminated. A pinch, on the other hand, evoked IPSCs, the activity of which lasted for only 1–2 s at its beginning and end, in 12 of the 15 neurons, as seen in the right panel of Fig. 1 (see also Fig. 3A). This transient response seemed to follow the movement of forceps. The remaining three neurons (20%) were insensitive to the brush while exhibiting a barrage of IPSCs without a decline in the frequency in response to the pinch, as seen for the brush (data not shown). They had an average amplitude and frequency, respectively, of 69.1 ± 2.7 pA and 15.3 ± 1.2 Hz (n = 3), values being significantly larger than those of spontaneous IPSCs (P < 0.05). With respect to inhibitory receptive fields of SG neurons, the points sensitive to stimulation were the lower leg, upper leg, and thigh and thus were unexpectedly wide, as seen for EPSCs (Furue et al. 1999).

IPSCs mediated by glycine and GABA_A receptors

In 10 (71%) of 14 SG neurons examined, spontaneous IPSCs were blocked by a glycine-receptor antagonist, strychnine (4 μM; A) or bicuculline (20 μM; B) blocked spontaneous IPSCs. Note that the strychnine-sensitive IPSC (inset in A) exhibits a shorter duration by about 3-fold compared with the bicuculline-sensitive one (inset in B). C and D: brush-evoked IPSCs (top in C and D) were blocked by strychnine (4 μM; bottom in C) or bicuculline (20 μM; bottom in D). Each of the records in A–D was obtained from a different SG neuron; V_H = 0 mV.
**DISCUSSION**

The present study revealed in SG neurons of the adult rat spinal cord in vivo that IPSCs occur spontaneously or in response to cutaneous mechanical stimuli and that they are mediated by either glycine or GABA. This is the first report showing mechanical stimulus-evoked IPSCs in spinal dorsal horn neurons in vivo, although Light and Willcockson (1999) have reported spontaneous IPSPs in a similar preparation. Such a glycinerergic or GABAergic transmission has been observed in in vitro slice preparations (Yoshimura and Nishi 1995). When compared between the in vivo and in vitro preparation, the IPSCs were similar in that glycinerergic IPSCs exhibit a shorter duration than GABAergic ones (see Yoshimura and Nishi 1995). On the other hand, the percentage of in vivo SG neurons exhibiting glycerine or GABA receptor-mediated IPSCs was different from that obtained in slices. Although Yoshimura and Nishi (1995) have reported that GABAergic glycine- and both-mediated evoked IPSPs are observed in 47, 37, and 16% of the SG neurons examined, respectively, we could not note SG neurons exhibiting IPSCs mediated by both glycinerergic IPSCs were mainly observed. Such a difference would be due to a distinction in the number of the inhibitory fiber inputs between SG neurons in vivo and in slices.

Our present results revealed in the majority (80%) of SG neurons that a brush produces a persistent activity of IPSCs during the stimulus while a pinch elicits IPSCs only at its beginning and end. The pinch-evoked activity may have been caused by a touch response that occurs at the on/off time of pinching the skin. Alternatively, it is possible that an activation of peripheral receptors on the skin by pinch is rapidly accommodated. This is, however, unlikely since EPSCs persist during pinch. When examined in the same SG neuron, both brush and pinch evoked a persistent activity of EPSCs during the stimuli, while brush stimulus only induced a similar persistent activity of IPSCs. It is suggested that both excitatory and inhibitory information are transmitted to SG neurons when a brush is applied to the skin, while a pinch evokes excitatory but not inhibitory responses. This idea seems not to be applied to all SG neurons, because pinch evoked persistent IPSCs in 20% of the SG neurons examined.

Our previous studies using spinal cord slices have suggested that primary-afferent Aδ fibers innervate glycinerergic and/or GABAergic interneurons, the activation of which results in the production of IPSPs in SG neurons (Yoshimura and Nishi 1995). So the IPSCs evoked by cutaneous mechanical stimuli are likely to be due to the activation of Aδ fibers. Figure 3C demonstrates our hypothesis that a touch, whose information is conveyed through Aδ fibers to the spinal dorsal horn, may activate inhibitory interneurons, resulting in the inhibition of nocuous transmission from the periphery to SG neurons. This hypothetical circuitry may explain the well-known behavioral observation that touching near the skin where a pain occurs leads to its alleviation.

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**REFERENCES**


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