

Synaptic interaction between GABAergic terminals and substance P receptor-positive neurons in rat spinal superficial laminae¹

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ABSTRACT

AIM: To identify synaptic relationship between γ -aminobutyric acid (GABA)-containing terminals and substance P receptor-positive neurons in the superficial dorsal horn of rat spinal cord. **METHODS:** A combination of preembedding immunoperoxidase staining for substance P receptor with postembedding immunogold staining for GABA was employed. **RESULTS:** Intense substance P receptor-expressing neurons were concentrated in medial half of the lamina I and the outer lamina II of the spinal cord. Under electron microscopy, substance P receptor immunoreactivity was found mainly in perikarya and dendrites, and deposited along neuronal membranes, in Golgi complex, in rough endoplasmic reticulum and on the surface of mitochondria with patch- or granular-like staining. The double-labeling studies revealed that substance P receptor-expressing dendrites received symmetric synaptic contacts from axonal terminals labeled with immunogold particles indicating GABA. GABA also co-localized with substance P receptors in the dendrites. **CONCLUSION:** The synaptic contact between GABA-containing terminal and substance P receptor-expressing neuron provided important morphological evidence for previous pharmacological studies concerning antinociceptive function of GABA in the spinal cord.

INTRODUCTION

Accumulating evidence has indicated that the spinal cord contains not only transmission pathway of nociceptive information but also modulating circuits of nociceptive information. γ -Aminobutyric acid (GABA), an important inhibitory neurotransmitter, plays a key role in modulating nociceptive information via pre- and post-synaptic inhibition in the spinal cord^[1-3]. Substance P (SP) is believed to be an important signaling molecule in the spinal transmission of nociceptive information^[4]. Intrathecal administration of the GABA receptor agonists significantly attenuated neuronal activity induced by noxious stimulation and exogenous SP, which was completely reversed by the GABA receptor antagonists^[5-7]. It is likely that GABA-containing neurons may modulate the activity of SP receptor-expressing neurons in the spinal cord. SP receptor-expressing neurons have been demonstrated to locate mainly in the superficial laminae of the spinal dorsal horn^[8-10], at the sites of predominant distribution of GABA-containing neurons^[1-3]. Here, we provide the morphologic evidence that there is a synaptic contact between GABA-containing terminal and SP receptor-expressing neuron in the superficial laminae of the dorsal horn.

MATERIALS AND METHODS

Experiments were performed on male Sprague-Dawley rats ($n = 8$, 230 - 280 g, Grade II, Certificate No 005, Shanghai Animal Center, Chinese Academy of Sciences). Under deep anesthesia with sodium pentobarbital (60 mg/kg, ip), the animals were perfused transcardially with 100 mL of 0.01 mol/L phosphated-buffered saline (PBS, pH 7.4), followed by 400 mL of a fixative containing 1% paraformaldehyde, 2% glutaraldehyde, and 0.1% picric acid in 0.1 mol/L phosphate buffer (PB, pH 7.4). The lumbar segment was removed, postfixed in the same fixative for 4 h, and cut into 50 μ m

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thick transverse sections on a vibratome. The sections were processed immunocytochemically with the use of the affinity-purified antibody against substance P receptor (SPR) (gift from Dr N Mizuno). The sections were incubated sequentially with (1) 1 mg/L rabbit anti-SPR IgG diluted in PBS containing 3 % normal goat serum at 4 °C for 48 h, (2) goat biotinylated anti-rabbit antibody (1:200, Vector) for 1 h at 37 °C, and (3) avidin-biotin-peroxidase complex (1:100, Vector) at 37 °C for 1 h. The immunoreactivity was visualized by incubating in the Tris-HCl buffer containing 0.05 % 3,3-diaminobenzidine and 0.01 % H₂O₂.

For electron microscopy, sections were postfixed with 1 % osmium tetroxide in 0.1 mol/L PB at room temperature for 45 min, and dehydrated in graduated ethanol, and finally embedded in Epon. The ultrathin sections were mounted on Formvar-coated nickel grids, and then immunostained for GABA. The grids were washed with Tris-buffered 0.9 % saline containing 0.2 % Triton X-100 (TBST, pH 7.6), and then incubated with a mouse anti-GABA serum (1:1500, Sigma, St Louis, MO, USA) overnight at room temperature. After being rinsed twice with TBST (pH 7.6) for 30 min, the grids were transferred into TBST (pH 8.2), and then incubated with a goat anti-mouse IgG-conjugated to 10 nm gold particles (Sigma) diluted at 1:20 with TBST (pH 8.2) at room temperature for 2 h. Finally, the ultrathin sections were counterstained with uranyl acetate and lead citrate. The observation was made with Phillips CM-100 electron microscope.

In the control experiments, when the SPR and GABA antibodies were omitted or replaced with normal rabbit or mouse serum, no immunoreactivity was detectable.

RESULTS

In the lumbar cord segments, dense population of SPR-expressing neurons was found mainly in lamina I and the outer lamina II, particularly in their medial parts. The most common type of SPR-expressing neurons had fusiform cell bodies and extensive rostrocaudal dendritic trees, which were typically confined to lamina I and the outer portion of lamina II, and occasionally reached the inner portion of lamina II and lamina III. In contrast, the inner lamina II contained very few SPR-positive neurons, but it contained a lot of SPR-expressing processes that derived from SPR-expressing neurons in

lamina I and the outer lamina II, and the deep laminae of the dorsal horn. A few SPR-expressing neurons were sparsely distributed in the deep laminae. These neuronal processes often extended dorsally into the superficial laminae (Fig 1).

Electron microscopic observation was made on the ultrathin sections obtained from the superficial laminae of the dorsal horn. SPR immunoreactivity was observed to locate in perikarya and dendrites. In perikarya, immunoreactivity deposited along neuronal membranes, in Golgi complex, in rough endoplasmic reticulum and on the surface of mitochondria with patch- or granular-like staining. The nucleus was never stained. SPR immunoreactivity was most encountered in the dendritic profiles (Fig 2-4). Immunoreactivity was distributed in a disconnected fashion along the dendrite membrane, and deposited not only in postsynaptic sites but also in non-synaptic areas. Synaptic contacts onto these SPR-expressing dendrites were asymmetrical and/or symmetrical (Fig 2-4). The presynaptic terminals exhibited numerous clear and/or a few dense core vesicles ones. The dense core vesicles were located near or away from the synaptic junction membrane.

Postembedding immunogold staining for GABA showed that a lot of GABA-containing terminals were found in the superficial laminae of the dorsal horn, most of which formed synaptic contacts with the dendrites, and some with neuronal bodies and axonal terminals. GABA-containing terminals contained pleomorphic vesicles with/without varying number of dense core vesicles. Immunogold particles coding for GABA were randomly distributed over extrasynaptic membranes, and only a few were found close to the synapses (Fig 3,4). GABA-containing terminals were observed to synapse with SPR-expressing dendrites (Fig 3,4). A few GABA-containing dendrites were also seen in the superficial laminae, some of these dendrites received synaptic contacts from axonal terminals. It was found that GABA-containing dendrites were also positive for SPR (Fig 5).

DISCUSSION

In conformity with the previous studies^[8-10], the present data showed that SP receptor immunoreactivity was distributed mainly in the superficial laminae of the spinal cord, where GABA-containing neurons were predominantly located^[1-3]. The overlap of SP receptor with GABA in the superficial laminae suggests that there is an interaction between GABA and SP.

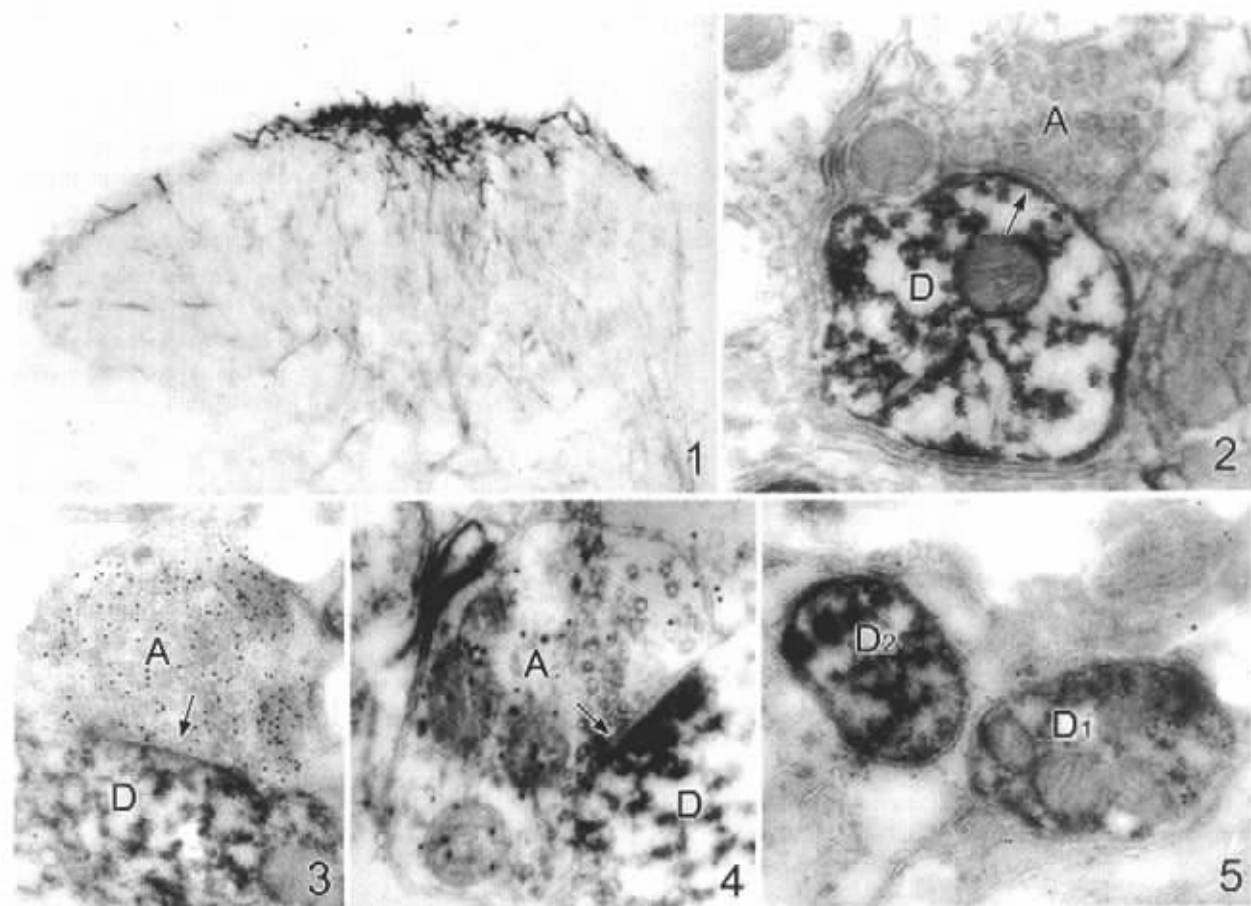


Fig 1. The distribution of substance P (SPR) immunoreactivity in rat spinal dorsal horn. Note that the SPR-positive neurons are located mainly in the medial part of laminae I. $\times 100$.

Fig 2. A substance P receptor (SPR)-positive dendrite (D) receiving synaptic contact (arrow) from unstained terminals (A) of the rat spinal dorsal horn. $\times 28\ 500$.

Fig 3. An axonal terminal (A), containing many GABA-labelled gold granules, synapsing (arrow) with an SPR-positive dendrite (D) of a spinal dorsal horn neuron of the rat. $\times 53\ 000$

Fig 4. An axonal terminal (A), containing a few GABA-labelled gold granules, synapsing (arrow) with an SPR-positive dendrite (D) of a spinal dorsal horn neuron of the rat. $\times 65\ 000$.

Fig 5. A SPR-positive dendrite (D1) positive for GABA-labelled gold granules, showing co-localization of SPR and GABA. Another SPR-positive dendrite (D2) is negative for GABA-labelled gold granules. $\times 49\ 000$.

GABA exerts its actions via GABA_A and GABA_B receptor subtypes. Both types of GABA receptors are located on the presynaptic primary afferent fibers and on the postsynaptic neurons and dendrites within the spinal cord^(11,12). Therefore, GABA exerts regulatory effects in transmission of spinal noxious stimulation via pre- and post-synaptic mechanisms. The previous pharmacological studies showed that intrathecal administration of GABA or its receptor agonists abolished pain behaviors induced by exogenous SP^(5,6). This suggests that GABA-containing neurons probably establish inhibitory postsynaptic connections in the neurons receiving SP terminals in the spinal cord. The present results demonstrated for first time that

GABA-containing axonal terminals synapsed with SP receptor-expressing dendrites in the superficial laminae, which provides strong morphological evidence for the pharmacological results mentioned^(5,6). Further studies indicated that GABA_A receptor agonist, muscimol, selectively blocked nociception involving excitatory amino acid receptors⁽⁷⁾ whereas GABA_B agonist, baclofen, selectively blocked SP spinal activity⁽⁶⁾. Combined with the present data, it is likely that GABA_B receptors but not GABA_A receptors co-localize with SP receptors in spinal cord neurons.

Another important finding in the present observations is the co-localization of GABA with SP receptors in the

dendrites. Since SP-expressing fibers in the superficial laminae originate mainly from the primary afferents^[13], it is indicated that GABA-containing neurons in the superficial laminae may receive inputs from the primary afferent fibers containing SP. This result further supports our previous observations that SP receptor antagonist, spantide, blocked the release of GABA triggered by capsaicin in the spinal dorsal horn^[14].

REFERENCES

- 1 Barber RP, Vaughn JE, Saito K, McLaughlin BJ, Roberts E. GABAergic terminals are presynaptic to primary afferent terminals in the substantia gelatinosa of the rat spinal cord. *Brain Res* 1978; 141: 35-55.
- 2 Carlton SM, Hayes ES. Light microscopic and ultrastructural analysis of GABA-immunoreactive profiles in the monkey spinal cord. *J Comp Neurol* 1990; 300: 162-82.
- 3 Magoul R, Onteniente B, Geffard M, Calas A. Anatomical distribution and ultrastructural organization of the GABAergic system in the rat spinal cord. An immunocytochemical study using anti-GABA antibodies. *Neuroscience* 1987; 20: 1001-9.
- 4 Otsuka M, Yanagisawa M. Does substance P act as pain transmitter? *Trends Pharmacol Sci* 1987; 8: 506-10.
- 5 Beyer C, Banas C, Gonzalea-Florez O, Komisaruk BR. Blockage of substance P-induced scratching behavior in rats by the intrathecal administration of inhibitory amino acid agonists. *Pharmacol Biochem Behav.* 1989; 34: 491-95.
- 6 Hwang AS, Wilcox GL. Baclofen, γ -aminobutyric acid_B receptors and substance P in the mouse spinal cord. *J Pharmacol Exp Ther* 1989; 248: 1026-33.
- 7 Aanonsen LM, Wilcox GL. Muscimol γ -aminobutyric acid_A receptors and excitatory amino acids in the mouse spinal cord. *J Pharmacol Exp Ther* 1989; 248: 1034-38.
- 8 Brown JL, Liu H, Maggio JE, et al. Morphological characterization of substance P receptor-immunoreactive neurons in the rat spinal cord and trigeminal nucleus caudalis. *J Comp Neurol* 1995; 356: 327-44.
- 9 Tao YX, Li YQ, Zhao ZQ, Shigemoto R, Kaneko T, Mizuno N. Colocalization of substance P receptor-like and Fos-like immunoreactivities in the neurons within the spinal dorsal horn of the rat. *Acta Anat Sin* 1997; 28: 346-49.
- 10 Tao YX, Li YQ, Zhao ZQ. Ultrastructural localization of substance P receptor and its synaptic relationship with the primary afferents in the superficial dorsal horn of the rat spinal cord. *Acta Anat Sin* 1998; 29: 228-31.

- 11 Price GW, Wilkin GP, Turnbull MJ, Bowery NG. Are baclofen-sensitive GABA-B receptors present on primary afferent terminals of the spinal cord? *Nature* 1984; 307: 71-4.
- 12 Sawynok J, Mochhala SM, Pillay DJ. Substance P, injected intrathecally, antagonizes the spinal antinociceptive effect of morphine, baclofen and noradrenaline. *Neuropharmacology* 1984; 23: 741-47.
- 13 Lawson SN. Neurochemistry of cutaneous nociceptors. In: Belmonte C, Cervero F, editors. *Neurobiology of nociceptors.* Oxford: Oxford University Press; 1996. P 72-91.
- 14 Wei F, Zhao ZQ. Blockade of capsaicin-induced reduction of GABA-immunoreactivity by spantide in cat spinal superficial dorsal horn. *Neuroscience* 1996; 71: 277-83.

大鼠脊髓背角浅层 GABA 能轴突与 SP 受体阳性神经元的突触联系¹

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关键词 GABA; P 物质受体; 突触传递; 共存; 脊髓

目的: 研究大鼠脊髓背角浅层内 GABA 能神经元与 P 物质受体 (SPR) 阳性神经元之间的相互关系. **方法:** 应用 SPR 包埋前免疫电镜结合 GABA 胶体金技术. **结果:** SPR 样免疫阳性神经元主要分布于脊髓背角 I 层和 II 层背侧部的内侧半. 电镜下, 浅层内 SPR 样免疫反应阳性产物主要定位于细胞体和树突. 免疫反应产物在树突呈片或颗粒状, 常常与线粒体外膜表面、粗面内质网、高尔基氏体、树突膜、胞浆膜内面和核膜外表面等相连. 双标记显示 SPR 样免疫反应树突接受 GABA 样免疫金标记的轴突传入, 形成对称性轴-树突触. 此外, GABA 还可与 SPR 共存于同一树突中. **结论:** GABA 和 P 物质受体在脊髓背角神经元的突触联系, 为以往 GABA 调制脊髓伤害感受功能的药理学研究提供了形态学证据.

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