Role of 5-HT$_3$ Receptors in the Mechanisms of Central Pain Syndrome

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 6, pp. 647-651, June, 2003
Original article submitted February 7, 2003

Electrophysiological and behavioral studies showed that spinal 5-HT$_3$ receptors are involved in the regulation of pain sensitivity in rats. Intrathecal administration of the 5-HT$_3$ receptor antagonist tropine (200 μg) produced allodynia, reduced the threshold, decreased the latency, and increased the number of spikes in the late component of the nociceptive flexion reflex. Intrathecal administration of 5-HT$_3$ receptor agonist quipazine (200 μg) abolished nociceptive flexion reflex and alleviated spinal pain syndrome produced by impairment of GABAergic inhibition in the lumbar spinal segments. Our results indicate that spinal 5-HT$_3$ receptors are involved in the modulation of pain sensitivity: activation of these receptors inhibits nociceptive reactions, while blockade of 5-HT$_3$ receptors potentiates the nociceptive response via modulation of excitability of GABAergic interneurons.

Key Words: *central pain syndrome; nociceptive flexion reflex; allodynia; 5-HT$_3$ receptors; tropine; quipazine*

Recent studies showed that the serotoninergic system of the brain plays an important role in the regulation of pain sensitivity [5,8]. Serotonin possesses not only analgesic, but also pronociceptive properties. The dual effects of serotonin on pain sensitivity were observed in experiments with its intrathecal administration and activation of descending supraspinal serotoninergic systems [3,6,12]. This discrepancy is related not only to methodical differences (various doses of exogenous serotonin and specific methods for studying nociceptive reactions), but also to heterogeneity of serotonin receptors (5-HT receptors). Four types of serotonin receptors were identified in the spinal cord (5-HT$_1$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ receptors). Moreover, these receptors have a variety of subtypes [5,8,10,11]. Published data show that 5-HT$_{1B}$ receptor agonists inhibit nociceptive neurons, while activation of 5-HT$_{1A}$ receptors augments the nociceptive response [4,5,13]. 5-HT$_2$ and 5-HT$_3$ receptor agonists produce pro- and antinociceptive effects. Activation of peripheral 5-HT$_2$ and 5-HT$_3$ receptors augments the nociceptive response [13], while spinal 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ receptors promote the increase in nociceptive thresholds [4,5,8].

Supraspinal serotoninergic neurons activate GABAergic and glycinergic inhibitory interneurons. GABA$_A$ receptor antagonists block the analgetic effect of 5-HT$_3$ receptor agonists [4]. The GABAergic inhibitory system of the spinal cord plays a role in the pathogenesis of central pain syndromes [1,2,9].

Here we evaluated the role of spinal 5-HT$_3$ receptors in the mechanisms of spinal pain syndrome (SPS) resulting from impairment of GABAergic inhibition.

MATERIALS AND METHODS

Experiments were performed on 56 male Wistar rats (220-250 g) according to ethical principles for studying experimental pain syndrome in animals in electrophysiological and behavioral experiments. 5-HT$_3$ receptor agonist quipazine (Sigma) and antagonist tropine (Sigma) were used.

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0007-4888/03/1356-0552$25.00

In behavioral studies we evaluated the effects of 5-HT_3 receptor agonists and antagonists on SPS. SPS was modeled under ether anesthesia after laminectomy. An agar plate (6×1.5×2 mm) containing penicillin sodium salt (50,000 U/ml) was applied to the dorsal surface of lumbar spinal segments (L4-L6). This agent impaired GABAergic inhibition and induced the formation of an aggregate of sensitized nociceptive neurons in the dorsal horns. The intensity of SPS was evaluated using a 3-point scale. Six parameters of SPS were taken into account: vocalization, general locomotor activity during pain attack, local reaction (licking and biting of hindlimbs), allodynia (nociceptive response to tactile stimulation), and incidence and duration of pain attacks. The effects of quipazine and tropine on SPS were studied on 3 animal groups (10 rats per group). In group 1 rats an agar plate containing penicillin was unilaterally applied to the dorsal surface of spinal segments L4-L6. The plates applied to groups 2 and 3 rats contained penicillin with 200 μg quipazine or with 200 μg tropine, respectively.

In electrophysiological experiments the effects of tropine and quipazine on nociceptive flexion reflex (NFR) were evaluated. The animals were narcotized with sodium ethaminal (40 mg/kg intraperitoneally). Bioelectrical activity of the biceps femoris induced by nociceptive stimulation of *n. suralis* receptors was recorded via bipolar needle electrodes using a VC-9 wide-band amplifying oscillograph (Nihon Kohden) and processed using a Micolink computer-assisted data analysis system (Biodata Limited). NFR was recorded before and after intrathecal administration of tropine and quipazine (200 μg in 10 μl) in the zone of spinal segments L4-L6. The substances were introduced via a hole in the lumbar vertebrae. We compared NFR thresholds, latencies and durations of the early and late components in NFR, and frequency and type of NFR spikes before and after intrathecal administration of tropine and quipazine. Test substances were dissolved *ex tempore*. Control experiments were performed with physiological saline.

The results were analyzed by nonparametric Wilcoxon test and Student's *t* test.

RESULTS

Electrical stimulation of tissues in the zone innervated by *n. suralis* induced NFR consisting of 2 components: the early and late responses characterized by latencies of 12.50±1.78 and 214.28±22.27 msec, respectively (Fig. 1, *3*; Fig. 2, *1*). The early response was stable and included 2-5 spikes with the total duration of 61.57±6.57 msec. The duration of the late response varied from 380 to 620 msec depending on the intensity of stimulation. The threshold of the early response was much lower than the threshold of the late response (1.56±0.37 and 5.05±0.27 mA, respectively). Intrathecal administration of physiological saline (n=5) had no effect on NFR. Intraspinal administration of tropine in a dose of 10 μg (n=6) did not modulate NFR. Increasing the dose of tropine to 200 μg (n=6) induced spontaneous electrical activity of the muscle for 2 min followed by a significant decrease of the threshold of

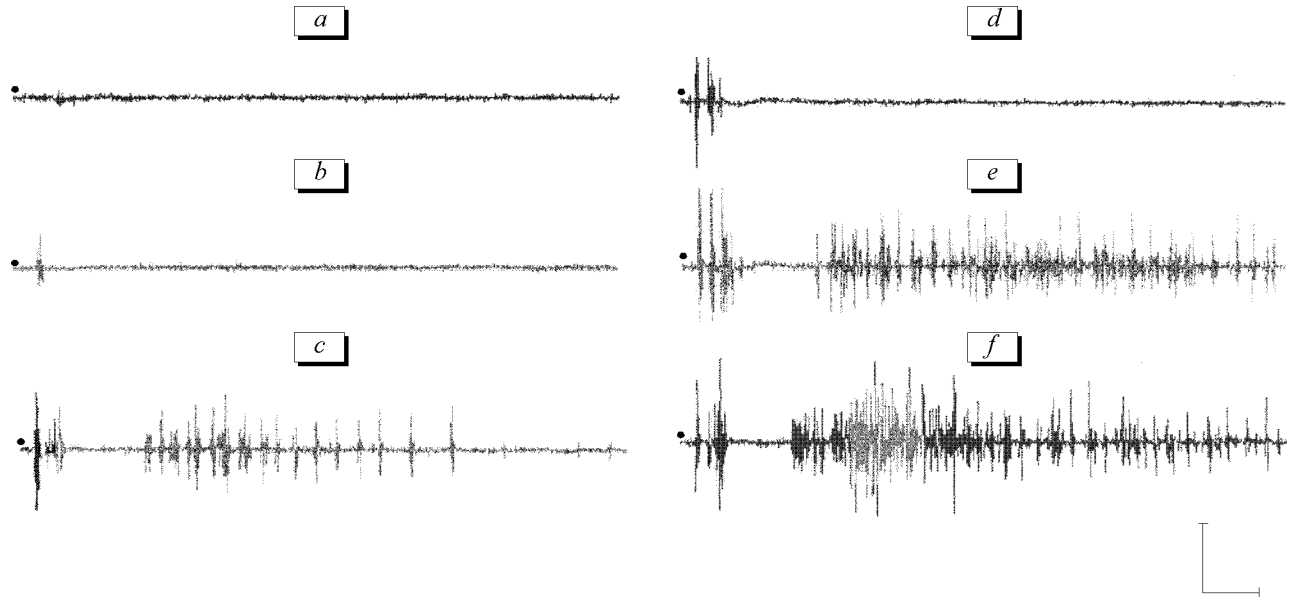

Fig. 1. Effect of the 5-HT_3 receptor antagonist tropine on nociceptive flexion reflex (NFR). Recording of NFR in rat biceps femoris during electrical stimulation of *n. suralis* receptors before (*a*, 1 mA; *b*, 2 mA; *c*, 15 mA) and after (*d*, 1 mA; *e*, 2 mA; *f*, 15 mA) intrathecal lumbar administration of 200 μg tropine. Here and in Fig. 2: moments of stimulation are shown by points. Calibration: 200 μV, 100 msec.

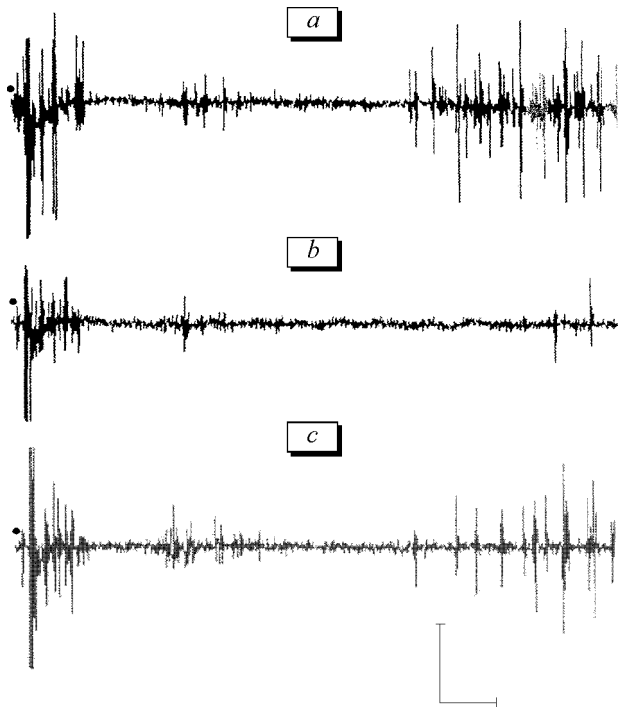

Fig. 2. Effect of the 5-HT_3 receptor agonist quipazine on NFR. Recording of NFR in rat biceps femoris during electrical stimulation of *n. suralis* receptors with 15-mA current before (*a*) and 10 (*b*) or 15 min after intrathecal administration of 200 μg quipazine (*c*).

the late response from 5.05±0.27 to 2.40±0.12 mA ($p<0.01$, Fig. 1, *4*). The threshold of the early response also decreased and the total duration and frequency of spikes increased. Changes in NFR persisted over 15-20 min.

Intrathecal administration of quipazine in a dose of 200 μg ($n=9$) produced opposite changes: elevated the threshold of the late response and reduced its duration to its complete disappearance (Fig. 2). The antinociceptive effect of quipazine persisted for 15-20 min.

Behavioral studies showed that the first manifestations of SPS in group 1 rats (licking and biting of topographically corresponding zones on the ipsilateral hindlimb) developed 5-10 min after application of penicillin, the drug impairing GABAergic inhibition. The severity and frequency of attacks increased with time, tactile stimulation produced allodynia. These changes were most pronounced 20-30 min after penicillin application (Fig. 3, *1*). At this term the degree of six characteristics of SPS was maximum and corresponded to 3 points.

SPS significantly decreased in group 2 rats after application of penicillin and quipazine ($p<0.01$). It should be emphasized that the severity of SPS characteristics decreased to a different degree (Fig. 3, *2*). The frequency of attacks and local and general locomotor activity decreased most significantly (70.1±6.2, 73.3±4.4, and 73.30±4.44%, respectively). The degree of vocalization and allodynia decreased to a lesser extent (78.0±5.4 and 86.6±5.4%, respectively).

Unilateral application of tropine to the dorsal surface of the spinal cord in group 3 rats (60%) induced mechanical allodynia on the ipsilateral side (back of fingers and shank).

Activation of 5-HT_3 receptors with quipazine suppressed the late component of NFR and abolished pain

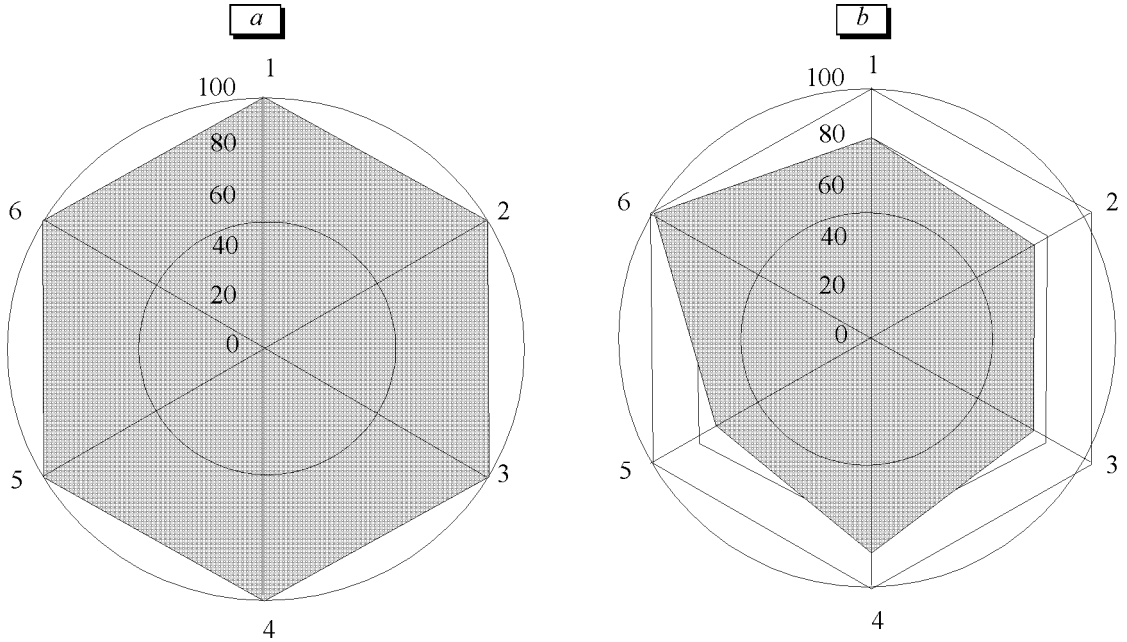

Fig. 3. Effect of the 5-HT_3 receptor agonist quipazine on spinal pain syndrome produced by application of penicillin (*a*) and penicillin+quipazine (200 μg, *b*) to lumbar spinal segments in rats. Characteristics of pain syndrome (%): vocalization (*1*), general locomotor activity in attack (*2*), local reaction (licking and biting of tissues in the painful zone, *3*), allodynia (pain response to tactile stimulation, *4*), frequency of pain attacks (min, *5*), duration of attacks (sec, *6*).

syndrome produced by application of penicillin. Intrathecal administration of 5-HT_3 receptor antagonist tropine potentiated the late component of NFR and induced allodynia.

The development of the early component of NFR is related to activation of A-δ-fibers and excitation of specific nociceptive neurons in the dorsal horns of the spinal cord. The late component of NFR reflects activation of C-afferent fibers and excitation of wide dynamic range nociceptive neurons in dorsal horns of the spinal cord [7]. In our experiments intrathecal administration of 5-HT_3 receptor agonists and antagonists produced opposite changes only in the late component of NFR. The data suggest that 5-HT_3 receptors primarily modulate excitability of wide dynamic range neurons. Intraspinal administration of tropine led to the development of mechanical allodynia. These findings confirm our hypothesis that the pathophysiological basis of allodynia is hyperactivity of wide dynamic range neurons in dorsal horns of the spinal cord related to a deficiency of GABAergic inhibition [1,2, 9]. Quipazine abolished central pain syndrome produced by impairment of GABAergic inhibition after application of penicillin to the dorsal surface of the spinal cord. Therefore, spinal 5-HT_3 receptors modulate excitability of GABAergic interneurons. Activation of spinal 5-HT_3 receptors initiates secretion of GABA and inhibits wide dynamic range neurons. 5-HT_3 receptor blockade attenuates GABAergic inhibition and promotes the appearance of central pains.

This work was supported by the Regional Social Foundation for Russian Medicine.

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