Role of Cannabinoid Receptor Agonists in Mechanisms of Suppression of Central Pain Syndrome

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We studied the effect of cannabinoid receptor agonists anandamide and WIN 55,212-2 on the central pain syndrome induced by intraspinal injection of penicillin sodium salt in rats. Cannabinoids suppressed allodynia and spontaneous attacks in rats with the central pain syndrome. The analgesic effect was most pronounced after intrathecal injection of cannabinoid receptor agonist in a dose of 100 μg in 10 μl. After systemic treatment the analgesic effect was produced by only WIN 55,212-2 in a dose of 1 mg/kg. WIN 55,212-2 was superior to anandamide by the duration and intensity of the effect on allodynia and spontaneous attacks.

Key Words: *central pain syndrome; analgesia; cannabinoidergic system; anandamide; WIN 55,212-2*

Neurogenic pain syndromes develop as a result of primary dysfunction or damage to structures of the peripheral or central nervous system. Disturbances in pulse generation in nociceptors and impaired control of excitability of central nociceptive neurons underlie the development of neurogenic pain syndromes [2]. Abnormal electrogenesis leading to the appearance of ectopic impulses is caused by increased number of tetrodoxin-resistant Na channels on the membrane of damaged nerve fibers and appearance of new receptor formations atypical for nerve fibers [2,4]. Impairment of local and descending inhibition in the spinal dorsal horns promotes the formation of aggregations of hyperactive neurons with increased excitability and reactivity [1]. These features of the pathogenesis of neurogenic pain syndrome make them resistant to classical analgesics and necessitate the use of anticonvulsants, antidepressants, and NMDA receptor antagonists [15]. Recently, a principally new possibility in the treatment of neurogenic pain syndromes through cannabinoidergic system appeared.

Cannabinoid receptors expressed primarily on peripheral and central neurons belong to type 1 (CB_1), while type 2 receptors (CB_2) are located mainly on membranes of splenic macrophages, monocytes, B- and T-lymphocytes, and microglial cells [3]. Numerous CB_1 receptors were detected in the key structures responsible for the regulation of pain sensitivity: on central terminals of primary afferents of the dorsal horn, in superficial lamina of the spinal dorsal horns, rostroventromedial compartment of the medulla oblongata, periaqueductal gray matter of the brain stem, and thalamic nuclei [3,7,8]. Inhibition of activity of spinal nociceptive neurons in response to pain stimuli was demonstrated for systemic and intrathecal injection of cannabinoids [11]. The analgesic effect of cannabinoids was demonstrated in animals with carrageenan- and formalin-

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induced inflammation [12,13], visceral pain [9,12], neuropathological pain syndrome [5,6,10]. On the other hand, the role of endogenous cannabinoidergic system in the mechanisms of central pain suppression remains unstudied. We studied the effects of cannabinoid receptor agonists anandamide (endogenous agonist) and WIN 55,212-2 (exogenous agonist) on behavioral manifestations of the central pain syndrome (CPS) in animals.

MATERIALS AND METHODS

The study was carried out on 35 male Wistar rats (220-250 g) in accordance with ethical requirements of International Association of Pain Research in Behavioral and Neurophysiological Experiments on Animals. The animals were kept under standard vivarium conditions with natural day/night regimen and free access to water and food.

The CPS was modeled as follows. An agar plate (10×3×1.5 mm) containing penicillin in initial solution (50,000 U/ml) was applied homolaterally to the dorsal surface of the spinal lumbar compartment (L4-L6) of ether-narcotized animals after laminectomy. This plate disturbed GABAergic inhibition and led to the development of aggregation of hyperactive nociceptiove neurons in the spinal dorsal horns [1]. The main characteristic manifestations of CPS in animals were allodynia (pain after weak mechanical stimulation) and spontaneous pain attacks (SPA).

The severity of allodynia was evaluated in points by the type of vocalization and motor reaction in response to tactile stimulation of the skin with a hair brush at 40 dermatome points topographically corresponding to spinal segments L4-L6: 0: no reaction to weak tactile stimulation of the skin ; 1 point: weak peep, motor reaction (hind paw shirking); 2 points: repeated vocalization and moderate motor reaction (avoidance); 3 points: intensive incessant vocalization, stormy avoidance reaction followed by chaotic running over the cage or biting the brush. The zone of allodynia propagation was determined by the number of points where tactile stimulation caused pain reaction.

Spontaneous pain attacks manifesting in spontaneous vocalization and motor activity were also evaluated in points: 0: no spontaneous vocalization and motor reaction; 1 point: spontaneous (not caused by stimulation) weak single vocalization and local spontaneous motor reaction presenting by scratching of the hind paw skin ipsilaterally to the site of penicillin application; 2 points: spontaneous moderate repeated vocalization, local scratching and biting of the corresponding zone, chaotic jumping, running; 3 points: intensive spontaneous incessant vocalization, intensive biting of the respective zone, rapid spontaneous running and rotation.

The total duration of SPA was estimated by summing up the duration of all attacks over 1 min. Experimental animals were divided into 6 groups. Group 1 animals (controls; n=6) before application of the agar plate with penicillin to the dorsal surface of the spinal lumbar compartment (L4-L6) received intrathecal injection of 0.9% NaCl (10 μl); group 2 (n=6) received intraperitoneal injection of anandamide (20 mg/kg); group 3 (n=6) were intraperitoneally injected with WIN 55,212-2 (1 mg/kg); group 4 (n=6) were intrathecally (L4-L6) injected with 100 μg anandamide (10 μl) before application of the agar plate; group 5 (n=6) received 100 μg WIN 55.212-2 (10 μl) intrathecally (L4-L6) before application of the agar plate. Anandamide and WIN 55,212-2 solutions of the needed concentration were prepared on the day of the experiment by diluting concentrated solutions with 0.9% NaCl. Since solutions of anandamide and WIN 55,212-2 contained DMSO, group 6 animals (control group 2; n=5) were intrathecally injected with DMSO (10 μl) before application of the agar plate with penicillin.

Accumulative index of allodynia was calculated by the formula A=B×N, where B was the score and N number of points. Accumulative index of SPA was calculated by the formula A=B×T, where B was the score and T total duration of attacks over 1 min. The significance of intergroup differences was evaluated by F test using one-way dispersion analysis (ANOVA).

RESULTS

The initial manifestations of CPS presenting as allodynia and SPA emerged in group 1 rats 5-10 min after penicillin application to the dorsal surface of the spinal lumbar compartments (L4-L6). After tactile stimulation of the skin in the innervation area of the sciatic nerve the rat peeped. Spontaneous pain attacks manifested during the same period; the rats suddenly peeped and performed abrupt motor reactions (running, rotation, biting the skin on hind paws). The intensity of allodynia and SPA increased with time and peaked 15-30 min after application of penicillin. The zone of allodynia was extended, the duration and frequency of SPA increased. After 60 min CPS decreased. In group 6 animals CPS was not suppressed, augmentation of allodynia and SPA was observed after 60 min.

Systemic treatment of animals with CPS with anandamide in a dose of 20 mg/kg intraperitoneally

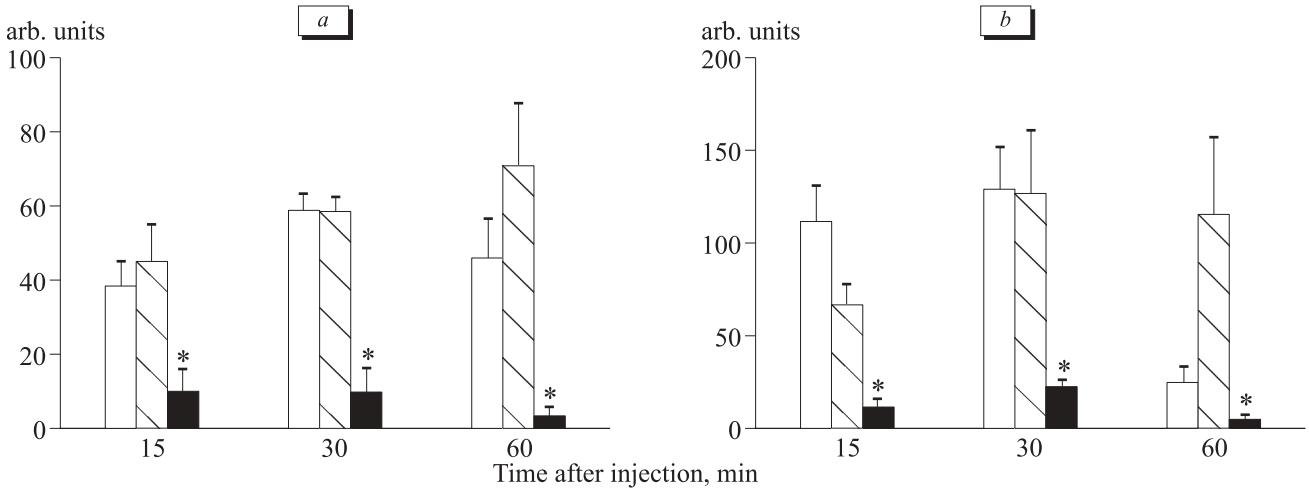

Fig. 1. Effect of systemic treatment by cannabinoids on allodynia (*a*) and spontaneous pain attacks (*b*) in rats with central pain syndrome. Here and in Fig. 2: light bars: 0.9% NaCl (control); cross-hatched bars: anandamide; dark bars: WIN 55,212-2. *$p<0.06$ compared to the control.

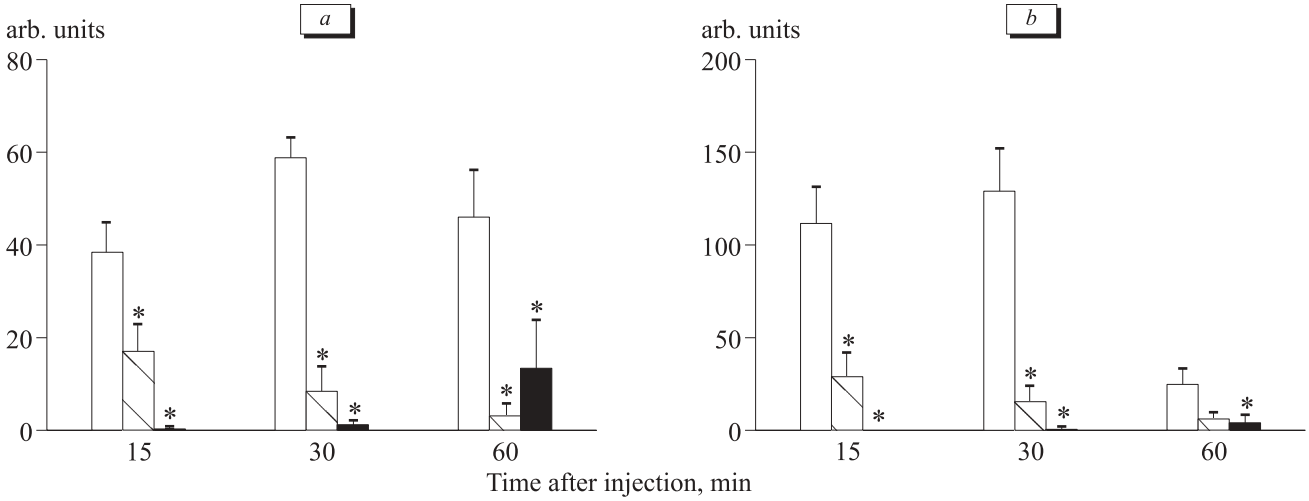

Fig. 2. Effect of intrathecal cannabinoid treatment on allodynia (*a*) and spontaneous pain attacks (*b*) in rats with central pain syndrome.

(group 2) virtually did not suppress allodynia or SPA (Fig. 1); only a trend to SPA alleviation was observed 15 min after injection of anandamide (Fig. 1). Systemic treatment with WIN 55,212-2 in a dose of 1 mg/kg significantly suppressed allodynia and SPA (the main symptoms of CPS); CPS was completely arrested in 50% animals.

The analgesic effect of cannabinoids clearly manifested after intrathecal injections of anandamide and WIN 55,212-2 (Fig. 2). Anandamide in a dose of 100 μg significantly decreased the intensity and area of allodynia 30 and 60 min after injection and decreased the intensity, frequency, and duration of SPA 15 and 30 min after injection (Fig. 2). The analgesic effect of intrathecal injection of WIN 55,212-2 in a dose of 100 μg was more pronounced; it manifested after 15 min and persisted throughout the observation; allodynia and SPA were completely arrested in 80% cases.

These results indicate that intrathecal injection of anandamide and WIN 55,212-2 to animals with CPS arrested allodynia and SPA. The decrease in the analgesic effect of anandamide after systemic treatment seemed to be due to its rapid inactivation by fatty acid amide hydrolase [3].

Simultaneous suppression of stimulus-dependent (allodynia) and stimulus-independent (SPA) pain with anandamide and WIN 55,212-2 is in line with the data indicating that these agents can interact with pre- and postsynaptic CB_1 receptors simultaneously modifying neurotransmitter secretion from central terminals of primary afferents and activities of the dorsal horn postsynaptic neurons [6, 11,14]. Electrophysiological studies showed that

local injection of cannabinoid receptor agonists into the dorsal horns of the spinal lumbar segments reduced activities of nociceptive neurons in response to thermal and electric stimuli [6,11].

Potentiation of the inhibitory effect of cannabinoid receptor agonists under conditions of tonic pain during hyperactivation of nociceptive neurons with repeated stimuli (excitation phenomenon) [6] suggests that cannabinoids can be used for the treatment of chronic pain syndromes.

Hence, our findings evidence that endogenous cannabinoid system of the brain actively participates in the mechanisms of CPS arrest.

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