GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

A New Animal Model of Allodynia

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A new rat model of allodynia is developed in which penicillin, an inhibitor of GABAergic inhibition, is applied to the dorsal surface of lumbosacral segments of the spinal cord. Correlation is found between abnormal pain reactions in rats with allodynia and alterations of evoked potentials in their dorsal horns. Stimulation of the sciatic nerve decreases threshold and increases amplitude and duration of evoked potentials.

Key Words: *allodynia; dorsal horns of spinal cord; evoked potentials; penicillin; GABAergic inhibition*

Allodynia is a term used for pain caused by a non-noxious stimulus applied to the body surface and felt at the site of application [10]. Allodynia arises in various neurogenic pain syndromes. Models of allodynia are necessary for studies of pathological pain, since allodynia is one of the characteristics of such a pain. Several models have been described [3,5, 11,13,14]. The present study is based on the theory of generator and systemic mechanisms of pain syndromes [1,7] that maintain that these syndromes are based on generators of pathologically enhanced excitation in the pain sensitivity system. Our previous studies showed that such a generator created in the dorsal horns (DH) of the spinal cord with the use of a convulsant gives rise to pain syndrome of spinal origin [2,8]. It was suggested that relatively small doses of convulsant induce allodynia instead of pain syndrome.

MATERIALS AND METHODS

Twenty-four Wistar rats weighing 320-350 g were used. Unilateral laminectomy was performed under ether anesthesia to expose the dorsal surface of the spinal cord in the L_{IV}-L_{VI} or L_{VI}-S_{II} region and dissect the meninges. Penicillin (sodium salt), which is known to impair GABAergic inhibition [6], was used as a convulsant. Its effect was prolonged by the agar plate method [2]. The antibiotic was dissolved in 1% warm liquid agar, and 6×2×1.5 or 4×2×1.5 mm plate containing penicillin (12,500 U/ml agar) was cut after agar solidification. The wound was sutured, and rat was placed in a chamber for observations. Pain in response to a tactile stimulus (touching with the blunt end of a pencil or with a hair brush) was scored in points as follows: 0, no response; 1, squeaking and the tail withdrawal reaction; 2, squealing, biting of the brush or the stimulation site, and the avoidance response. It was more convenient to evaluate allodynia by holding the rat suspended in the researcher's hands.

Bioelectrical activity in the DH was recorded under urethane anesthesia (1.4 g/kg intraperitoneally) in artificially ventilated rats immobilized with the muscle relaxant Myo-Relaxin (50 mg/kg intramuscularly) and secured in a stereotaxis apparatus with the lumbar region being fixed at several sites. The skin, muscles, and spinous processes at the sites of

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fixation were treated with 0.5% Novocain. Before and after application of penicillin-containing agar plate, evoked potentials (EP) in the DH of L_V segment in response to electrostimulation of the sciatic nerve were recorded at a depth of 800 μ from the dorsal surface (which corresponds to lamina V [12]) using glass microelectrodes (tip diameter 8-10 μ, filled with 2.5 M NaCl) without changing their position. The potentials were amplified with an MZ-4 microelectrode amplifier and a VC-9 wideband amplifier (Nihon Kohden) and then averaged by 10-15 presentations in a computer. The sciatic nerve electrostimulation with 0.1 msec rectangular pulses was carried out using bipolar silver electrodes.

RESULTS

The initial manifestations of allodynia (1 point) appeared 5-10 min after penicillin application to the dorsal surface of the lumbosacral (L_{IV}-L_{VI}) region: the rat squeaked when tactile stimulus was applied to the skin in the area of the saltatory joint. The severity of allodynia increased with time and reached the maximum (2 points) 15-30 min after penicillin application: the rat responded to tactile stimulation by squealing and biting the brush or the stimulation site. Between provoked attacks of pain rats held the affected paw suspended in a flexed position (paw-sparing posture). This phase lasted 90 min.

It is noteworthy that the area of allodynia expanded with time (Fig. 1). Initially, allodynia was confined to the saltatory joint (at the site of its attachment to the Achilles tendon), then it expanded to the paw, digits, and thigh. After 60-90 min, allodynia involved virtually the entire leg. Then it began to decrease with reduction in the pain zones in a reverse order. There were no signs of allodynia 3-4 h after application of agar plate.

When the smaller (4×2×1.5 mm) penicillin-containing agar plate was applied to segments L_{VI}-S_{II}, allodynia was localized at the tail root, and the application of tactile stimuli of different sides of the tail induced absolutely different responses: stimulation from the side of penicillin application resulted in a squeal with immediate withdrawal of the tail, while contralateral stimulation generated no response.

Study of EP in the DH of segment L_V in response to sciatic nerve electrostimulation revealed pronounced changes in these potentials. Electrostimulation with 0.08 mA current (the mean current being 0.09±0.02 mA) before penicillin application had no effect (Fig. 2, *a*, *1*), while 20 min after it the same current induced a primary negative EP with an amplitude of 0.6 mV (0.50±0.07 mV) and a secondary depolarization wave of about 70 msec long (Fig. 2, *a*, *2*). This complex EP was more pronounced 50 min after penicillin application: the primary response had an amplitude of 1.1 mV (1.2± 0.1 mV) and the secondary negative wave had a high amplitude and, merging with the primary response, lasted more than 75 msec (Fig. 2, *a*, *3*).

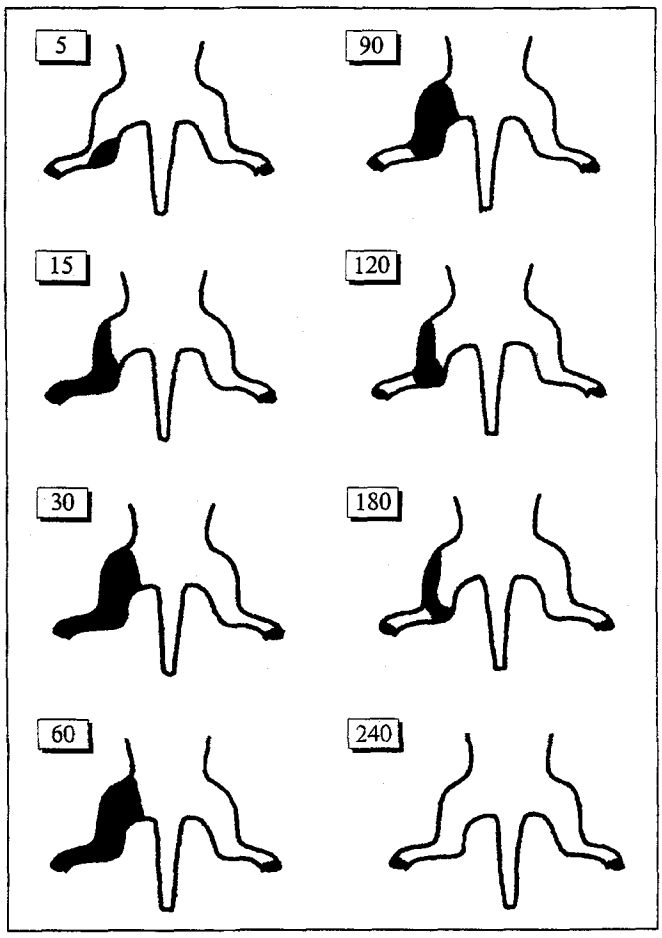

Fig. 1. Zones of abnormal pain sensitivity (black areas) after application of penicillin-containing agar plate to the dorsal surface of ipsilateral L_{IV}-L_{VI} spinal cord segments in rats. Figures are the times (min) after the plate application.

Stimulation of the sciatic nerve with 0.12 mA threshold current (0.12±0.02 mA) before penicillin application induced a primary EP with an amplitude of 0.5 mV (0.50±0.08 mV) followed by 25-30 msec hyperpolarization wave (Fig. 2, *b*, *1*), while stimulation 20 min after penicillin application generated a primary response with an amplitude of 1.1 mV (0.9± 0.1 mV) with the secondary depolarization wave lasting more than 70 msec (Fig. 2, *b*, *2*). Fifty minutes after penicillin application, the same current caused a high-amplitude primary response of 1.4 mV (1.3±0.1 mV), followed by a high-amplitude and long-lasting (>90 msec) depolarization wave merging with the primary response (Fig. 2, *b*, *3*).

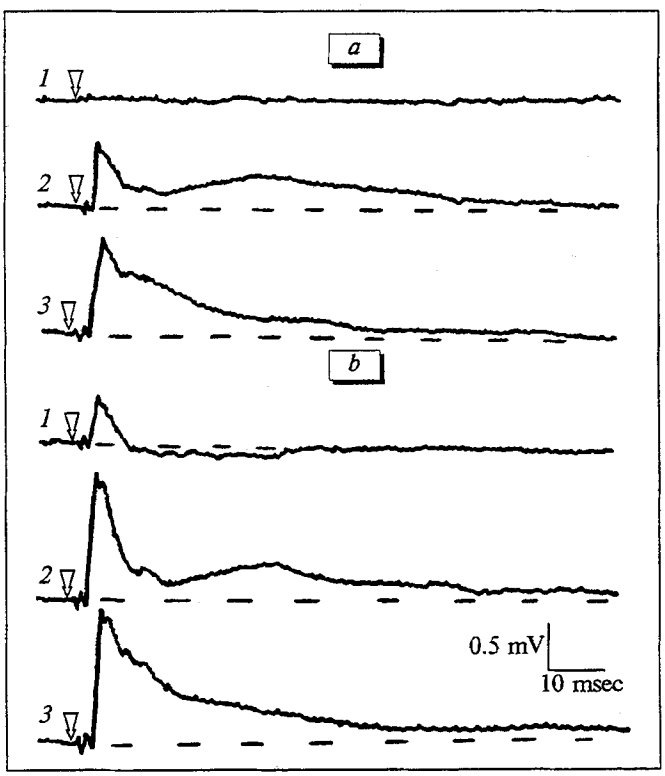

Fig. 2. Altered ipsilateral evoked potential in L_V segment of dorsal horns after application of the penicillin-containing agar plate to the dorsal spinal cord surface upon sciatic nerve electrostimulation with a current of 0.08 mA (*a*) and 0.12 mA (*b*). *1*) before agar plate application; *2*) 20 min postapplication; *3*) 50 min postapplication. The arrows indicate the times when stimulation was started.

These findings indicate that specific features of EP in the DH of rat spine evoked by electrostimulation of the sciatic nerve coincide with those of behavioral responses to tactile stimulation. Subthreshold electrostimulation of the sciatic nerve and tactile stimulation of the hind limb prior to penicillin application elicited no response, while after it electrostimulation EP in the DH and tactile stimuli generated a response in the form of allodynia.

It can be hypothesized that pulse propagation along A_β-fibers is the mechanism triggering pain upon tactile stimulation and EP upon subthreshold electrostimulation of the sciatic nerve [9]. Prior to penicillin application, these pulses evoke no response from nociceptive neurons (specifically, from those with a wide dynamic range) because the inhibitory control is preserved. When this control is disrupted by penicillin (which is confirmed by the disappearance of hyperpolarization wave from the evoked response), the disinhibited and sensitized neurons respond to the same stimulation as before by generating EP. Since the inhibitory control is impaired, these neurons acquire a capacity to produce relatively synchronized responses to electrostimulation of A_β fibers of the sciatic nerve, as evidenced by primary EP. Stimulation with stronger current (0.12 mA) induces higher EP, indicating that more neurons are involved in the response.

The increased amplitude of the primary EP component, the disappearance of the hyperpolarization wave, and the emergence of a secondary prolonged high-amplitude depolarization wave suggest that a large population of uninhibited neurons with self-sustained excitation is involved in electrogenesis. Neurons of a wide dynamic range are capable of producing excitation during a long time period [4,9]. If these neurons form an aggregate, the latter may develop a long-lasting self-sustaining activity. This aggregate is a generator of pathologically enhanced excitation which in response to triggering stimulation produces a pulse flow capable of involving other, including higher, components of the pain sensitivity system, thus causing the emergence of a short-lived (while the attack of pain lasts) pathological algesic system responsible for the pain reaction. However, the generator thus established is not powerful enough to be activated spontaneously, which explains why spontaneous pain attacks characteristic of complete pain syndrome are absent in this form of allodynia.

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