Peculiarities of the Development of Analgesic Effect during Transcutaneous Dynamic Electrical Stimulation

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The effect of transcutaneous dynamic electrical neurostimulation on the development of analgesia was studied in behavioral and electrophysiological experiments on rats. A 30-min dynamic electrical stimulation elevated the nociception threshold in tail-flick and hot plate tests, increased the threshold of the late nociceptive flexor reflex, and decreased the number of bursts in the response. Intraperitoneal injection of naloxone (2 mg/kg) abolished the analgesic effect of dynamic electrical neurostimulation. It is concluded that the key role in reflex analgesia during dynamic electrical neurostimulation is played by the endogenous cerebral opioid system, which inhibits the nociceptive signals traveling to CNS via unmyelinated C-fiber afferents.

Key Words: *transcutaneous dynamic electrical neurostimulation; analgesia; nociceptive flexor reflex*

Increasing prevalence of pain syndromes [4,5,7] necessitates the search for new efficient and safe analgesic preparations. At present, complex therapy of pain syndromes widely uses the methods of reflex therapy, such as transcutaneous electrical neurostimulation and electroacupuncture, which have sufficient analgesic potency and produce no side effects [1,8,9,11,14]. However, an important disadvantage of transcutaneous electrical neurostimulation is the development of tolerance of somatosensory receptors to stimulation during this procedure [1,12]. It is suggested that dynamic electrical neurostimulation (DENS), a new method of transcutaneous electrical stimulation, can solve this problem. This method is based on stimulation of cutaneous reflex zones with short-term current pulses, which persistently modify their shape in response to changes in electrical resistance of the skin under the electrodes, thereby decreasing the adaptation of neural elements to electrical stimulation [3]. Our aim was to study the effect of DENS on pain sensitivity and nociceptive flexor reflex (NFR) in experimental animals.

MATERIALS AND METHODS

Experiments were performed on 32 albino male Wistar rats (220-250 g) in compliance to ethic requirements of the International Association of Study of Pain (IASP) for study of experimental pain and neurophysiologic investigations in animals. The animals were maintained under standard vivarium conditions at natural illumination and food and water *ad libitum*. Pain reactions were assessed in the tail-flick and hot plate tests. For evaluation of the tail-flick reaction, a special device (Ugo Basile) was used, which focused light on the tail and recorded the moment of nociceptive response. For evaluation of the paw-licking response to heat, the rats were placed on a hot plate (55°C) of an Ugo Basile algesimeter and the latency of the response was determined. The latencies of noci-

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ceptive reactions were measured before and 5 and 30 min after DENS.

The rats were subjected to DENS for 30 min with the help of a DiaDENS setup (a modified version of serial DENAC device). During the procedure the animals freely moved in a special chamber (25×40×30 cm) with electroconducting floor.

Electrical pulses generated by DiaDENS had a bipolar asymmetrical shape without constant component. The amplitude of the first trapezoidal phase of the pulse was fixed at 30 V, while the intensity of stimulation was controlled by the duration of this phase from 0 to 500 μsec. The second phase of the stimulus was a transient process consisting of damping sine oscillations. The amplitude of the second phase was one order of magnitude higher than that of the first phase, and it was the most load-variable portion of the stimulation pulse. The intensity of stimulation was chosen individually for each animal and reduced after appearance of the first signs of anxiety (squeak and/or grooming). Groups 1 and 2 rats were stimulated at 10 and 77 Hz, correspondingly.

NFR in narcotized animals (40 mg/kg sodium etaminal, intraperitoneally) was recorded with needle electrodes inserted into *musculus biceps femoris*. NFR was evoked by electrical stimulation of *n. suralis* receptive field with electrical pulses (1 msec, 15 mA). The bioelectrical signals were fed into a wide-band amplifier of a VC-9 oscilloscope (Nihon Kohden), digitized at the sampling period of 0.8 msec, and fed into PC. Analysis of electrical activity was carried out using a Microlink software (Biodata Limited). NFR was examined before and after DENS. Transcutaneous stimulation was performed with a DiaDENS device in the skin projection area of *n. suralis*. Electrical stimuli were applied for 30 min at a rate of 77 Hz. Stimulating current was reduced when electromyographic activity appeared in *musculus biceps femoris*. In some experiments, naloxone was injected intraperitoneally (2 mg/kg) 10 min after the end of DENS. The data were processed statistically using nonparametric Wilcoxon and Student *t* tests.

RESULTS

Behavioral experiments showed that 30-min DENS delivered at rates of 10 and 77 Hz increased the nociceptive sensitivity thresholds in both tests (Table 1). The duration of analgesic effect was about 30 min. The increase in pain sensitivity threshold was similar in both groups. However, in contrast to group 1 rats subjected to 10-Hz DENS, in group 2 rats the changes in nociceptive threshold assessed by the tail-flick test remained significant up to minute 30 after 77-Hz DENS.

NFR recording showed that nociceptive electrical stimulation of *n. suralis* skin projection induced electromyographic activity in *musculus biceps femoris*, which consisted of two components: the early and late responses with latencies of 26.0±2.4 msec and 284.28±36.74 msec, respectively. The early response was stable and consisted of 2-5 spikes, which appeared at stimulation intensity of 1.56±0.34 mA (Fig. 1). The increase in stimulation intensity did not change the number of spikes in the early response. The late re-

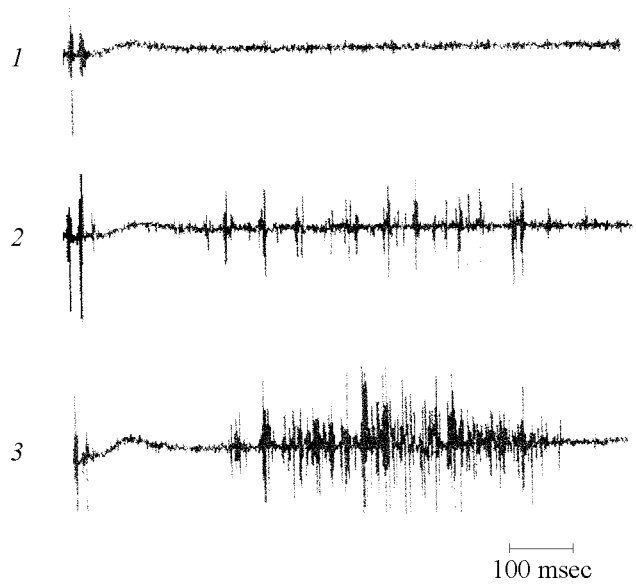

Fig. 1. Nociceptive flexor reflex in rat *musculus biceps femoris* evoked by stimulation of *n. suralis* skin receptive field with electrical current of increasing amplitude. Electrical stimulation: *1*) 1.5 mA; *2*) 5 mA; *3*) 10 mA.

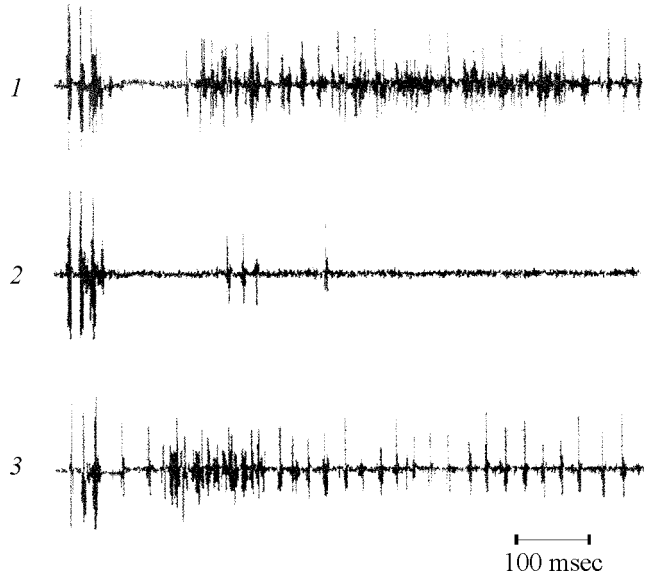

Fig. 2. Effect of dynamic electrical neurostimulation (DENS, *2*) and naloxone (*3*) on nociceptive flexor reflex (NFR) evoked by stimulation of *n. suralis* skin receptive field with 10-mA electrical current pulses. *1*) NFR before DENS; *2*) NFR 2 min after DENS; *3*) NFR 2 min after naloxone injection.

TABLE 1. Effect of 10- and 77-Hz DENS on Nociceptive Thresholds in Rats (*M*±*m*)

Stimulation		Control	Time after DENS, min	
			5	30
10 Hz				
	tail-flick test	3.52±0.95	4.65±1.01**	3.67±0.64
	hot plate test	18.00±3.94	23.70±4.57*	20.80±3.96
77 Hz				
	tail-flick test	3.06±0.64	4.87±0.71*	5.00±0.65*
	hot plate test	15.90±3.26	23.7±3.4*	16.00±3.57

Note. *p<0.01, **p<0.05 compared to the control.

sponse was multiphase. Its threshold (5.05±0.47 mA) far surpassed the threshold of the early response, while the number of spikes increased with increasing the intensity of stimulation (Fig. 1). Electrical stimulation produced a significant increase in the threshold of the late response (12.6±2.4 mA, p<0.01) and decreased the number of discharges in it (Fig. 2).

In different rats, the duration of suppression of the late NFR phase by DENS was 40-90 min. Naloxone abolished the analgesic effect of DENS. Moreover, the threshold of the late NFR response returned to the control values, and the number of discharges in this response increased (Fig. 2).

These data suggest that the analgesic effect of DENS is predominantly related to inhibition of signals entering CNS via fine unmyelinated C-afferents. This conclusion is corroborated by selective inhibition of the late phase of NFR by electrical stimulation. According to calculations [10], the early component of NFR with the latency of 5-56 msec results from activation of fine myelinated Aδ afferents, while activation of unmyelinated C-fiber afferents is responsible for the appearance of the late NFR component with a latency of 85-425 msec. Taking into consideration that activation of Aδ afferents produces primary, fast, acute, and epicritic pain, while activation of C-fibers leads to perception of secondary, delayed, diffuse, and protopathic pain [15], it can be assumed that the analgesic effect of DENS is predominantly related to inhibition of distressing protopathic component of pain.

The observed effect of naloxone-dependent inhibition of the late NFR phase suggests that activation of inhibitory mechanisms by DENS similarly to activation induced by transcutaneous electrical stimulation or electroacupuncture is mediated by cerebral opioidergic antinociceptive system [1,2,6,10,13].

Therefore, behavioral and electrophysiological studies showed that the analgesic effect of DENS is caused by reflex activation of endogenous opioid cerebral system, which leads to inhibition of nociceptive signals delivered to CNS via fine unmyelinated C-fiber afferents.

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