14. T. Nagatsu et al., in: Catecholamines and Stress (International Symposium), Oxford (1976), p. 47.
15. J. M. Saavedra, H. Grobecker, and J. Axelrod, Circ. Res., 42, 529 (1978).

ANALYSIS OF ANALGESIA EVOKED BY CREATION OF AN EXCITATION GENERATOR IN DORSAL RAPHE NUCLEUS

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An excitation generator was created in the dorsal raphe nucleus of the mesencephalon in experiments on albino rats by microinjection of tetanus toxin into the nucleus. During formation of the excitation generator electrical activity was found to change in this nucleus, with a sharp increase in the primary negative component (N_1) and a change in the general configuration of the evoked potentials (EP), and an increase in the frequency of spontaneous paroxysmal activity. These changes in EP coincide in time with the appearance of deep and increasing analgesia. The type of analgesia described is not abolished by naloxone.

KEY WORDS: Analgesia; excitation generator; tetanus toxin; evoked potentials; dorsal raphe nucleus.

The raphe nuclei of the midbrain and medulla play a specially important role in the central mechanisms of analgesia induced both by electrical stimulation of these nuclei and by injection of substances interacting with opiate receptors into them [1, 9-11, 14]. It was shown previously [4] that injection of tetanus toxin (TT), which disturbs inhibitory mechanisms and so evokes the formation of a generator of enhanced excitation [2-7], into the dorsal raphe nucleus of the midbrain leads to deep analgesia.

In order to continue the analysis of the mechanisms of this analgesia, it was decided to study evoked potentials in the dorsal raphe nucleus, into which TT was injected, and also to make a comparative analysis of the type of analgesia described above and that evoked by morphine.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male albino rats weighing 200-300 g. Analgesia, determined by behavioral tests, was induced by microinjection of 1-3 MLD of purified TT in a volume of 0.05 ml, at a point whose coordinates were taken from the atlas [12]. Full details of the method were described previously [4]. Evoked potentials were recorded in the dorsal raphe nucleus before injection of TT and after the appearance of definite analgesia. Evoked potentials were derived in the usual way by a monopolar method with nichrome electrodes (diameter 0.2 mm) in glass insulation, connected to the micropipet for TT injection. The electrode and micropipet were inserted under hexobarbital anesthesia (100 mg/kg body weight). Processes to be recorded were photographed from the monitor screen of a BC-9 dual-beam oscilloscope (Japan) by means of a photorecorder. Nociceptive electrical stimulation was applied to the skin of the tail and limbs through needle electrodes from an ÉSU-1 stimulator. The positive and negative components of the evoked potential were assessed quantitatively and the experimental results subjected to statistical analysis. Experiments were carried out on rats hypophysectomized under ether anesthesia by a suction method through a burr-hole in the base of the skull (these experiments were carried out jointly with É. R. Bagramyan). Morphine was injected systemically (15 or 50 mg/kg body weight) or into the dorsal raphe nucleus (5 μg in 1 μl). Naloxone also was injected either systemically (0.1 mg/kg body weight) or into the dorsal raphe nucleus (5 μg in 1 μl).

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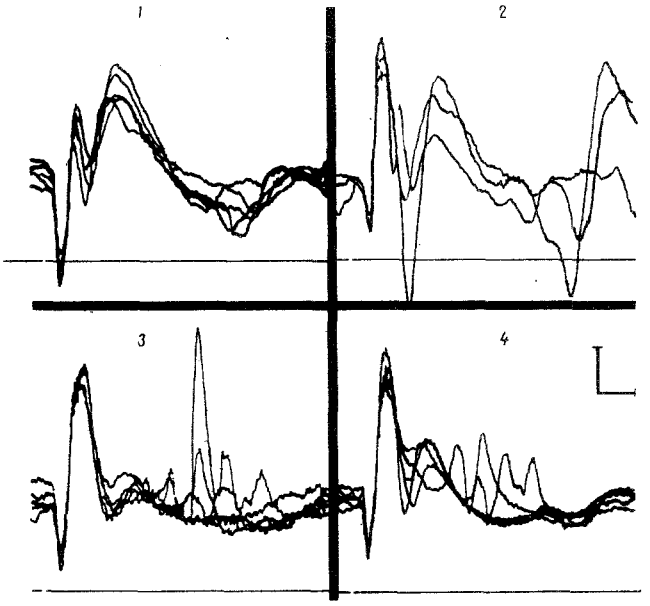

Fig. 1. Evoked potentials in dorsal raphe nucleus in response to electrical stimulation of skin of tail before (1) and 8 h after (2-4) injections of tetanus toxin into the same nucleus. 2, 3, 4) Examples of evoked responses recorded in the same nucleus. Negativity upward. Calibration: 50 μV, 50 msec.

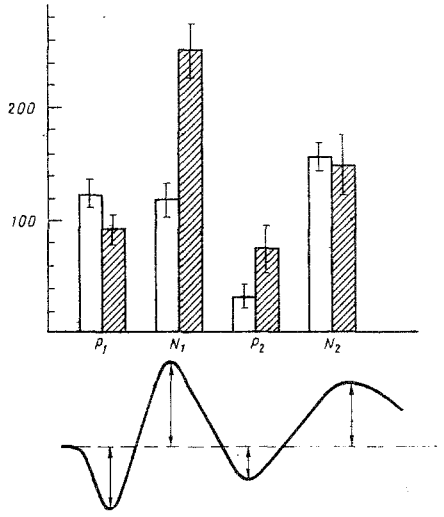

Fig. 2. Changes in amplitude of components of evoked potential in dorsal raphe nucleus 8 h after injection of tetanus toxin into it. Unshaded columns) before injection of toxin, shaded columns) after injection. Ordinate, amplitude of components of evoked potential (in μV); abscissa, components of evoked potential. Scheme of measurement of components of evoked potential given on the following page.

EXPERIMENTAL RESULTS

The evoked potentials recorded before injection of TT consisted of several components: P_1) the first positive wave with a latent period of 11.7 ± 0.2 msec, N_1) the first negative wave, appearing after 32 ± 1 msec, P_2) the second positive wave, appearing after 51 ± 2 msec, and N_2) the second negative wave, appearing after 66 ± 2 msec. By the time of appearance of analgesia, significant changes were observed in the evoked potentials (Fig. 1): The N_1-wave was larger than normal, on average by 100% ($P < 0.05$) but meanwhile, the first positive wave was reduced on average by 25% ($P < 0.05$). The configuration of the evoked potentials also was substantially changed: Whereas normally the P_2 wave was of low amplitude and sometimes was absent altogether, 6-8 h after injection of TT, when analgesia developed, this component of the evoked potential could be seen to be enlarged. Sometimes component N_2 was reduced or absent, but the changes were not significant in character, possibly because of the great dispersion of the amplitude. In some cases the evoked potentials suffered interference from potentials arising in the nucleus without electrical stimulation. This spontaneous activity was observed only after injection of TT.

Central analgesia is associated with the action of endogenous opiates: endorphines and enkephalins [8]. The pituitary gland is one source of endorphines. Acupuncture analgesia is also due to liberation of pituitary endorphine [13]. Experiments were carried out to study whether the central analgesia, due to formation of a generator in the midbrain, can develop after removal of this source of endorphines. For this purpose, a generator was created in the dorsal raphe nucleus of three rats 24 h after complete hypophysectomy and in another three rats five days after the same operation. These experiments showed that the animals did develop analgesia, and in the time of its development the character of its manifestation was completely indistinguishable from analgesia induced by the same method in control animals.

Microinjection of minimal doses of morphine and endogenous opiates into the central gray matter of the spinal cord and, in particular, into the region of the dorsal raphe nucleus is known to give rise to analgesia, which can be abolished by subsequent injection of the morphine antagonist naloxone [9, 14]. Analgesia induced by electrical stimulation of the brain can also be suppressed in some cases by naloxone, a drug which blocks opiate receptors. These results confirm the role of opiate receptors in these forms of analgesia. To study the role of opiate receptors in central analgesia induced by the generator in the dorsal raphe nucleus, the effect of naloxone was compared on analgesia following injection of morphine and that following creation of a generator in the nucleus. Analgesia appears 10-20 min after systemic injection of morphine and lasts 5-6 h or more. The rats did not respond to nociceptive stimulation of the skin of the tail and limbs. Besides analgesia produced by morphine in the doses used, in smaller doses (15 mg/kg body weight) evidence of catalepsy was observed, and in larger doses motor activity was increased. Injection of naloxone (0.1 and 1 mg/kg body weight) completely abolished the analgesia. A similar pattern was observed after microinjection of morphine (5 μg in 1 μl) into the dorsal raphe nucleus. Analgesia appeared after 5-10 min and lasted 5-6 h. Injection of the equivalent dose of naloxone completely abolished the analgesia. The rats responded to nociceptive stimulation of the skin of the limbs and tail by vocalization and a motor reaction.

A different picture was observed when naloxone was injected against the background of central analgesia evoked by creation of a generator in the raphe nucleus. Naloxone, if injected into the nucleus or intraperitoneally, did not weaken this type of analgesia: The rats did not respond to nociceptive stimulation of the skin of the limbs and tail. Spontaneous vocalization was observed in some rats for 30-40 min after injection of naloxone, but this vocalization was not connected with nociceptive stimulation. These experiments showed that analgesia induced by the generator was resistant to the agents blocking opiate receptors.

It was thus shown that when an excitation generator is created in the dorsal raphe nucleus sharp changes are observed in electrical activity in that nucleus, as shown by a marked increase in amplitude of the first negative component. This activation of the nucleus may take place as the result of disinhibition of cells in the nucleus, since TT, if injected into the nucleus, disturbs inhibitory mechanisms by blocking the secretion of inhibitory transmitters. As a result of disinhibition of the cells of the nucleus and, in particular, of serotoninergic cells working under tonic conditions and exerting an inhibitory influence on cells at the spinal and supraspinal levels, activation of the antinociceptive system takes place and long-lasting analgesia develops. This system can be triggered by impulsation in the nociceptive system.

Experiments to study the role of opiate receptors in the mechanisms of this type of analgesia showed that pituitary endorphines do not affect analgesia evoked by the creation of a generator in the midbrain, and that naloxone, which blocks opiate receptors, does not abolish this analgesia. These facts suggest either that this form of central analgesia is unconnected with opiate receptors or that naloxone does not abolish the effects of TT, which blocks the secretion of mediator by nerve endings.

LITERATURE CITED

1. S. I. Igon'kina and G. N. Kryzhanovskii, Byull. Éksp. Biol. Med., No. 7, 16 (1977).
2. G. N. Kryzhanovskii, Zh. Nevropatol. Psikhiatr., 76, 1090 (1976).
3. G. N. Kryzhanovskii, Zh. Nevropatol. Psikhiatr., 76, 1730 (1976).
4. G. N. Kryzhanovskii and S. I. Igon'kina, Byull. Éksp. Biol. Med., No. 2, 145 (1978).
5. Yu. S. Sverdlov, Neirofiziologiya, No. 1, 25 (1969).
6. V. B. Brooks, D. P. Curtis, and J. C. Eccles, J. Physiol. (London), 136, 655 (1957).
7. D. P. Curtis, D. Felix, C. J. A. Gane, et al., Brain Res., 51, 358 (1973).
8. H. W. Kosterlitz and J. Hughs, in: Advances in Pain Research and Therapy, Vol. 1, New York (1976), p. 641.
9. F. D. Olson, D. A. Twombly, and J. C. Liebeskind, Pain, 4, 2139 (1978).
10. J. L. Oliveras, F. Redjemi, G. Guilbaud, et al., Pain, 1, 139 (1975).
11. D. J. Mayer and J. C. Liebeskind, Brain Res., 68, 73 (1974).
12. L. J. Pellegrino and A. J. Cushman, A Stereotaxic Atlas of the Rat Brain, Plenum Press, New York (1967).
13. B. Pomeranz, R. Cheng, and P. Law, Exp. Neurol., 54, 172 (1977).
14. T. L. Yaksh, J. C. Yeung, and T. A. Rudy, Brain Res., 114, 83 (1976).

ANALYSIS OF HEMODYNAMIC RESPONSES TO HYPOTHALAMIC STIMULATION IN WAKING ANIMALS

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Experiments on waking cats showed that electrical stimulation of "protective" zones of the hypothalamus leads to development of hypertension and inhibits baroreceptor reflexes. In animals with divided carotid sinus and aortic nerves, threshold hypothalamic stimulation leads to the appearance of depressor responses, whereas above-threshold stimulation evoked depressor-pressor responses. It is suggested that depression of baroreceptor reflexes is one of the mechanisms of the development of hypertension in response to hypothalamic stimulation.

KEY WORDS: Hypothalamic stimulation; hypertension; baroreceptor reflexes.

Electrical stimulation of "protective" zones of the hypothalamus is known to evoke hypertension due to an increase in cardiac output and constriction of regional vessels. Acute experiments have shown [2, 4] that the developing hypertension is accompanied by inhibition of baroreceptor reflexes.

The object of this investigation was to study the character of hemodynamic responses and to analyze the mechanisms of onset of hypertension during hypothalamic stimulation in chronic experiments on waking animals.

EXPERIMENTAL METHOD

Altogether 25 experiments were carried out on 14 cats. A week before the experiment, under sterile conditions, nichrome electrodes 150-180 μ in diameter were inserted into the ventromedial nucleus of the hypothalamus, and fluorine-plastic catheters filled with heparin were introduced into the external jugular vein and aorta (through the carotid artery). The distal ends of the catheters were brought out under the skin and fixed in special cocks, secured to the skull. The following were measured: the arterial pressure (BP, by a manometer incorporating mechanotrons), the interval between two adjacent systoles (by a cardiotachometer), and respiration (with a carbon detector); the baroreceptor reflexes were tested by the method described previously [3, 6] at rest and also during electrical stimulation (0.5-1.5 msec, 80-100 stimuli/sec, 50-500 μA,

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