EFFECT OF NEUROMEDIATORS ON THE ANTINOCICEPTIVE EFFECT OF MESENCEPHALIC STIMULATION IN RATS

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The action of L-tryptophan (25 mg/kg, intraperitoneally) and microinjection of serotonin (20 μg), dopamine (10 μg), and neostigmine (5 μg) into the circum aqueductal gray matter on the antinociceptive effect of stimulation of the same points of the mesencephalon was studied in rats with implanted cannula-electrodes. L-tryptophan, serotonin, and neostigmine (after preliminary injection of methylatropine) potentiated the effect of subthreshold antinociceptive stimulation, tested with respect to changes in thresholds of appearance of individual components of the complex nociceptive response to electrical stimulation of the rat's tail. Dopamine did not possess this action. The potentiating effect of serotonin is not abolished by naloxone.

KEY WORDS: antinociceptive action; neuromediators; nociceptive response.

Recent investigations have revealed the presence of structures stimulation of which induces analgesia in various parts of the CNS. Antinociceptive effects have been well studied from the neurophysiological aspect [1], but their neurochemical mechanisms have been studied much less fully.

The object of this investigation was to study the effect of various neuromediators on the development of the antinociceptive effect, taking into consideration the dynamics of individual components of the nociceptive response. Microinjections of serotonin, dopamine, and neostigmine were given into points electrical stimulation of which is accompanied by analgesia.

METHODS

Experiments were carried out on 32 noninbred male rats with cannula electrodes implanted into the region of the circumaqueductal gray matter of the mesencephalon at coordinates taken from the atlas [6]. The method of electrical stimulation of the rat's tail was used, with our own modification of the method of assessing the nociceptive response, by means of which different components could be estimated quantitatively from the threshold of perception of the aversive stimulus to a generalized pain-induced emotional behavioral response (Table 1). Points whose stimulation was accompanied by an antinociceptive effect were identified beforehand. Microinjections of serotonin creatine-sulfate (20 μg), dopamine hydrochloride (10 μg), and neostigmine (5 μg) were given into the same zones; L-tryptophan (25 μg/kg) was injected intraperitoneally. Control tests showed that these substances, in the above doses, do not induce visible changes in behavior and do not change the response to pain. The effect of the substances was assessed during subthreshold stimulation of the antinociceptive points, not causing any changes in the nociceptive response, 1-5, 10, 20, 40, and 60 min after microinjection and 45-60 min after intraperitoneal injection. Each of the substances was injected into the same animal at the same point. The interval between injections was 2-3 days. The location of the tip of the cannula electrode was verified in frontal sections of the brain after fixation and staining. Van der Waerden's criterion of signs method was used for statistical analysis of the data [3].

EXPERIMENTAL RESULTS

The principal experimental results are summarized in Table 1.

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TABLE 1. Changes in Structure of Generalized Nociceptive Response (appearance of its individual components, in % of the total number of observations, used as the criterion) under the Influence of Drugs with Neuromediator-Type Action against the Background of Subthreshold Stimulation of Antinociceptive Zones of Periaqueductal Gray Matter

Substance	Number of observations	Component of nociceptive response								
		shivering, lifting tail	tapping with paws	turning head toward electrode	touching electrode	biting electrode	squeaking	rotation	crying	chewing electrode
Control	32	100	100	100	100	100	100	100	100	100
L-tryptophan	32	100	100	100	100	100	62	0*	0*	0*
Serotonin	24	100	100	100	100	100	100	50	33*	0*
Dopamine	32	100	100	100	100	100	75	100	75	100
Neostigmine	32	100	100	100	100	88	75	37*	0*	0*

*P < 0.05 (by Van der Waerden's criterion of signs)

L-tryptophan, in a dose not causing changes in the structure of the original nociceptive response, led to the appearance of an analgesic effect in all animals during subthreshold stimulation of the antinociceptive structures. The potentitating effect was manifested as a significant rise of the thresholds of appearance of features of emotional behavior in response to pain. Microinjection of serotonin into the same antinociceptive zones likewise caused the appearance of analgesia in six of the eight rats. Subthreshold stimulation of antinociceptive points 2-5 min after microinjection was manifested as weakening of the response to pain (suppression of the electrode chewing response). Maximal potentiation of the antinociceptive effect developed after 10-15 min: All affective features (vocal response, turning, chewing the electrodes) disappeared from the composite nociceptive response. To produce them, the intensity of stimulation of the base of the tail had to be increased by 2-3 thresholds. During the next 20-30 min the intensity of the potentiating effect of serotonin was reduced, and after 60 min subthreshold antinociceptive stimulation was not accompanied by any change in the structure of the composite nociceptive response.

The histological control verified that the antinociceptive effect developed predominantly in response to stimulation of the ventral part of the periaqueductal gray matter of the mesencephalon. The potentiating action of serotonin on the antinociceptive effects, so far as the emotional behavioral components of the pain response were concerned, was exhibited after microinjections into a region with serotonin-containing neurons and terminals [5], but not into more dorsal zones, where these were much less numerous. Our previous investigations showed [2] that the antinociceptive effect, as a manifestation of the adaptive self-defensive (antistress) response, is effected through two mechanisms: elevation of the pain threshold at the segmental level and suppression of the affective behavioral response to pain. An important role in the development of this latter mechanism is played by ascending serotoninergic projections [10]. Serotonin itself, if injected in a large dose (60 µg) into the zones of the periaqueductal gray matter, abolishes affective behavioral components without changing the threshold of the nociceptive response. esponse.

Evidence has been obtained that antinociceptive effects are due to the secretion of endogenous morphine-like peptides (encephalins, endorphines) [4, 7]. However, potentiation by serotonin of the antinociceptive effect with respect to the affective behavioral components of the nociceptive response is evidently not brought about through the intervention of these neuroactive peptides. Naloxone (5 µg), which specifically blocks opiate receptors, if injected into the same point immediately after serotonin (20 µg), did not prevent the effect of the latter from developing, even if accompanied by subthreshold or threshold stimulation of the antinociceptive zones.

Dopamine had no effect on development of the antinociceptive effect if injected into the same brain structures of the same animals. Only in two of eight rats did the vocal response disappear 12-15 min after microinjection of dopamine and in response to subthreshold of the antinociceptive point, but chewing of the electrode and turning were actually intensified, and the animal licked its tail vigorously. An essential role in the development of the analgesic effect is ascribed to dopaminergic mechanisms [9, 11]. In those investigations,

however, substances changing the neuromediator level were injected parenterally and, what is particularly important, analgesia was judged according to a change in only one feature (withdrawal of the tail in response to temperature stimulation, squeaking in response to electrical stimulation of the tail). According to our own observations, after injection of a larger dose of dopamine (40 μg) into the periaqueductal gray matter, in the absence of antinociceptive stimulation the threshold of the vocal response was raised, but motor-affective manifestations of the nociceptive response occurred more intensively. Consequently, during a detailed assessment of the structure of the nociceptive response no clear change in the effect of antinociceptive stimulation (and also in the analgesic effect of morphine) could be found in response to microinjection of dopamine into the zone of central stimulation.

Potentiation of the effect of subthreshold stimulation of the antinociceptive point after microinjection of neostigmine into this zone (after preliminary intraperitoneal injection of methylatropine) appeared only after a delay of 15 min; it was manifested as significant depression of affective behavioral components of the nociceptive response. However, the action of neostigmine was of short duration, and it was no longer detectable after 5-10 min. The effect of neostigmine was not dose-dependent. There is evidence [8] that microinjection of muscarinic cholinomimetic agents into a zone of the central gray matter of the mesencephalon is accompanied by an antinociceptive effect, but the efferent mechanisms of this effect have not been adequately studied. According to our own observations, microinjection of neostigmine (10-15 μg) into the periaqueductal gray matter after preliminary injection of methylatropine leads to suppression of the responses of chewing and biting the electrode, but squeaking and crying develop even to stimulation of lower intensity. Physostigmine (0.5 mg/kg, intraperitoneally) facilitated the response to pain during a similar evaluation of the whole structure of the emotional-stress nociceptive response. Although in the present experiments neostigmine enhanced the response to stimulation of the antinociceptive zones, because of the considerable delay in the development of this effect no definite conclusion can be drawn regarding the direct involvement of acetylcholine in the mechanism of analgesia during activation of antinociceptive points in the midbrain.

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