ROLE OF GROUP A5 CATECHOLAMINERGIC NEURONS IN ANALGESIA INDUCED BY AURICULAR ELECTROACUPUNCTURE AND BY ELECTRODERMAL NOCICEPTIVE STIMULATION

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Anterior structure of the brain (the hypothalamus, septum, and limbic formations) [1-3] are involved in the formation of antinociceptive responses to the action of various painful and painless stimuli (pain-induced stress, acupuncture, morphine, electrical stimulation of the brain). These structures are innervated from caudal noradrenergic (NA) nuclei of the brain stem through ascending ventral and dorsal bundles. Axons of group A5 neurons, at the level of the superior olive [9], take part in the formation of the first of these bundles. Destruction of the ascending ventral NA bundle [5, 6, 9] causes disappearance of many NA terminals in these structurs. Group A5 NA neurons, it can be tentatively suggested, exert a definite influence on the formation of antinociceptive responses to various stimuli.

To test this hypothesis, experiments were carried out on rats to study the role of group A5 neurons in mechanisms of analgesia during auricular electroacupuncture (AEA) and electrodermal footshock (FS).

EXPERIMENTAL METHOD

Experiments were carried out on 57 rats weighing 200-250 g. The rats were anesthetized with chloral hydrate (360 mg/kg) and group A5 neurons were destroyed bilaterally by means of electrodes inserted into the brain at coordinates: AP −1.6, VD −5.4, L ±2.0, according to the atlas [8]. Local damage in this region was caused by passing a direct current of 5 mA for 20 sec. Rats undergoing mock operations served as the control. Nociceptive sensitivity (NS) was estimated by measuring the latent periods (LP) of the paw licking response (PLR) to placing the rats on a hot plate (t = 55°C), and the tail withdrawal response (TWR) to application of a focused beam of light from a 150 W projection lamp on it. LP was measured before and at various times after stimulation. To study analgesia several techniques were used: 1) footshock (FS) with a direct current of 2.5 mA, 5 min, 8 pulses/min; 2) auricular electroacupuncture (AEA), induced by stimulating zones of the "lung" acupuncture points on the concha auriculae through clip-on electrodes, using a current of 0.6-1.0 mA, frequency 4 Hz, pulse duration 0.4 msec, applied for 15-20 min. After the experiments the brain was removed and the noradrenalin concentration determined in a tissue homogenate from the anterior part of the brain, located rostrally to the caudal border of the hypothalamus, by the trihydroxyindole fluorometric method [4]. The brain region with local coagulation was fixed in 10% neutral formalin solution and sections were cut on a freezing microtome and damage to A5 neurons was verified morphologically. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Biochemical determination of noradrenalin in brain tissues located rostrally to the caudal border of the hypothalamus revealed a significant decrease by 40% ($p < 0.001$) in the experimental rats compared with the control level (83.7 ± 3.3 and 117.4 ± 1.3 mg/g brain tissue respectively).

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In the experiments of series I the effect of blocking NA neurons of group 5 on spontaneous NS and on the time course of analgesia during AEA stimulation was studied (Table 1). In animals of the experimental group, after destruction of group A5 neurons the duration of LP of PLR was 1.86 ± 1.5 sec, compared with 12.5 ± 1.2 sec in the control. Comparison of these parameters showed that they differed statistically significantly ($p < 0.05$). This fact can be interpreted as evidence of an essential influence of the NA systems of group A5 neurons in the regulation of background values of LP of PLR. Measurement of LP of TWR showed no significant difference between the control and experimental values. For instance, in the first group LP was 4.0 ± 0.3 sec and in the second group 3.8 ± 0.3 sec. These results indicate that destruction of group A5 neurons does not cause changes in LP of TWR. It is therefore easy to conclude that NA neurons of this group exert a significant activating effect on the formation of PLR to pain, which are more complex in their neurofunctional structure than TWR to pain.

In rats of the control group, AEA induced a significant increase in LP of PLR at the 1st, 5th, and 10th minutes compared with the initial level. In the experimental group, AEA did not lead to any lengthening of LP of PLR. On the contrary, they were shorter than the background level. Significant shortening, evidence of the development of hyperalgesia, was observed in this case at the 10th and 20th minutes of the recovery period. Comparative analysis of LP in rats of the experimental and control groups demonstrated the presence of significantly shorter LP of PLR in rats after destruction of group A5 nuclei throughout the duration of the experiment.

The time course of LP of TWR after AEA can be seen in Table 1 also. In control rats AEA caused lengthening of LP throughout the experiment. It was shown that the increase in LP were significant. In rats of the experimental group the duration of the nociceptive responses was not increased after AEA, but showed a tendency to decrease. Comparison of LP of TWR showed that they were significantly shorter in rats of the experimental group than in the control rats at the 1st and 5th minutes after the end of stimulation. Hence it can be concluded that blocking the group A5 nuclei has a significant influence on depression of NS after AEA, when measured by various methods.

In the experiments of series II the role of NA neurons of group A5 in mechanisms of analgesia during FS was studied (Table 1). Initial values of LP of PLR in rats of the control group were 15.8 ± 1.2 sec, but in the experimental group they were significantly longer, namely 27.6 ± 2.5 sec. Estimation of NS by the TWR test, just as in the previous experiments, showed no difference in the duration of LP. In the control, for instance, its value was 4.2 ± 0.2 sec, compared with 4.6 ± 0.4 sec in the experimental rats. FS induced a significant increase in LP of PLR in rats of the control group. LP exceeded its initial length and was significantly longer until the 10th minute of the recovery period. During subsequent time intervals LP remained higher than the background values, but these differences were not significant. In the experimental group, significant lengthening of LP also was observed during the first 5 min after FS. However, at the 20th minute and at later intervals of the recovery period the duration of PLR became significantly shorter than initially, and this can be interpreted as a development of hyperalgesia after the action of FS.

Comparison of these parameters in the experimental and control groups shows that after destruction of group A5 nuclei in rats the development of analgesia in the first 10 min after FS was considerably and significantly weaker than in the control. During subsequent intervals of the recovery period, reliable signs of hyperalgesia began to appear compared with the control rats. Investigation of the duration of LP of TWR showed that in control rats FS causes a marked increase in TWR for 10 min after the end of stimulation. After 20 min and until the 40th minute TWR approximated to its initial values. In the experimental group, FS caused virtually no increase in LP of TWR during the first 5 min, but starting with the 10th and until the 30th minute, LP became significantly shorter than the background value. Statistical comparison of LP showed that the duration of this response was significantly shorter in the experimental than in the control group. Just as in the case of LP of PLR, in rats of the experimental group starting from the 10th minute of the recovery period, the development of considerable hyperalgesia was observed in the TWR test compared with the control.

It can accordingly be concluded from the results of this series of experiments that group A5 NA nuclei are involved in the formation of antinociceptive mechanisms during FS in the first minutes of the recovery period, and that starting with the 10th-20th minutes of the recovery period this brain structure in intact rats inhibits the development of hyperalgesic responses.

TABLE 1. Duration of LP of PLR and TWR (ΔC) in Rats after Destruction of Group A5 Neurons and Rats Undergoing Mock Operation after AEA and FS

Animal's response	Experimental conditions	Initial value of LP	Time after stimulation, min					
			1	5	10	20	30	40
PLR	AEA							
	Control	12,5±1,2	4,6±1,1*	4,7±0,7*	4,9±1,6*	—0,4±1,9	—	—
	Experiment	18,6±1,5+	—3,3±3,1+	—5,8±3,0+	—4,3±1,7*+	—7,4±1,3*+	—	—
TWR	Control	4,0±0,3	1,2±0,25*	1,0±0,25*	—0,8±0,4*	—1,0±0,4*	—	—
	Experiment	3,8±0,3	0,07±0,5+	—0,6±0,3+	—0,3±0,3	—0,7±0,3		
PLR	FS							
	Control	15,8±1,2	33,4±1,5*	23,6±3,6*	15,2±4,6*	6,7±4,8	3,1±4,0	1,2±3,3
	Experiment	27,6±2,5+	19,7±2,8*+	13,9±3,6*	0,95±3,1+	—7,0±3,9*+	—10,6±2,2*+	—12,7±2,0*+
TWR	Control	4,2±0,2	2,0±0,5*	1,3±0,6*	1,5±0,5*	0,3±0,5	0,04±0,4	—0,18±0,3
	Experiment	4,6±0,4	0,8±0,6	0,13±0,5+	—0,6±0,6+	—1,2±0,3*+	—1,3±0,35*+	—0,66±0,6

Legend. *$p < 0.05$ Compared with initially, +$p < 0.05$ compared with control.

A qualitatively different result was obtained in experiments [7] on rats, in which the ventral NA bundle was destroyed. The authors cited showed that FS induces a significant increase in LP of TWR in experimental and control rats, and no difference was found in the value of LP of TWR between rats of the experimental and control groups. These workers accordingly concluded that the ventral ascending NA bundle plays no part in stress-induced analgesia. The contradictions between the results of our own experiments and those examined above can be explained by differences in the parameters of FS. In our experiments stress was induced for 5 min by a current of 2.5 mA, whereas in the work cited it was induced for 30 min by a current of 3.5 mA. On the other hand, destruction of the ventral NA bundle and not of individual nuclei forming it may lead to blocking of both activating and inhibitory influences on the formation of mechanisms of antinociception from other brain formations.

The results thus suggest that the NA system of group A5 nuclei have an activating influence on the mechanisms of depression of NS during AEA and FS.

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