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LIBERATION OF THYREOTROPIN, THYROXINE
AND TRIIODOTHYRONINE IN THE CONTROLLABLE
AND UNCONTROLLABLE STRESS AND AFTER ADMINISTRATION
OF NALOXONE IN RATS

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The aim of this study was to investigate whether controllable stress, in which the animal can avoid a stressor, and uncontrollable stress, in which the animal can not avoid its influence change the activity of the endocrine pituitary-thyroid axis and whether these changes are modified by naloxone — the antagonist of opioid receptors. The experiments were carried out on rats using electric foot shock as a stressor and the concentration of thyreotropin, thyroxine and triiodothyronine in the blood was determined with radioimmunoassay. The uncontrollable stress caused the inhibition of secretion of the hormones mentioned above, but the controllable stress did not influence their secretion. Naloxone administered centrally to lateral ventricle of the brain decreased the inhibitional influence on the pituitary-thyroid axis. The obtained results suggest that change of pituitary-thyroid axis activity during the stress depends not only on the stressor but also on the animal's abilities to control that stressor.

Key words: *stress, thyreotropin, thyroxine, triiodothyronine, naloxone.*

INTRODUCTION

It is assumed that the stress situation in an animal organism involves the activation of the hypothalamus-pituitary-adrenocortical axis (1, 2) and the hypothalamus-sympathetic-adrenomedullary system (3, 4). It has been proved lately that during the stress reaction there is also the modification of the activities of the hypothalamus-pituitary-thyroid axis. Both stimulation (5, 6) and inhibition (7), or the lack of changes in the function of this axis (8, 9), have been discussed in the studies so far. That is why the question whether this reaction depends on the stressor features or on the psychosocial state of the test animal should be formulated. To answer it two stress models were used in the

experiment: controllable stress i. e. a situation in which the animal may avoid the stressor — by breaking it off and uncontrollable stress when the animal is not able to avoid the stressor (10). Because of the opioids participation in both the regulation of the hypothalamus-pituitary-thyroid axis activities (11—14) and in the behavioural mechanism, it has been also decided to investigate whether opioids mediate the possible changes. This was done by the use of naloxone — the opioid receptors blocker.

MATERIAL AND METHODS

The experiments were carried out on 60 mature male Levis rats weighing 225—250 g. The stressor was an electrical stimulus of alternating current with 1 mA intensity, 50 Hz frequency and 10 millisecond action. The electric stimulus was conducted through the metal bars which formed the floors of the test cages. In each cage there was one animal from a couple. The floors of the cages were connected with an electric stimuli generator by a switch which was situated in only one of these cages. An animal situated in the cage with the switch, when it jumped from one into the other part of the cage, broke off the stressor activity in both cages. The animal in the cage with the switch was under controllable stress, while the animal in the cage without the switch was under uncontrollable stress. The animals were divided into three groups. In the first group, after the two-week-adaptation period with handling, 2 ml of blood were taken in 20 rats; this was done by the puncture of the orbital plexus using heparinized capillary tubes. This procedure was performed in the light ether anesthesia. After 72 hours, for the three consecutive days the rats were subjected to stress lasting 10 minutes. One part of the animals was subjected to controllable stress, while the other part to the uncontrollable one. Immediately after the end of the last stress session the blood was taken again. The animals from the second group and the third group were exposed to stress in the same way but there was one difference: before the stress the rats from the second group were administered naloxone intraperitoneally in doses of 3 mg per kg of body mass in 0,5 ml 0,9% NaCl and the rats from the third group were administered naloxone into the lateral cerebral ventricle in doses of 0,5 mg in 5 ul 0,9% NaCl through a canule implanted two weeks before. The serum TSH, T_4 and T_3 concentrations in the tested animals were determined using radioimmunological methods, TSH (15), T_4 (16), T_3 (17).

RESULTS

The serum thyreotropin (TSH), thyroxine (T_4) and triiodothyronine (T_3) concentrations in rats under controllable and uncontrollable stress are shown in *Table 1*. Controllable stress did not change the serum TSH, T_4 and T_3 levels, while uncontrollable stress caused a significant decrease in these hormones concentrations ($p < 0.001$). The serum TSH, T_4 and T_3 concentrations in rats after administering naloxone intraperitoneally and into the lateral cerebral ventricle and, subsequently, after controllable and uncontrollable stressor are shown in *Figs 1, 2 and 3*. Neither naloxone given intraperitoneally nor the controllable stressor altered the serum TSH, T_4 and T_3 levels, whereas the uncontrollable stress caused the decrease in the concentrations of all the

investigated hormones: TSH, T_4 and T_3 ($p < 0.05$), *Figs. 1, 2, 3*. The serum TSH, T_4 and T_3 levels were decreased after intraventricular administration of naloxone and the controllable stressor did not change these values. Uncontrollable stress situation increased the TSH level in comparison with that after administering naloxone only, while the T_4 and T_3 levels remained unchanged ($p < 0.05$).

Table 1. Concentrations of thyreotropin (TSH), thyroxine (T_4) and triiodothyronine (T_3) in the rat serum during controllable and uncontrollable stress

		TSH in $\text{ng} \times \text{ml}^{-1}$	T_4 in $\text{ng} \times \text{ml}^{-1}$	T_3 in $\text{ng} \times \text{ml}^{-1}$
Controllable Stress N = 10	Before Stress	7.48 ± 0.85	65.7 ± 9.23	0.95 ± 0.15
	After Stress	7.49 ± 0.86	61.9 ± 11.23	0.96 ± 0.11
Uncontrollable Stress N = 10	Before Stress	7.57 ± 1.00	61.9 ± 7.78	1.02 ± 0.31
	After Stress	\neq 3.70 ± 0.77	\neq 56.8 ± 8.33	\neq 0.73 ± 0.15

$\neq p < 0.05$

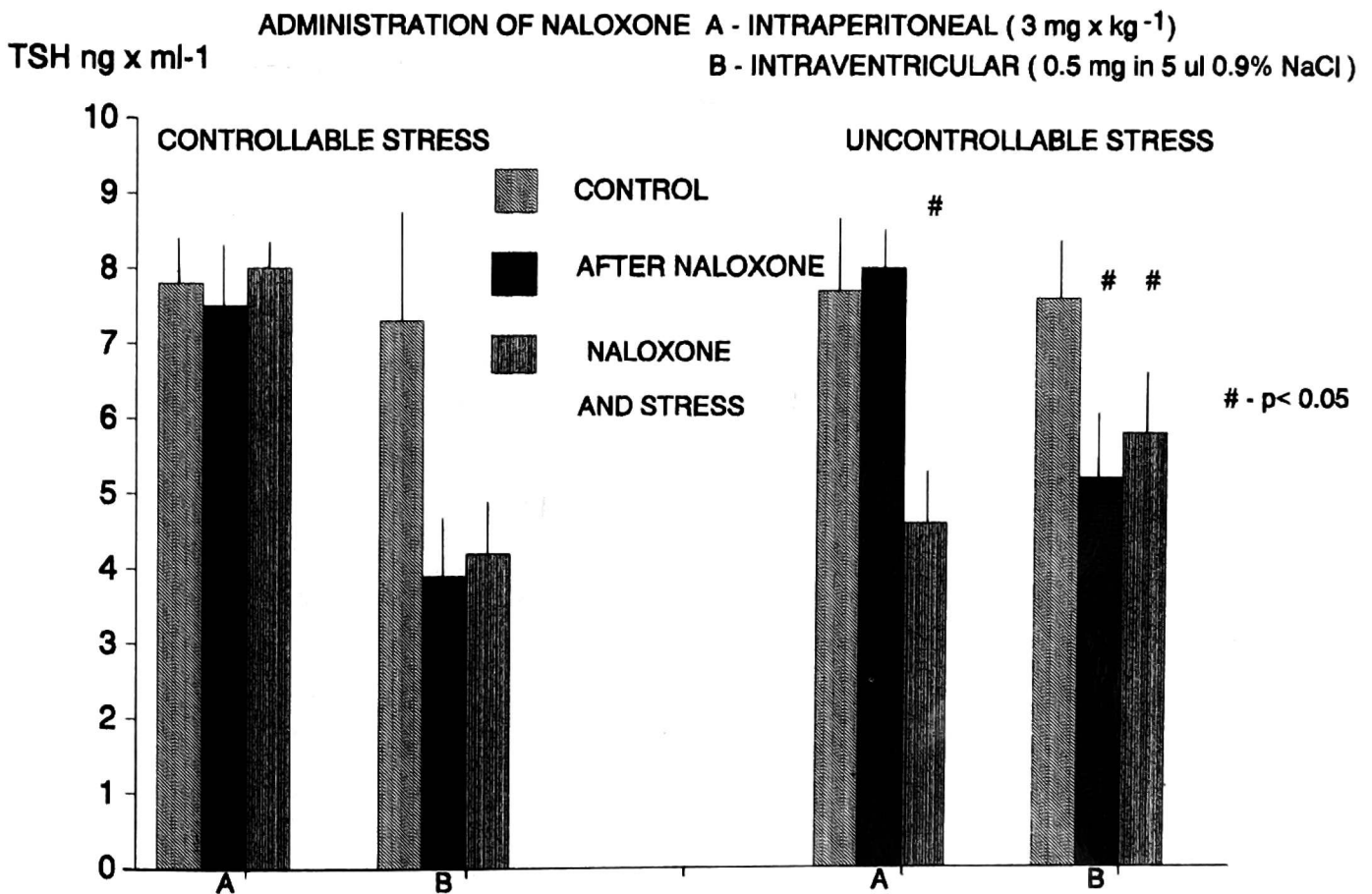


Fig. 1. The influence of naloxone administered intraperitoneally and intraventricularly on the serum thyreotropin (TSH) concentration during controllable and uncontrollable stress.

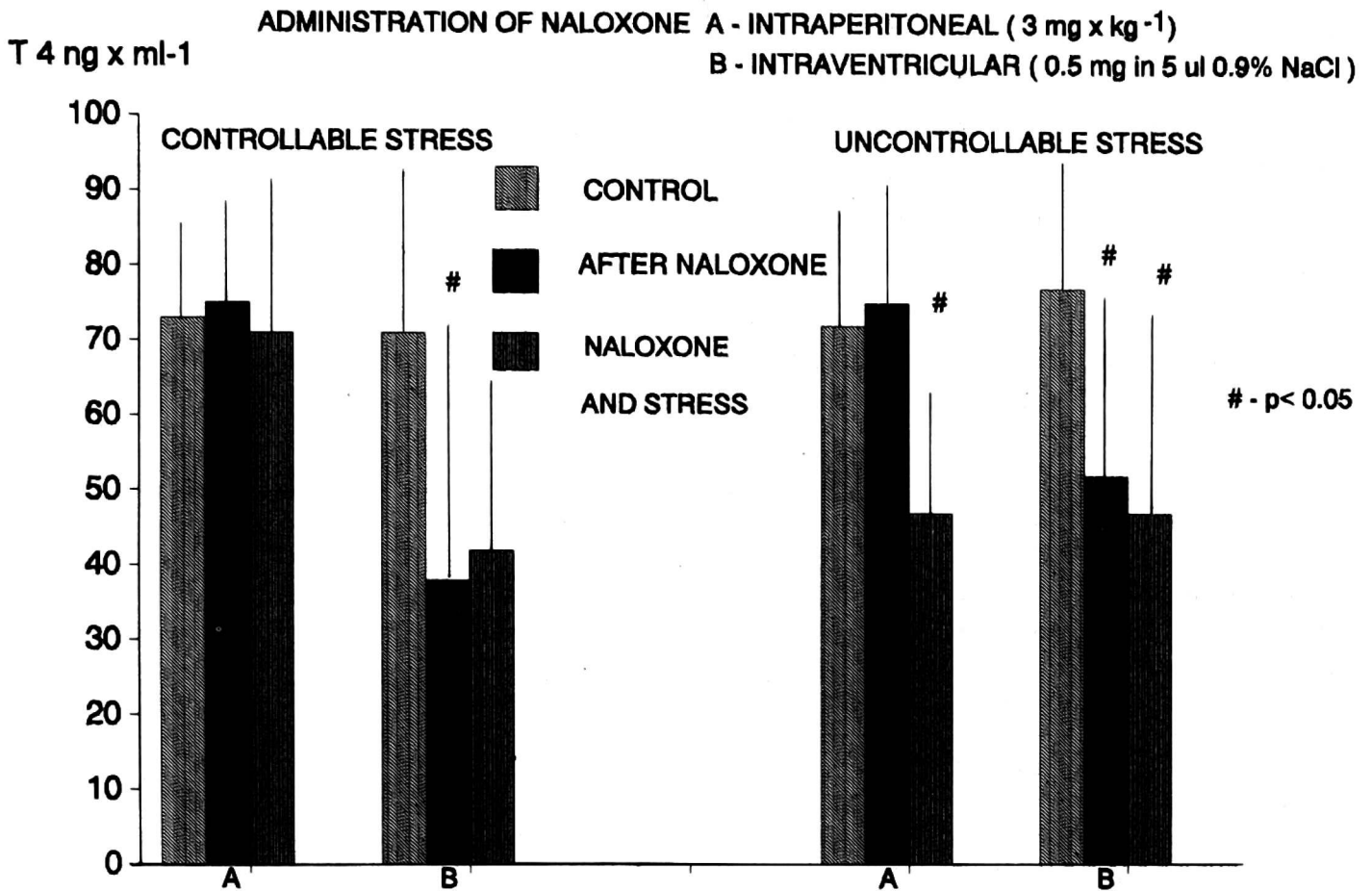


Fig. 2. The influence of naloxone administered intraperitoneally and intravenicularly on the serum thyroxine (T₄) concentration during controllable and uncontrollable stress.

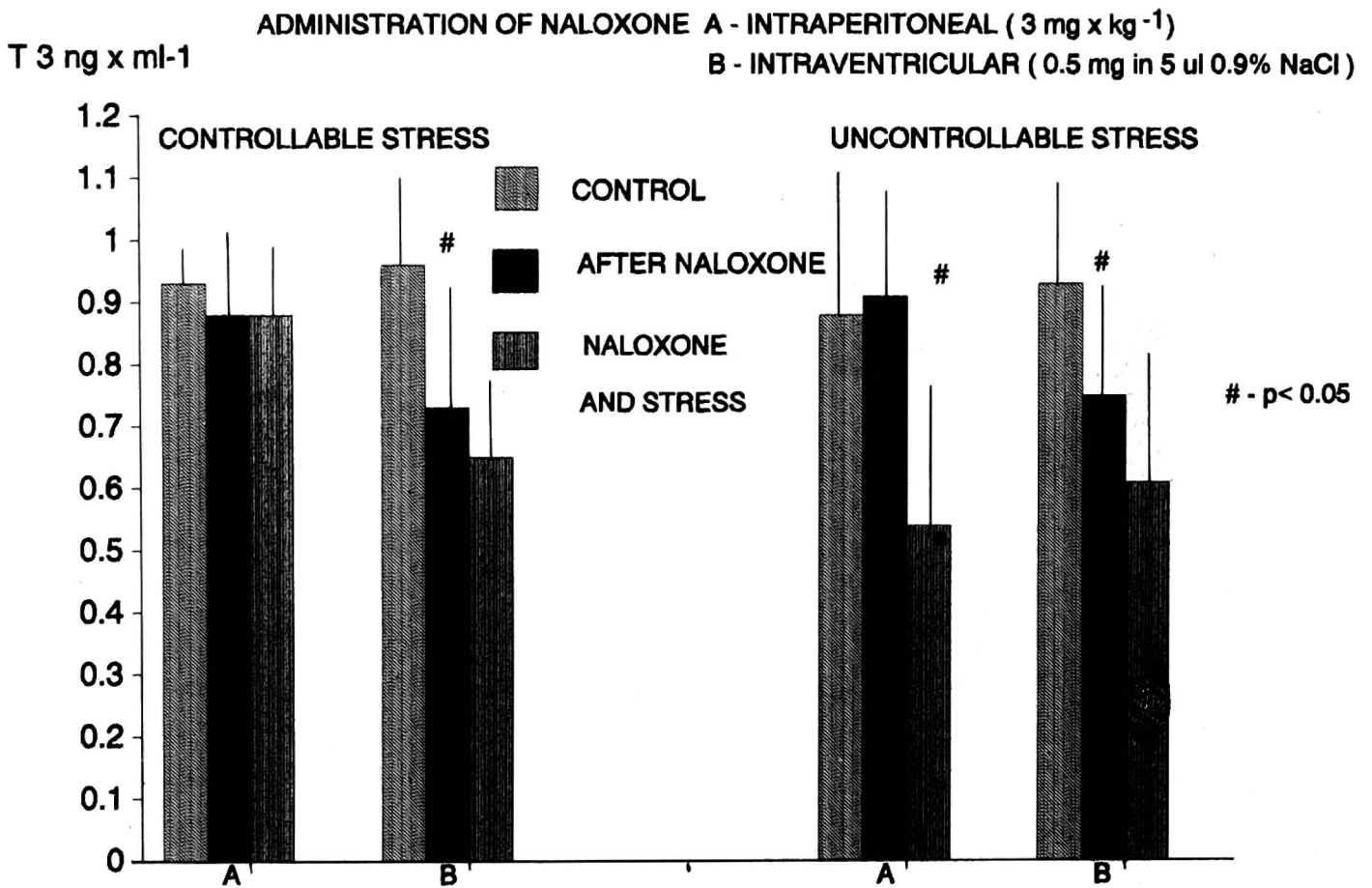


Fig. 3. The influence of naloxone administered intraperitoneally and intravenicularly on the serum triiodothyronine (T₃) concentration during controllable and uncontrollable stress.

DISCUSSION

The obtained results indicate that the changes in the pituitary-thyroid axis activity in the stress situation caused by the intermittent electric stimuli („foot shock”) depend on the animal's ability to avoid the action of stressor. The pituitary-thyroid axis activity did not change in the animals which could break off the stressor action — i. e. in those under the controllable stress. This was proved by the lack of changes in the serum TSH, T_4 and T_3 concentrations. On the other hand, in animals which could not avoid the stressor action — i. e. in those under the uncontrollable stress — the serum TSH, T_4 and T_3 concentrations significantly decreased, which indicate the inhibition of the endocrine function of the pituitary-thyroid axis.

According to Mason's hypothesis (18) the psychogenic factors responsible for the stress reaction intensity are superior to the intensity and time of duration of the stressor. The above mentioned results prove that they also apply to the mechanism of the hypothalamus-pituitary-thyroid axis response. In other stress models the opioids are released in large amounts (19, 20) and they are able to inhibit the TRH, TSH, T_4 and T_3 release (21—23). These data prompted us to investigate whether opioids mediate the above mentioned inhibition caused by the uncontrollable stress. Our results show that only blocking of central opioid receptors by icv naloxone decreases the secretion of TSH, T_4 , T_3 even in unstressed animals. Peripheral administration of naloxone did not influence the response of the pituitary-thyroid axis in rats under controllable or uncontrollable stress. However different kind of stressors cause significant increase of beta-endorphin levels in blood but not brain; the inhibition of peripheral opioid receptors is not able to affect the TSH, T_4 and T_3 levels in controllable stress, no matter of dose and possibility of transporting naloxone through blood-brain barrier (11, 24). Probably, noradrenergic system plays an important role in uncontrollable stress, by causing changes in vegetative reactions, including the secretion of thyroid hormones. Quite different results were obtained after administering naloxone to the right lateral cerebral ventricle, that is after blocking the central opioid receptors. Inhibition in the TSH, T_4 and T_3 secretion was noted, which was proved by their lower serum concentration. Other studies on this subject suggest that dopaminergic system inhibits TRH liberation from hypothalamic neurons (25—28). Thus, there is a possibility that naloxone exerts a direct, stimulating effect on this system. So far, however, one cannot also exclude the direct, inhibitory effect of naloxone on the hypothalamic neurons secreting TRH. Because the hypothalamic noradrenergic system is the indispensable promotor in the TRH liberation (4), there is a possibility of inhibiting its promoting function by naloxone. Answering this question requires further investigations. Blocking the central opioid receptors by naloxone did not change the reactivity

of the pituitary-thyroid axis in the controllable stress condition. However, the pituitary-thyroid axis response to the uncontrollable stress was considerably changed.

The uncontrollable stress did not cause any decrease in the serum TSH, T_4 and T_3 levels and in the case of TSH there was even an increase in its level. This finding may indicate that intraventricularly administered naloxone abolishes the inhibitory effect of the uncontrollable stress on the pituitary-thyroid axis endocrine activity. Inhibition of the endocrine activity under the influence of the uncontrollable stress and the lack of such inhibition under the controllable stress may be explained by different actions of the hypothalamic opioidergic system. This phenomenon was described by Meier et al. (29) and they proved that only the uncontrollable stress increases the opioid production in the hypothalamus. Rosier et al. (19) showed that the electrical controllable stress can even decrease the opioid concentration in the hypothalamus. Central opioids exert an inhibitory effect on the hypothalamic neurons secreting TRH (22). This process leads to a decrease in the TSH secretion and, consequently, to the decrease in the T_4 and T_3 secretion too. Supposedly, there is also the possibility that endogenous opioids indirectly inhibit TSH release, which is regulated by central dopaminergic system. It can be concluded that the uncontrollable stress caused by the intermittent electric "foot shock" inhibits the activity of the pituitary-thyroid axis; it is suggested that such inhibition is mediated by the central opioid system.

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