# THYROID-STIMULATING EFFECT OF EXOGENOUS VASOPRESSIN AND OXYTOCIN IN HYPOPHYSECTOMIZED RATS DURING IMMOBILIZATION STRESS

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Many previous invetigations have shown that during exposure to different kinds of stress the content of Gomori-positive neurosecretory material, the morphological equivalent of the hypothalamic nonapeptide neurohormones vasopressin (VP) and oxytocin (OT), is substantially reduced in the posterior lobe of the mammalian pituitary gland [1, 6]. This fact is regarded as an indicator of intensive release of these neurohormones into the general blood stream. Effects of the hypothalamic nonapeptide neurohormones under these circumstances are not confined to their well known action on the renal epithelium and smooth muscles. The hypothesis relating to the possibility of a direct, para-adenohypophyseal effect of these neurohormones on peripheral endocrine glands was formulated by Polenov [5] as long ago as in 1962. Evidence has recently been obtained of the presence of receptors for VP and OT in the adrenal cortex and testes [9, 14], confirming this hypothesis. The possibility that nonapeptide neurohormones may have a direct influence on the thyroid gland has been examined only in sporadic studies in recent years [2, 10, 13], and the results proved contradictory.

The aim of this investigation was to study the effect of VP and OT on the response of the thyroid gland under stress conditions in hypophysectomized rats (HER), i.e., in animals deprived of their anterior pituitary hormones, which under normal conditions play a leading role in the regulation of the peripheral endocrine glands.

## EXPERIMENTAL METHOD

Altogether 90 male Wistar rats weighing 120-140 g were studied in two series of experiments. Hypophysectomy was performed by the trans-sphenoidal method 6-7 days before exposure to stress. Emotional stress was induced by immobilizing the rats for 20 min in the supine position, with their limbs and head fixed.

The following groups of animals were studied: 1) HER, no other procedure; 2) HER and exposed to immobilization stress; 3) HER without stress, decapitated 20 min after intraperitoneal injection of VP (5 mg/100 g), diluted in 1.0 ml physiological saline; 4) HER, receiving VP in the same dose before immobilization; 5) HER, decapitated 20 min after injection of OT (15 mg/100 g), in 1.0 ml physiological saline; 6) HER, receiving OT in the same dose before immobilization. In addition, rats not undergoing hypophysectomy, but subjected to the same procedures as the HER in groups 1, 2, 3, and 5, were used for comparison.

All the animals were quickly decapitated and blood was collected for radioimmunoassay of VP (Kits from Bühlmann Lab., Switzerland), of thyroid-stimulating hormones (TSH, from "Mallinckrodt Diagn.," West Germany), and tri-iodothyronine ($T_3$, Institute of Bio-organic Chemistry, Academy of Sciences of the Belorussian USSR). For histological investigation the pituitary and thyroid glands were fixed in Bouin's fluid. Sections through the pituitary gland were stained with paraldehyde-fuchsine after Gomori-Gabe, and immunohistochemically by the PAP method with antiserum to OT. The height of the thyrocytes and the size of the follicles were measured, under a magnification of 10 × 90, in sections through the thyroid gland stained with Ehrlich's hematoxylin.

The numerical results were subjected to statistical analysis by Student's t test and the Mann–Whitney–Wilcoxon U test.

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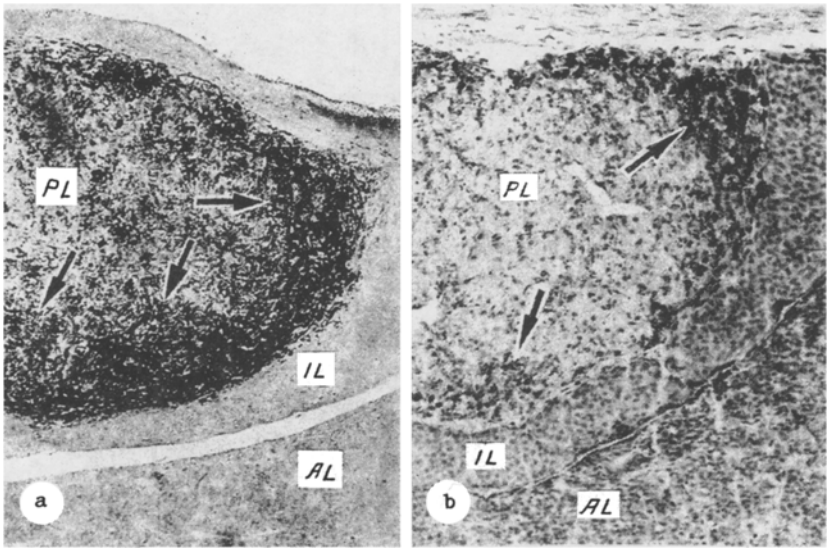


Fig. 1. Posterior lobe of rat pituitary gland: a) control; immobilization stress content of OT-immunoreactive substance appreciably reduced (arrows); PL) posterior lobe of pituitary; IL) intermediate lobe; AL) anterior lobe of pituitary; immunohistochemical reaction reaction with antiserum to OT, counterstained with Ehrlich's hematoxylin. Magnification: 12.5 × 3.

## EXPERIMENTAL RESULTS

In intact rats exposed to immobilization stress the content of Gomori-positive neurosecretion in the posterior lobe of the pituitary gland was somewhat reduced, especially so on the boundary with the intermediate lobe, where most terminals of OT-ergic fibers are located [3]. The decrease in the content of OT-immunoreactive substance in the posterior lobe of the pituitary during immobilization stress was clearly visible (Fig. 1), evidence of intensive release of OT from the posterior lobe of the pituitary into the general blood steam during immobilization stress. It was shown by radioimmunoassay that release of VP into the blood stream also was increased under these circumstances (intact rats 8.3 ± 0.42 pmoles/liter, stress 20.4 ± 3.54 pmoles/liter; $p < 001$). The blood BP level in HER fell to 30% of the control value, and remained unchanged during immobilization, even if VP was given to the HER before stress (Fig. 2). Injection of OT likewise had no effect on the blood level of VP in HER.

The blood TSH level of the intact rats exposed to stress fell somewhat 20 min after exposure (control 1.1 ± 0.1 mU/liter, stress 0.94 ± 0.08 mU/liter), and injection of VP or OT had no effect on the blood TSH level. The TSH concentration in HER did not change significantly, possibly as a result of functioning of the thyrotrophocytes in the tuberal part of the adenohypophysis, which remain intact in HER [11]. Immobilization stress caused a small fall of the blood TSH level in HER (0.69 ± 0.24 mU/liter; $p > 0.05$).

No significant changes could be found in the state of the thyroid gland of the nonhypophysectomized rats during immobilization stress as regards morphologic and functional criteria (height of the epithelium 92%; plasma $T_3$ concentration 118%; $p > 0.05$). Injection of VP into nonhypophysectomized rats led to some elevation of the $T_3$ level (up to 159%), but this increase remained not significant. In HER, despite the presence of THS in the blood, the thyroid gland was characterized by a state of depressed function, the follicles were dilated and filled with dense colloid, and the height of the thyrocytes was reduced to 43% of the level in intact animals ($p < 0.01$). Immobilization stress caused no significant changes in the state of the thyroid gland of these rats, and injection of VP or OT led to only a very small increase in height of the thyrocytes. A combination of injection of nonapeptide neurohormones with exposure to stress provides a model of the hormonal changes that are charcteristic of nonhypophysectomized animals during stress, but in such a model hormones of the anterior pituitary and the effects connected with them are absent. In such a situation it is possible to examine changes in the thyroid gland caused by the direct in-

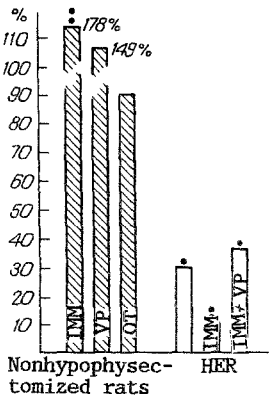


Fig. 2

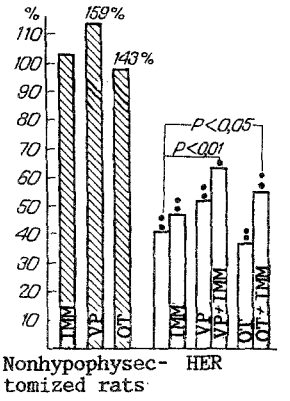


Fig. 3

Fig. 2. Blood plasma VP concentration (in % of intact control, taken as 100%) of nonhypophysectomized rats and of HER. Here and in Fig. 3: IMM) immobilization stress; VP) injection of VP; OT) injection of OT; VP + IMM) injection of VP coupled with IMM; OT + IMM) injection of OT coupled with IMM; *$p < 0.05$ compared with intact control.

Fig. 3. Parameters of functional state of thyroid gland in nonhypophysectomized rats and HER (expressed in % of corresponding intact control, taken as 100%). Shaded columns – plasma $T_3$ concentration; unshaded columns – height of thyrocytes.

fluence of neurohormones entering the blood stream during stress. In fact, the simultaneous action of stress and of VP or OT led to activation of the thyroid gland in HER, as reflected in an increase in height of the thyrocytes after injection of VP up to 154% ($p < 001$) and after injection of OT to 126% ($p < 0.05$) compared with HER not exposed to any additional procedures (Fig. 3). Thus both VP and OT, under conditions of stress, can stimulate the thyroid gland of HER, the effect of OT in this case being rather weaker.

An important role in regulation of the thyroid gland is played by the autonomic nervous system [1], and there is also evidence that catecholamines may have a direct influence on the thyroid gland [7, 15]. In immobilization stress the blood adrenalin and noradrenalin levels rise, but nevertheless no response of the thyroid gland of HER to stress could be found. Consequently, involvement of the sympathicoadrenal system alone is insufficient to cause activation of the thyroid gland. A combination of exposure to stress with injection of nonapeptide neurohormones, simulating their release into the general blood stream during stress, leads to significant activation of the thyroid gland, confirming the hypothesis of dual (through monoamine and peptide neurohormones) control of the visceral organs during stress [6]. In the absence of anterior pituitary hormones, whose action evidently masks the phylogenetically older para-adenohypophyseal mechanisms of regulation of the peripheral endocrine glands [5, 6], interaction of hypothalamic nonapeptide and catecholamine neurohormones (chromaffin tissue) in the mechanism of the response of the body to stress becomes clear. Similar interaction between monoamine and peptide neurohormones to that described in this paper in regulation of the response of the adrenal cortex of HER during cold-induced stress [4] was noted by the writers previously.

Increased thyroid activity during immobilization accompanied by injection of VP or OT in HER is at first glance difficult to explain, if it is recalled that the blood level of nonapeptide neurohormones rises during stress, but activation of the thyroid gland does not take place under these circumstances. This contradiction can probably be explained on the grounds that the blood level of somatostatin, the neurohormone which usually inhibits the reaction of the TSH–thyroid gland system [16], is depressed in HER [17].

The results of this investigation thus showed that hypothalamic nonapeptide neurohormones in HER may have a direct stimulating effect on the thyroid gland which, under conditions of stress, is intensified, probably due to interaction with catecholamines of chromaffin tissue and, in particular, of the adrenal medulla.

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# ROLE OF INHIBITION OF CELL $Na^+/H^+$ EXCHANGE AND GLYCOLYSIS IN ANTIVIRAL ACTION

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Antiviral agents include compounds with the ability to change the mechanism of interaction between virus and cell that has become established in evolution [3]. The antiviral action of substances can be realized through action on the structural and functional state of cells, both before and after infection. Inhibition of the early stages of infection is most promising, for in this case the development of pathological processes in the cell is prevented. We know that many viruses penetrate into the cytoplasm by viropexis (a variant of receptor endocytosis) – a complex physiological mechanism developed by the cell in the course of evolution in order to seize macromolecules [1, 14]. To realize the next stage, namely uncoating of the virus, a considerable fall of the intraendosomal pH is required [10, 13]. The writers previously discovered the nature of acidification of the internal medium of virus-containing endosomes, effected by membrane-bound systems of the cell [7, 8]. As a tool to act upon these systems we used remantadine, which has a prophylactic and early therapeutic action [10] and possesses membranotropic properties [9]. By this approach it is possible not only to discover the mechanism of the anti-influenzal action of remantadine at the stage of uncoating of the virus, but also to determine to what degree suppression of processes responsible for reduction of the intraendosomal pH is coupled with inhibition of viral activity.

We therefore decided to study the effect of remantadine, in different doses and the time of preliminary treatment of the cells on the process of acidification of the incubation medium of chick embryonic fibroblasts.

## EXPERIMENTAL METHOD

Cell suspensions of chick embryonic fibroblasts obtained by removal of a monolayer culture from the substrate by treatment with 0.1% trypsin solution for 30 sec were used in the experiments. Changes of pH in the cell suspension were recorded potentiometrically [7] at 25°C in medium of the following composition: 0.15 M NaCl, 1 mM HEPES/KOH (pH 7.4) with the addition of either KCl (10 mM) or glucose (1%); 0.25 ml of a thick cell suspen-

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