The *In Vitro* Effect of Thyreostimulating Hormone on the Functional State of Nonapeptidergic Cells in Rat Hypothalamus

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In persistent rat hypothalamic slices thyreostimulating hormone activates only the oxytocinergic cells of the supraoptic and paraventricular nuclei. This effect manifests itself in increased volume of nucleoli in oxytocinergic cells and in markedly increased content of c-Fos-immunoreactive cells in both nuclei.

Key Words: *thyreostimulating hormone; vasopressin; oxytocin; paraventricular and supraoptic nuclei of the hypothalamus*

The interactions between the nonapeptidergic neurosecretory system of the hypothalamus and the hypothalamus-hypophysis-thyroid gland system, which produces thyroliberin, thyreostimulating hormone (TSH), and the thyroid gland hormones, have been extensively investigated [1,5,13]. It was demonstrated that vasopressin stimulates the thyroid gland both *in vivo* and *in vitro*. There is evidence that thyroid hormones modify the activity of nonapeptidergic cells [6]. The effects of thyroliberin on the function of neurosecretory cells in the supraoptic (SON) and paraventricular (PVN) nuclei of rat hypothalamus were studied [2-4,11,17].

The interactions between the nonapeptidergic neurosecretory system of the hypothalamus and TSH-cells of the adenohypophysis have not been studied in sufficient detail. Nevertheless, it is known that vasopressin and oxytocin affect TSH secretion [12,14]. It remains unclear whether TSH has any effect on the activity on nonapeptidergic cells. In the *in vivo* experiments TSH increased blood concentration of nonapeptide hormones and it was impossible to distinguish between its effects and those of the thyroid gland hormones. We managed to demonstrate a direct effect of TSH on vasopressin- and oxytocinergic cells in experiments on hypothalamic slices.

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MATERIALS AND METHODS

Experiments were performed on adult male Wistar rats weighing 120-140 g. The animals were maintained under the standard vivarium conditions. All the experiments were started at 12:00-13:00. The rats were decapitated, the brain was rapidly isolated under sterile conditions, and hypothalamic slices (400-μ thick) containing SON and/or PVN were prepared. The slices were preincubated for 90 min at 37°C in Earle's growth medium or in medium 199 with Hanks' balanced salts saturated with carbogen (95% O_2+5% CO_2). The cells were then transferred to the culture medium containing 50×10^{-6} U/ml TSH and incubated for 30 or 60 min under the same conditions.

After the incubation the slices were fixed in Bouin's fluid and processed according to the standard histological techniques.

Parallel hypothalamic sections were incubated with unlabeled antivasopressin or antioxytocin antibodies and the peroxidase-antiperoxidase complex, using 3,3'-diaminobenzidine for visualization of the

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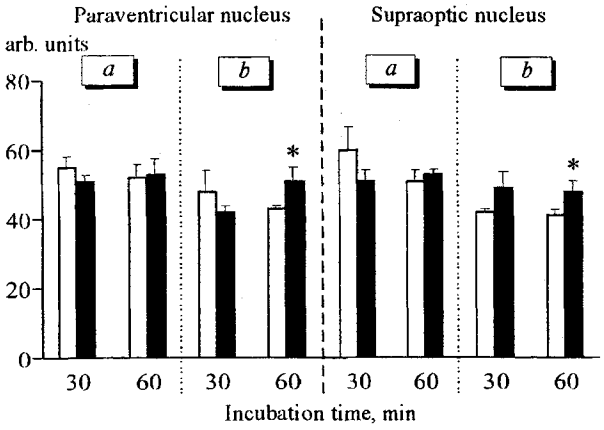

Fig. 1. Volume of the nuclei in nonapeptidergic cells in the control (white bars) and under the effect of thyreostimulating hormone (black bars). Vasopressinergic (*a*) and oxytocinergic (*b*) cells. Here and in Fig. 2: *$p<0.05$ in comparison with the control.

immunoreactive product and subsequent counterstaining with Ehrich's hematoxylin.

After 30 min of incubation, some control and experimental slices were fixed with 2% paraform for 5 days and embedded in paraffin by the standard technique. Histological sections 6-μ thick were processed as described [16].

The functional state of nonapeptidergic cells of SON and PVN was assessed by the content of c-Fos-immunoreactive cells and by the content of so-called polynucleolar neurosecretory cells, bearing in mind that the size of the neurosecretory cell nucleolus correlates with the intensity of protein synthesis [7,9]. We determined the nucleolus size in 35 oxytocin- and vasopressinergic cells of SON and PVN in each rat.

Statistical analysis of the results was performed using the Student *t* test.

RESULTS

After 30- or 60-min incubation in medium containing TSH, the intracellular content of vasopressin or oxytocin-immunoreactive product and the shape of cells, their nuclei, and nucleoli did not differ from the control. The same held true for the number of polynucleolar cells, i.e., cells containing nucleolus-like bodies in the nucleus. The volume of the nucleoli in oxytocinergic cells increased. After a 30-min incubation with TSH, in SON cells it increased to 115% ($p<0.05$), while in PVN cells it increased to 117% ($p<0.05$) after 60 min of incubation with TSH (Fig. 1).

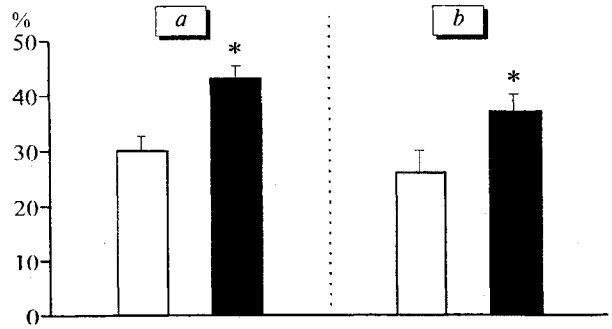

Fig. 2. Percentage of c-Fos-immunoreactive cells crates in supraoptic (*a*) and paraventricular (*b*) nuclei of the hypothalamus in the control (white bars) and after 30-min incubation in the presence of thyrostimulating hormone (black bars).

After 30 min of incubation in the presence of TSH, the number of cells positively staining for c-Fos protein, which is an indicator of the initial stage in the activation of neurosecretory cells [10,16], increased significantly to 43.3% in SON and to 37.4% in PVN (control: 30.4% in SON and 26.4% in PVN, $p<0.05$, Fig. 2). It should be noted that the content of c-Fos-protein-positive cells was greater in the dorsal regions of SON departments and ventromedial regions of PVN, i.e., in the regions where oxytocinergic cells prevail.

There were no statistically significant changes in the nucleus size of the vasopressinergic cells of SON and PVN (Fig. 1), indicating that the intensity of hormone production by these cells does not change under the effect of TSH. However, there is evidence that vasopressin stimulates the release of TSH from the thyrotrophic cells of the adenohypophysis in the blood [14]. Elevation of blood TSH concentration stimulates the thyroid gland and, consequently, increases the secretion of thyroid hormones. Since thyroid hormones decrease the functional activity of hypothalamic vasopressinergic cells *in vitro* [6], it can be suggested that the interaction between TSH cells of the adenohypophysis and vasopressinergic cells of the hypothalamus is mediated by the thyroid gland hormones.

Thyreostimulating hormone activates oxytocinergic cells of both SON and PVN, as evidenced by increased size of the nuclei and content of c-Fos immunoreactive cells in the corresponding regions of SON and PVN. Taken together, the inhibitory effect of oxytocin on the stimulation of TSH-cells in the adenohypophysis [12] and activating effect of TSH on oxytocinergic cells suggest that the relationship between oxytocinergic and thyrotrophic cells obeys the negative feedback principle.

The possibility of delivering TSH to the hypothalamus is determined by retrograde blood flow from the hypophysis to the median eminence [15]. Since the capillaries of the primary portal plexus are fenestrated [8] and their walls are permeable for adenohypophyseal hormones, it is likely that TSH from the adenohypophysis reaches the hypothalamus and regulates the interactions between TSH-cells of

the adenohypophysis and the large-cell centers of the hypothalamus.

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