Effect of Thyroid Hormones on Neurosecretory Cells of Supraoptic and Paraventricular Nuclei of Rat Hypothalamus *in Vitro*

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The direct effect of thyroid hormones on oxytocin- and vasopressinergic cells is studied *in vitro* on cultured slices of rat hypothalamus containing the paraventricular and/or supraoptic nuclei. It is shown that 30- and 60-min exposure to thyroxine leads to considerable shrinkage of the nucleoli of oxytocin- and vasopressin-secreting cells in the supraoptic and paraventricular nuclei, which reflects the decrease in their functional activity. The addition of triiodothyronine leads to stimulation of neurosecretory cells (30-min incubation) followed by a drop in their functional activity.

Key Words: *thyroid hormones; vasopressin; oxytocin; paraventricular nucleus; supraoptic nucleus*

The direct effect of the hypothalamic hormones vasopressin (VP) and oxytocin (OT) on functional activity of the thyroid gland is well established [1,2]. However, the possibility of feed-back regulation of VP- and OT-cells of the hypothalamus by thyroid hormones (TH) remains unsolved. TH receptors were identified in many areas of the brain, including the hypothalamus [7]. Conceivable TH influence the VP cells since different thyroid statuses are accompanied by shifts in the blood VP level [5,6]. However, under conditions of long-term hormone imbalance it is very difficult to determine which factor plays a key role in the regulation of endocrine organs. It has been shown that TH participate in the regulation of the OT gene *in vitro* [3,4].

In the present study we examined the effect of short-term direct exposure of cells of paraventricular and supraoptic nuclei to TH under conditions of *in vitro* incubation of hypothalamic sections in the presence of TH.

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MATERIALS AND METHODS

Sections of the hypothalamus were obtained from male Wistar rats (120-140 g). After decapitation, the brain was promptly removed and 400-μ sections containing supraoptic and/or paraventricular nuclei were prepared from the hypothalamic area. The sections were preincubated in Earle's medium saturated with carbogen (95% O_2+5% CO_2) at 37°C for 90 min. The medium was replaced every 30 min.

Two experimental series were carried out. In both series binding of triiodothyronine (T_3) and thyroxine (T_4) was measured using radiolabeled hormones: ^{125}I-T_3 and ^{125}I-T_4.

After preincubation, the sections were transferred to a medium containing 2.7×10^{-9} M ^{125}I-T_3 (74×10^{15} Bq/mol, series I) or 4.5×10^{-9} M ^{125}I-T_4 (140×10^{15} Bq/mol, series II). Control sections were incubated without TH. After 30- and 60-min incubation, the sections were fixed in picric acid and formalin (3:5) at 37°C for 7 days and processed by standard histological methods.

Histological sections (6 μ) were stained with paraldehyde-fuchsin by the Gomori—Gabu method and poststained with azan by the Heidenhain method.

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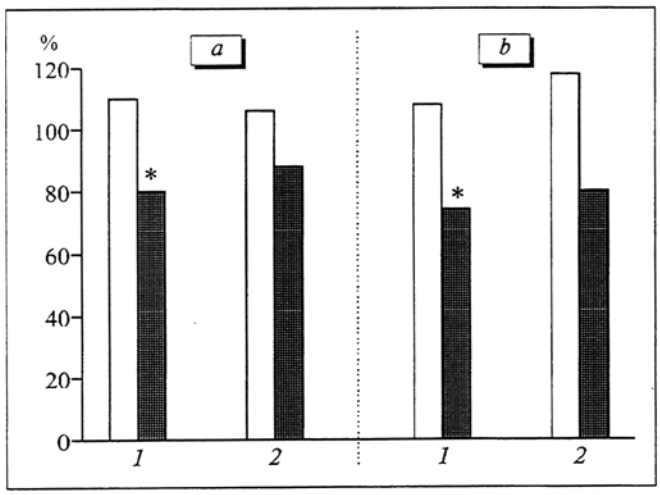

Fig. 1. Diameter of nucleoli of nonapeptidergic cells in the supraoptic (*a*) and paraventricular (*b*) nuclei after incubation of hypothalamic sections in the presence of triiodothyronine (T_3). *p<0.05 in comparison with 30-min incubation. Here and in Fig. 2: oxytocin- (*1*) and vasopressin-secreting (*2*) cells. Open and shaded bars correspond to 30- and 60-min incubation, respectively. Control values are taken as 100%.

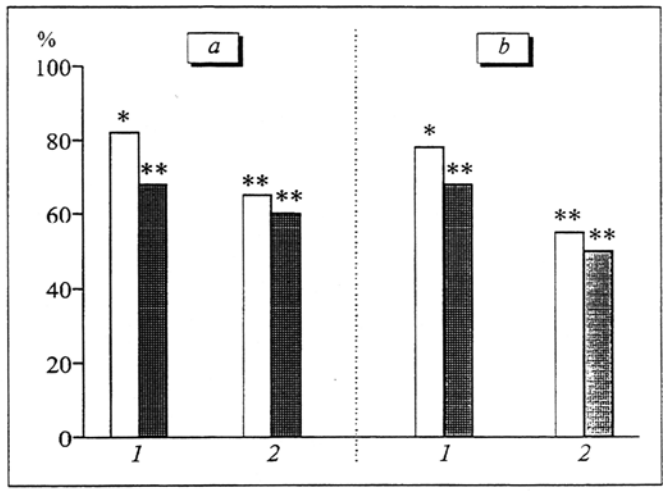

Fig. 2. Diameter of nucleoli of nonapeptidergic cells in the supraoptic (*a*) and paraventricular (*b*) nuclei after incubation of hypothalamic sections in the presence of thyroxine (T_4). *p<0.05, **p<0.01 in comparison with the control.

Immunohistochemical PAP-reaction was performed on parallel sections using anti-VP and anti-OT antiserum and poststaining with Ehrlich's hematoxylin. Functional activity of VP- and OT-ergic cells of the supraoptic and paraventricular nuclei were assessed by measuring the nucleolus diameter with an AM-9-2 screw ocular-micrometer at ×3375.

The significance of differences between the mean values was evaluated using the Student's *t* test.

RESULTS

Examination of histological preparations showed that incubation did not change morphological structure of the hypothalamus. The regular round shape of neurosecretory cells, the nuclei, and nucleoli characteristic of intact animals was preserved. In VP-cells the immunoreactive substance was evenly distributed in the cytoplasm, while in OT-cells it was concentrated

Fig. 3. Cells of the supraoptic nucleus after 60-min incubation in the presence of ^{125}I-T_3. Autoradiography: Arrows indicate silver grains; *NC*: nonapeptidergic cell; *GC*: glial cell. Ehrlich's hematoxylin poststaining. ×600.

in the perinuclear zone. Axonal fragments of both types filled with immunoreactive material were seen. In control sections the diameter of nucleoli in VP- and OT-cells remained unchanged in all experiments ($p>0.05$).

A 30-min incubation in the presence of T_3 resulted in a marked enlargement of nucleoli of the OT-cells (Fig. 1), while after a 60-min incubation the nucleolar diameter was below the control value and differed significantly from that observed after a 30-min incubation (Fig. 1), which implies suppression of cell functional activity. Visual analysis revealed no differences between sections incubated in the presence and absence of T_3 in the content of immunoreactive matter in the perikaryons of neurosecretory cells.

A 30-min incubation in the presence of T_4 led to a considerable decrease in the diameter of nuclei in both VP- and OT-cells, which was also observed after a 60-min incubation (Fig. 2).

On radioautograms, silver grain concentrated above the nuclei and perikaryons of nonapeptidergic cells in sections incubated with ^{125}I-T_3 and ^{125}I-T_4 in both hypothalamic centers (Fig. 3). The label was also seen above glial cells, which is consistent with active uptake of TH by glial cells [8,9].

Thus, our findings suggest that TH (both T_3 and T_4) are incorporated into neurosecretory cells of the supraoptic and paraventricular nuclei. No differences in incorporation of TH into VP- and OT-cells were found. T_3 stimulates functional activity of neurosecretory cells (30-min incubation). This effect was most pronounced in OT-cells of both centers. However, further incubation led to a drop in functional activity of OT- and VP-cells. The addition of T_4 to the incubation medium results in a progressive decrease in the functional activity of neurosecretory cells in both centers throughout the experiment. Our findings imply the possibility of a feed-back regulation of nonapeptidergic centers of the hypothalamus by TH.

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