Norepinephrine-Induced Inhibition of Thyroid-Stimulating Effects of Oxytocin in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 12, pp. 657-659, December, 1999
Original article submitted March 15, 1999

In vivo and *in vitro* experiments on rats and isolated fragments of the thyroid gland showed ^{3}H-oxytocin incorporation into thyrocytes followed by activation of the synthesis and release of thyroid hormones. Norepinephrine *in vivo* activates the thyroid gland, but combined action of norepinephrine and oxytocin suppressed *in vivo* and *in vitro* thyroid-stimulating effects of oxytocin.

Key Words: *thyroid gland; oxytocin; epinephrine*

Various stress factors increase blood content of some hormones, responsible for the reaction to specific and nonspecific stress components. The increase in blood concentrations of catecholamine neurohormones of the adrenal medulla, epinephrine and norepinephrine (NE), is the major component of the hormonal reaction to stress. Hypothalamic nonapeptide hormones vasopressin and oxytocin (OT) are also released into the blood under the effect of various factors [4-7]. In light of this, combined effects of various neurohormones on target organs during stress are of considerable interest. In our previous experiments, we studied the reactions of rat thyroid gland (TG) *in vivo* [1] and thyrocytes *in vitro* [8] to combined action of vasopressin and epinephrine. However, there are no published data on the effects of combined treatment with OT and NE on mammalian TG.

MATERIALS AND METHODS

Experiments were performed on adult male Wistar rats weighing 140-160 g.

In *in vivo* experiments, neurohormones and their combination were injected intraperitoneally 30 min before decapitation. The animals were divided into 4 groups (n=5) and injected with 1 ml physiological saline (control), 15 ng/100 g body weight OT, 30 ng/100 g body weight NE, and OT and NE in the same doses, respectively.

After decapitation, TG was removed, fixed in Bouin's fluid, and subjected to routine histological treatment. The height of thyrocytes was measured on slices stained with azan by Heidenhain's method (×900).

In vitro experiments were performed on 20 rats. Cross-sections (400 μ) from the central zone of TG lobes were incubated in medium 199M saturated with carbogen (95% O_2 and 5% CO_2) at 37°C. The medium was changed every 30 min. After 90-min preincubation, the slices were transferred into the medium containing test neurohormones (60 min). We analyzed 5 groups of TG fragments incubated in media containing various neurohormones (100 pg/ml ^{3}H-OT, 100 pg/ml OT, 10 pM NE hydrotartrate, or both neurohormones in the same concentrations) or without neurohormones (control).

All samples (except for those incubated with ^{3}H-OT) were initially incubated in the medium containing ^{3}H-leucine for 30 min. Some slices were then transferred into the medium without ^{3}H-leucine, and others were further incubated with ^{3}H-leucine. This method allows to assess the dynamics of thyroglobulin formation in thyrocytes and to determine the intensity of its transfer into the colloid or release from follicles [3,12,13].

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0007-4888/99/0012-1229$22.00

TG fragments were fixed in Bouin's fluid and subjected to routine histological treatment. Slices (6 μ) were stained with azan by Heidenhain's method or covered with type M photoemulsion, and autoradiographic samples were then prepared. The functional state of thyrocytes was assessed by their height and the mean number of silver grains above one thyrocyte (×900). The results were analyzed by Student's *t* and Mann—Whitney *U* tests.

RESULTS

In vivo experiments showed that the intraperitoneal injection of OT to rats caused hyperemia of TG; capillaries were enlarged and filled with blood cells. The height of thyrocytes increased (9.48±1.42 *vs.* 4.95±0.2 μ in the control, $p<0.05$), and they had cylindrical shape. Intracellular colloid drops and parietal resorption vacuoles appeared in follicles. These changes indicate considerable and rapid activation of TG. Injection of NE led to parietal vacuolization of the follicular colloid and increased the height of thyrocytes to 6.52±0.5 μ ($p<0.05$). After simultaneous administration of OT and NE, these signs of TG activation were less pronounced, and the height of thyrocytes increased to a lesser extent (6.4±0.17 *vs.* 9.48±1.42 μ in rats receiving OT alone, $p<0.05$).

In vitro incubation of isolated fragments of TG in a medium containing ^{3}H-OT revealed incorporation of labeled OT into follicular thyrocytes but not into interfollicular epithelial cells.

Incubation of TG fragments in the medium containing labeled leucine was not accompanied by accumulation of ^{3}H-leucine in the follicular colloid. Silver grains were found above thyrocytes and on their apical surfaces. These data suggest that newly synthesized thyroglobulin containing ^{3}H-leucine did not accumulate in the colloid and together with thyroxin and triiodothyronine was released from thyrocytes under the effect of neurohormones. Similar dynamics of the formation and release of thyroglobulin was previously observed during *in vitro* action of thyrotropin [13]. Incubation of TG fragments in a medium containing OT and ^{3}H-leucine increased the height of thyrocytes on the 30th and 60th minutes (6.34±0.67 and 7.8±0.56 *vs.* 5.19±0.23 and 5.26±0.14 μ in the controls, respectively, $p<0.05$). The mean number of silver grains above the thyrocyte, which reflected the formation of thyroglobulin in thyrocytes, also increased on the 60th minute of incubation (23.7±0.22 *vs.* 16.67±0.1 in the control, $p<0.05$, Fig. 2). The transfer of TG fragments from neurohormone-free (control) and OT-containing media with ^{3}H-leucine to the ^{3}H-leucine-free medium decreased the number of labels above thyrocytes to 78 and 49% (respectively) of their number

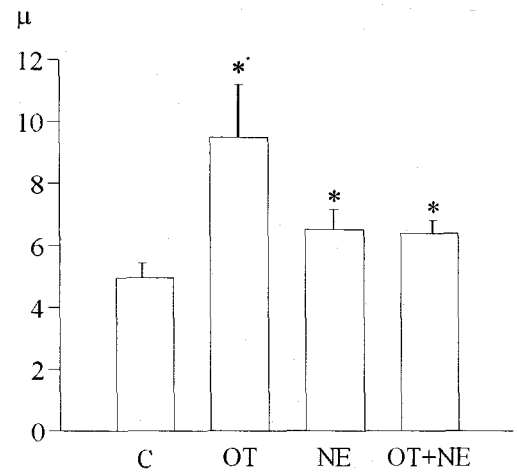

Fig. 1. Height of rat thyrocytes after intraperitoneal administration of neurohormones. Here and in Figs. 2 and 3: control (C); oxytocin (OT); norepinephrine (NE); and both neurohormones (OT+NE). *$p<0.05$ compared with the control.

noted after continuous incubation in ^{3}H-leucine-containing media. Therefore, the release of labeled compounds from thyrocytes under the effect of OT was more intensive than in the control (Fig. 3).

In the group of TG fragments incubated in the medium with NE, the intensity of ^{3}H-leucine incorporation into thyrocytes did not considerably change on the 30th and 60th minutes, while the height of thyrocytes increased to 123% of the control level on the 60th minute of incubation ($p<0.05$). After the transfer of these fragments to the ^{3}H-leucine-free medium, the number of silver grains above thyrocytes decreased to 56% of the level observed after continuous incubation in the ^{3}H-leucine-containing medium and was below the control level. Thus, NE primarily activates the release of hormones from thyrocytes (Fig. 3). However,

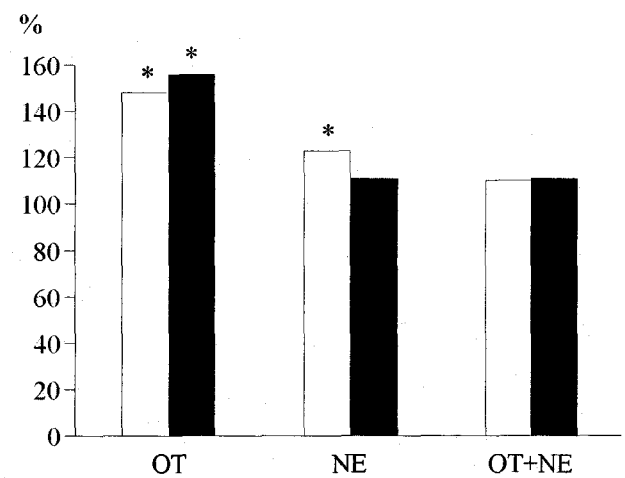

Fig. 2. Changes in functional state of thyrocytes after incubation with oxytocin, norepinephrine, or both neurohormones (compared with the control, 100%): height of thyrocytes (light bars) and mean number of silver grains above the thyrocyte after incubation with ^{3}H-leucine for 60 min (dark bars).

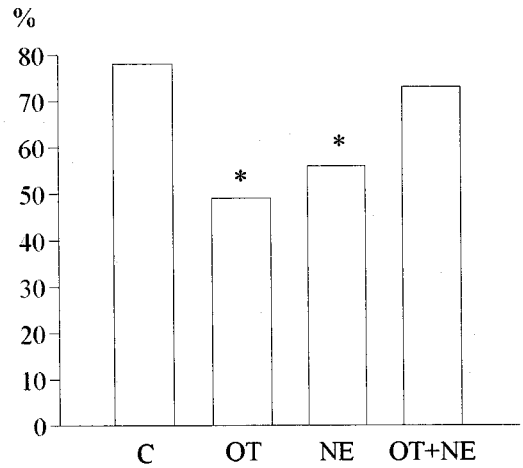

Fig. 3. Number of silver grains above the thyrocyte in thyroid gland fragments transferred from ^{3}H-leucine-containing to ^{3}H-leucine-free medium (total incubation time 60 min) compared with that after 60-min incubation in ^{3}H-leucine-containing medium (100%).

the height of thyrocytes and the intensity of ^{3}H-leucine incorporation observed 60 min after simultaneous addition of OT and NE were lower compared with those noted under the effect of OT alone (Fig. 2). In TG fragments transferred from ^{3}H-leucine-containing to ^{3}H-leucine-free media, the release of thyroglobulin from thyrocytes during combined action of OT and NE was lower than that induced by OT (Fig. 3).

In vitro experiments showed that thyroid-stimulating effects of OT were realized during its direct incorporation into thyrocytes. Combined action of OT and NE on thyrocytes markedly attenuated OT-induced activation of the synthesis and release of thyroglobulin. Interactions of NE and OT in thyrocytes probably proceeding after cAMP formation [10] remain to be studied. These mechanisms are similar to the inhibitory effects of NE on thyrotropin-stimulated thyrocytes [9,11].

The data suggest that under conditions simulating the stress-induced rise in blood contents of OT and NE, these hormones directly affect thyrocytes [2].

This work was supported by the Russian Foundation for Basic Research (grant No. 98-04-49921).

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