Effect of Thyroliberin on Nonapeptidergic Cells in Cultured Rat Hypothalamic Slices

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 5, pp. 526-528, May, 1999
Original article submitted April 16, 1998

The direct effect of thyroliberin on nonapeptidergic cells of the supraoptic and paraventricular nuclei was studied on cultured rat hypothalamic slices. Incorporation of labeled thyroliberin into vasopressinergic and oxytocinergic cells and a decrease in functional activity of these cells were observed in both hypothalamic nuclei.

Key Words: *thyroliberin; vasopressin; oxytocin; hypothalamus; paraventricular nucleus; supraoptic nucleus*

Most peptide hypothalamic hormones act as neurohormones affecting the target cells via blood and liquor and as neurotransmitters, *i.e.* affect other nerve cells (including those producing neurohormones) via the corresponding synapses [7]. There is evidence on the presence of corticoliberinergic synapses of cells of the paraventricular hypothalamic nucleus (PVN) that produce thyroliberin (TRH) [12]. Somatostatin- and somatoliberin-producing cells of the arcuate nucleus also interact via synaptic connections [11].

The interaction between the cells producing releasing-hormones, in particular between TRH-cells and nonapeptidergic cells in the hypothalamic PVN and supraoptic nucleus (SON) remains unclear. The existence of such connections is confirmed by the thyreostimulating effect of vasopressin and oxytocin [2-4] and by the effect of TRH on the functional state of PVN and SON cells *in vivo* [1,9]. However, the data obtained *in vivo* provide little information on the mechanism of TRH action on nonapeptidergic cells. Our aim was to carry out a morphofunctional study of vasopressin- and oxytocinergic cells in isolated hypothalamic slices exposed to TRH.

MATERIALS AND METHODS

The study was carried out on mature male Wistar rats weighing 120-140 g kept under standard vivarium conditions. After decapitation, the brain was rapidly removed and 400-μ slices containing SON and/or PVN were prepared from the hypothalamic area. The slices were preincubated in Earle's medium aerated with carbogen (95% O_2+5% CO_2) at 37°C. Preincubation was performed in 3 portions of the growth medium (30 min in each portion).

In the first experimental series we studied incorporation of ^{3}H-TRH into neurosecretory cells of PVN and SON. After preincubation, the hypothalamic slices were transferred to the growth medium with ^{3}H-TRH (1.75×10^{-8}M, 1.85×10^6 Bq/mol, NEN Product) and incubated for 15 and 30 min (the medium was changed every 15 min).

The slices were fixed in picric acid and formalin (3:5) and processed by standard histological methods. Serial sections (6 μ) were used in immunohistochemical PAP-reaction performed with nonlabeled antibodies to oxytocin. The sections were covered with an M-type photoemulsion (KhimFotoProekt, Moscow), exposed for 6 weeks, and developed in 2,4-diaminophenol dihydrochloride. Binding of ^{3}H-TRH in PVN and SON cells was accesses by the number of silver grains concentrated above one cell (30 vasopressin- and 30 oxytocinergic cells for each rat). The number of grains in the nervous tissue near the neurosecretory centers in the standard area was taken as the baseline value.

In the second experimental series we studied the effect of TRH on the functional state of vasopressin- and oxytocinergic cells in PVN and SON. The slices were incubated for 30 or 60 min in the medium with

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0007-4888/99/0005-0474$22.00

10^{-8} M TRH (experiment) or in a hormone-free medium (control). The media were changed every 15 min. All slices were fixed and processed by standard histological methods as in the first experimental series. Immunohistochemical PAP-reaction was performed on parallel sections using unlabeled antivasopressin and anti-oxytocin antibodies (a gift of Prof. G. Alonso, INSERM, Techniques Science University, France). Functional activity of PVN and SON cells was assessed by measuring the diameter of nucleoli with an ocular-micrometer at a magnification of 90×15×2.5; the volume was calculated by the formula for sphere volume. The results were analyzed statistically using Student's *t* test.

RESULTS

In series I, when the sections were incubated with ^{3}H-TRH, autoradiography revealed silver grains above the cytoplasm and nucleus of vasopressinergic cells only. Incorporation of ^{3}H-TRH was observed in both hypothalamic nuclei after 15-min incubation (Fig. 1) and increased during following incubation.

In oxytocinergic cells of the PVN and SON, the number of grains did not significantly differ from the baseline after 15- and 30-min incubation with ^{3}H-TRH.

In series II, nonapeptidergic cells of the PVN and SON and their nuclei maintained round shape after incubation with TRH. The nucleoli had regular spherical shape; the cytoplasm and fragments of neurosecretory cell filaments were filled with immunoreactive substance.

In PVN, the volume of nucleoli in vasopressin- and oxytocinergic cells decreased significantly after 30- and 60-min incubation with TRH (Fig. 2).

In SON, the volume of nucleoli in vasopressinergic cells decreased to 85 and 49% ($p<0.01$) after 30- and 60-min incubation with TRH, respectively (Fig. 2). The volume of nucleoli in oxytocinergic cells decreased to 73 and 65% ($p<0.05$), respectively (Fig. 2). Therefore, the inhibitory effect of TRH on nucleolus size, which reflects the intensity of protein synthesis, increased with prolongation of incubation.

The radioautography data indicate that labeled TRH was incorporated only in vasopressin- but not in oxytocinergic cells of SON and PVN. It may be considered as an indirect indication on the existence of TRH receptors in vasopressinergic cells. It can be hypothesized that TRH produces a direct neurohormonal effect on vasopressinergic cells in the PVN and SON. This hypothesis agrees with evidence on the existence of TRH-receptors in nerve cells of the hypothalamus [8,13,14]. TRH inhibits protein synthesis in vasopressinergic cells, which is manifested in a rapid decrease in the size of their nucleoli and indirectly supported by the data on a drop of blood vasopressin after TRH injection [1,9].

TRH also reduced functional activity of oxytocinergic cells (a decrease in nucleolus size). However, autoradiography revealed no incorporation of labeled TRH into oxytocinergic cells. It remains unclear how TRH affects functional activity of these cells. Presumably, the reaction of oxytocinergic cells to TRH was mediated by some TRH-sensitive cell elements. On the other hand, TRH can affect oxytocinergic cells not as a neurohormone, but as a neurotransmitter. This hypothesis agrees with our data on the absence of ^{3}H-TRH incorporation into the cytoplasm of these cells, as well as with the evidence on the decrease of TRH influence on the nonapeptidergic cells during *in vitro* blockade of synaptic transmission in PVN [15].

It cannot be ruled out that integration of reaction of oxytocin- and vasopressinergic cells is effected via

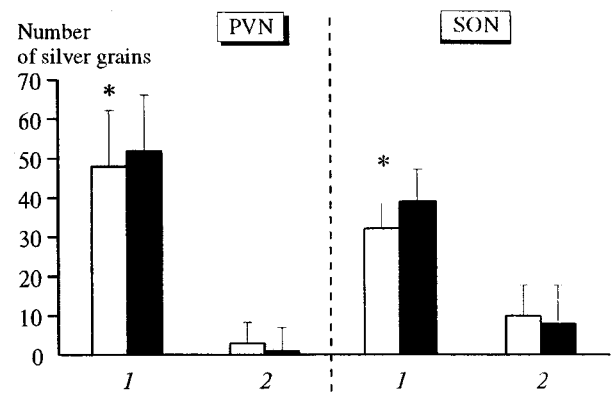

Fig. 1. Number of silver grains in nonapeptidergic cells of paraventricular (PVN) and supraoptic (SON) nuclei after 15- (light bars) and 30-min (solid bars) *in vitro* incubation with labeled thyroliberin. *$p<0.05$ compared to baseline values. Here and in Fig. 2: *1*) vasopressinergic cells; *2*) oxytocinergic cells.

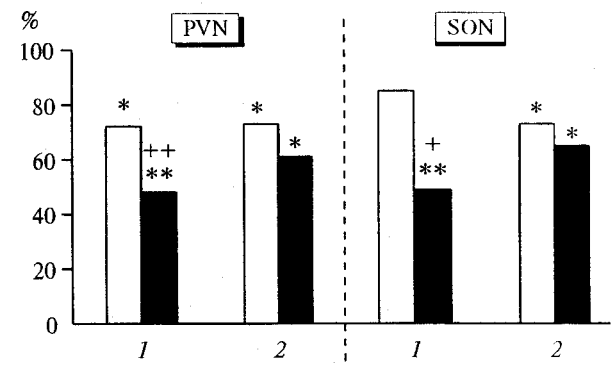

Fig. 2. Changes of nucleolus volume in nonapeptidergic cells from paraventricular (PVN) and supraoptic (SON) nuclei after 30- (light bars) and 60-min (solid bars) incubation with thyroliberin. *$p<0.05$, **$p<0.01$ compared to control (100%). +$p<0.05$, ++$p<0.01$ compared to 30-min incubation.

interneurons and intranuclear connections between PVN and SON [6] even *in vitro, i.e.* without participation of other physiological systems. The *in vitro* conditions determine the unidirectional response of vasopressin- and oxytocinergic cells to stimulation, while in the whole organism their reactions sometimes differ under various influences [10] and depend on the thyroid status [3,5], which is probably related to different afferentation of these cells [6,10].

Therefore, *in vitro* experiments showed that the direct action of TRH on nonapeptidergic cells induces a unidirectional reaction of vasopressin- and oxytocinergic cells in PVN and SON of rat hypothalamus; however, the mechanisms of the effect of this neurohormone on these cells are different.

REFERENCES

1. M. V. Glazova and I. A. Krasnovskaya, *Ros. Fiziol. Zh.*, **82**, No. 4, 65-69 (1996).
2. G. V. Dityateva, I. A. Krasnovskaya, and V. I. Skopicheva, *Byull. Eksp. Biol. Med.*, **110**, No. 10, 423-425 (1990).
3. I. A. Krasnovskaya, in: *Neuroendocrinology*, Pt. 2 [in Russian], St. Petersburg (1994), pp. 49-74.
4. I. A. Krasnovskaya, M. V. Glazova, D. M. Makina, and L. S. Voropanova, *Zh. Evoluts. Biokhim.*, **33**, No. 2, 205-211 (1997).
5. I. A. Krasnovskaya, G. V. Dityateva, and A. L. Polenov, *Probl. Endokrinol.*, **35**, No. 2, 67-70 (1989).
6. E. Lutsik, in: *Neuroendocrinology*, Vol. 2, Pt. 1 [in Russian], St. Petersburg (1993), pp. 270-299.
7. A. L. Polenov, M. S. Konstantinova, and P. E. Garlov, in: *Neuroendocrinology* [in Russian], Pt. 1, Vol. 1, St. Petersburg (1993), pp. 139-187.
8. L. Calza, L. Giardino, S. Ceccatelli, *et al.*, *Neuroscience*, **51**, No. 4, 891-909 (1992).
9. J. Ciosek, J. W. Guzec, and M. Orlowska-Majdak, *J. Physiol. Pharmacol.*, **44**, No. 3, 293-302 (1993).
10. T. A. Day, D. W. Smith, and K. M. Buller, in: *Neurohypophysis: Recent Progress of Vasopressin and Oxytocin Research*, Amsterdam (1995), pp. 215-225.
11. J. Epelbaum, E. Moyse, G. S. Tannenbaum, *et al.*, *J. Neuroendocrinology*, **1**, 109-115 (1989).
12. S. Hisano, Y. Fukui, M. Chikamori-Aoyama, *et al.*, *Brain. Res.*, **620**, No. 2, 343-346 (1993).
13. T. Satoh, P. Feng, U. J. Kim, and J. F. Wilber, *Brain Res. Mol. Brain Res.*, **19**, No. 1-2, 175-178 (1993).
14. T. L. Stanton, S. B. Caine, and A. Winokur, *Brain Res. Bull.*, **28**, No. 6, 877-886 (1992).
15. H. Yamashita, Y. Hattori, and M. Kasai, *Neurosci. Res. Suppl.*, No. 9, 66 (1989).