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Thyrotropin-releasing hormone (TRH) inhibits vasopressin and oxytocin release from rat hypothalamo-neurohypophysial explants *in vitro*

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Abstract. Incubation of hypothalamo-neurohypophysial explants in Locke's solution containing 28 nM/L thyrotropin-releasing hormone (TRH) resulted in an inhibition of vasopressin and oxytocin secretion during depolarization due to excess potassium. These data suggest the involvement of TRH in the regulatory mechanisms of vasopressin and oxytocin release; the inhibitory effect of TRH cannot be excluded.

Key words: thyrotropin-releasing hormone, vasopressin, oxytocin

INTRODUCTION

Thyrotropin-releasing hormone (TRH; p-Glu-α--His-α-Pro-NH_2) is the hypothalamic peptide that regulates the synthesis and secretion of thyrotropin and prolactin from the anterior pituitary gland (Murdoch et al. 1983, Carr et al. 1989). TRH has been shown to be involved in a number of various endocrine and biological functions in the central nervous system, acting as a neurotransmitter or neuromodulator (Griffiths 1985, Reichlin 1986, Siren and Feuerstein 1987). Much of the hypothalamic TRH is synthesized in the parvocellular subdivision of the paraventricular nucleus (Lechan and Jackson 1986, Liao et al. 1988) and transported *via* anterolateral and medial pathways to the median eminence to regulate anterior pituitary thyrotropin and prolactin secretion. Moreover, the high concentration of radioimmunoassayable TRH as well as many TRH-immunoreactive fibres have been found in the posterior pituitary (Jackson and Reichlin 1974, Lechan and Jackson 1982).

TRH has been reported to regulate vasopressin and oxytocin release (Horita and Carino 1977, Weitzman et al. 1979). Other authors, however, noted that neither oxytocin nor vasopressin was released following i.v. TRH injection in rats (Kasting 1988, Siren et al. 1988) and humans (Amico and Johnston 1985). We reported recently that intracerebroventricular (i.c.v.) infusions of TRH increased significantly the hypothalamo-neurohypophysial vasopressin content in dehydrated, haemorrhaged or salt-loaded rats (Ciosek and Guzek 1992, Ciosek et al. 1993a,b, Ciosek and Orłowska-Majdak 1995) but decreased the hypothalamo-neurohypophysial oxytocin content in haemorrhaged rats or in rats drinking hypertonic saline (Ciosek and Orłowska-Majdak 1995, Ciosek and Stempniak 1995).

In this study the influence of TRH on vasopressin and oxytocin release from the hypothalamo-neurohypophysial explants *in vitro* was evaluated.

METHODS

Animals

Fourty four adult male Wistar rats weighing 240 - 275 g served as donors of hypothalamo-neurohypophysial explants. They were fed normal pelleted laboratory diet and kept at room temperature about +20° C. A 14-h light, 10-h dark cycle was provided (artificial illumination 6.00 a.m. - 8.00 p.m.). Before experiments they were given tap water *ad libitum.*

Experimental design

The vasopressin and oxytocin release from the hypothalamo-neurohypophysial explants was estimated *in vitro,* TRH being added to the incubation media (L_3 and L_4; see below). In this experiment 10 incubation procedures using media not containing TRH (group A) as well as 11 incubation procedures using media enriched with TRH (group B) were included.

Experimental procedure

For each experiment *in vitro*, two hypothalamo-neurohypophysial explants were pooled. The animals were killed by decapitation. The explants were removed from the base of the brain within 3 min of decapitation and consisted of a 1.5-2.0 mm thick piece of hypothalamic tissue extending from the front of the optic chiasm to the mammillary bodies including the intact pituitary stalk and neural lobe of the pituitary with traces of the surrounding intermediate lobe. The single explant was approximately 5 mm wide and weighed approximately 35 mg. This preparation included the intact supraoptic nuclei with their axonal projections extending through the median eminence and terminating in the neural lobe (Sladek and Knigge 1977). Two explants were removed and immediately transferred to an incubation chamber containing 2 ml Locke's solution (L_1) at +37° C, constantly bubbled with O_2 and CO_2 (95% : 5%). The incubation chamber was washed twice and the solution was then changed every 10 min; next four periods (=40 min) being intended for equilibration only, these samples of incubation fluid were discharged. In series A, the explants were thereafter incubated for another 30 min (3 x 10 min; fractions 1-3) in Locke's solution (L_1) and then for 20 min (2 x 10 min; fractions 4-5) in the medium (L_2) containing 10-fold higher (i.e., 56 mM/L) con-

centration of potassium chloride (see below). Following potassium stimulation the explants were incubated for three further 10 min periods (=30 min; fractions 6-8) in normal Locke's solution (L_1). In series B, the solutions L_3 and L_4 containing TRH instead of solutions L_1 and L_2 were used.

The incubation programme is shown in Figs. 1-2.

Incubation media

The following incubation media, freshly prepared for each experiment, were used:
L_1 - modified Locke's medium, containing (mM/L): NaCl - 154; KCl - 5.6; $CaCl_2$ - 2.2; $MgCl_2$ - 1.0; $NaHCO_3$ - 6.0 and glucose - 10.0 (Douglas and Poisner 1964);
L_2 - Locke's medium (as L_1) containing 56 mM/L KCl; in order to maintain unchanged the osmolality NaCl concentration was appropriately reduced as recommended by Douglas and Poisner (1964);
L_3 - Locke's medium (as L_1) containing in addition 28 nM/L TRH (Sheppard and Shennan 1984);
L_4 - Locke's medium containing 56 mM/L KCl and additionally enriched with 28 nMol/L TRH.

The pH was controlled for each incubation fluid and was found within a range 7.36-7.50, 7.35-7.50, 7.32-7.61 and 7.40-7.45 for the media L_1 , L_2 , L_3 and L_4 , respectively. Osmolality (Knauer's semi-microosmometer: Halbmikro-Osmometer, Dr. Herbert Knauer, Wissenschaftliche Geräte KG, Berlin) was within a range of 282 -286 mOsm/Kg H_2O; 280 - 285 mOsm/Kg H_2O; 283 -287 mOsm/Kg H_2O and 280 - 284 mOsm/Kg H_2O for the media L_1, L_2, L_3 and L_4 , respectively.

Radioimmunoassay of vasopressin and oxytocin

Before radioimmunoassay (RIA), the incubation media were stored at -23° C; the assays were completed within at most two days. All specimens from individual experiments were measured in duplicate in the same assay.

CHARACTERISTICS OF ANTISERA

The antibody titer to be used in the RIA was 1:24,000 for anti-AVP and 1:80000 for anti-OT (both final dilutions). Antiserum to arginine vasopressin [Arg^8]AVP, purchased from Amersham International, was raised in rabbits against AVP covalently conjugated to bovine thyroglobulin. Cross-reaction with other related peptides, including oxytocin, was less than 1%. Cross reactivity with AVP for anti-OT antibodies (Amersham International) was 1.12%; with LH-RH, TRH, Leu-Enk and Ang II was <0.002%.

IODINATION OF VASOPRESSIN AND OXYTOCIN

Oxytocin (Peninsula Lab., No 027179) as well as arginine vasopressin (Peninsula Lab., No 032179) were iodinated with ^{125}I using the chloramine T-method (Greenwood et al. 1963). Unreacted iodide was removed by mixing the reaction mixture with Amberlite (Ion Exchange Resin, type CG-400). Further purification was carried out on a column with 0.05 M/L acetic acid. Labelled AVP and OT were identified in the third peak by their ability to bind to the corresponding antibodies (Landgraf 1981). The effectiveness of the iodination procedure was 70-90%. The top or the 1-st descending portion of this peak was used as the tracer in RIA. Labelled hormones retained their antibody bindability for up to four weeks.

The lower limits of detection for the AVP and OT assay was 1.25 pg per tube with intra- and inter-assay coefficient of variation of 3.7% and 6.3% for OT and 2.0% and 8.3% for AVP, respectively.

Statistical evaluation of the results

The neurohormonal release into the incubation fluid was finally expressed in nanograms for each 10 min period and two hypothalamo-neurohypophysial explants. All findings are reported as mean ± standard error of the mean (SEM). For statistical evaluation Student's "*t*" test was used.

RESULTS

Incubation of hypothalamo-neurohypophysial explants in Locke's solution containing TRH did

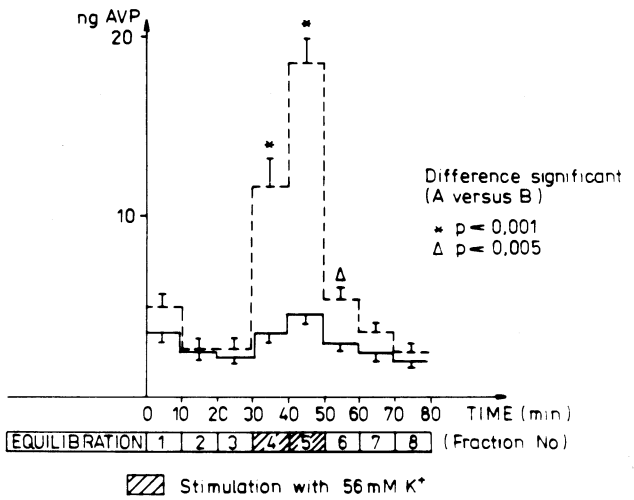

Fig. 1. Effect of TRH on the vasopressin release from isolated hypothalamo-neurohypophysial explants (HNE) *in vitro* (ng/2HNE/10 min); (mean ± SEM) — A, controls; B, —28 nM/L TRH.

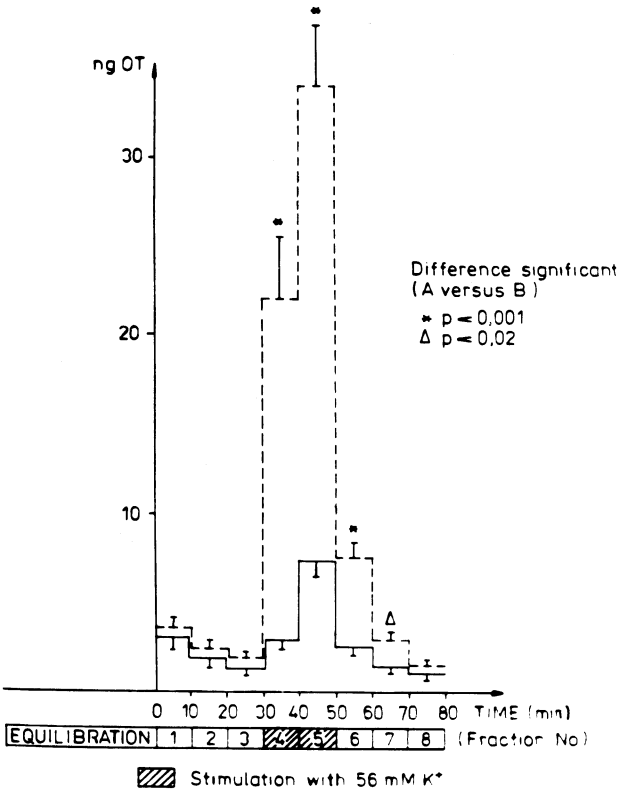

Fig. 2. Effect of TRH on the oxytocin release from isolated hypothalamo-neurohypohysial explants (HNE) *in vitro* (ng/2HNE/10 min); (mean ± SEM) — A, controls; B, —28 nM/L TRH .

not result in any significant change of AVP and OT release during basal incubation (Figs. 1 and 2: fractions 1-3). Incubation in Locke's solution enriched with TRH resulted in a strong decrease of both AVP and OT release during stimulation with excess potassium (Fig. 1: fractions 4-5: t_{19}=4.9390 and t_{19}=9.3464: P<0.001; Fig. 2: fractions 4-5: t_{19}=5.6140 and t_{19}=6.8205: P<0.001). During subsequent incubation in normal Locke's solution, treatment with TRH decreased the AVP as well as OT release (for AVP: Fig. 1: fraction 6: t_{19}=3.3340, P<0.005; for OT: Fig. 2: fractions 6-7: t_{19}=4.7000 and t_{19}=2.6596, P<0.001 and P<0.02, respectively).

DISCUSSION

The present study showed that TRH decreased the release of vasopressin as well as oxytocin from the hypothalamo-neurohypophysial system *in vitro*. These data seem to be only partly consistent with our previous findings *in vivo:* i.c.v. administration of TRH significantly increased the vasopressin content in the hypothalamus and neurohypophysis of the dehydrated, haemorrhaged or salt-loaded rats but decreased that of hypothalamo-neurohypophysial oxytocin under the same experimental conditions (Ciosek and Guzek 1992, Ciosek et al. 1993a,b, Ciosek and Orłowska-Majdak 1995, Ciosek and Stempniak 1995). It should be interjected, however, that the function of neurosecretory neurones *in vivo* is related to manifold influences connected with hypothalamic afferentation; these processes being apparently excluded by the method *in vitro.*

The mechanisms of effects exerted by TRH under conditions of this experiment are not clear. Earlier studies suggested that TRH was an inhibitory neuromodulator within the cerebral cortex (Renaud et al. 1979a,b). Other studies, on the contrary, indicated that it served as an excitatory transmitter on the spinal motor neurones (Winokur and Beckman 1978). What is more, it has been proposed that TRH acts on the brain neurones, changes in synthesis and secretion of dopamine being an intermediary event. It has namely been noted that TRH increased the rate of dopamine release from the brain slices (Maeda and Frohman 1980). The con-

tent of homovanillic acid (degradation product of dopamine) was shown to increase in the brain following treatment with TRH (Narumi and Nagawa 1983). Moreover, some authors suggest that TRH may be involved in the regulation of sympathetic outflow: Brown (1981) found that the concentrations of norepinephrine and epinephrine were increased in blood plasma after i.c.v. administration of TRH. Noradrenaline, in turn, is thought to inhibit the vasopressin and oxytocin release (Sklar and Schrier 1983), both *in vitro* (Yeo et al. 1972, Armstrong et al. 1983) and *in vivo* (Kimura et al. 1980). On the other hand, the presence of TRH-immunoreactive neuronal cells and fibres in the hypothalamus and in the neurohypophysis gives the possibility that TRH may act on vasopressin and oxytocin biosynthesis and release immediately, i.e., without any intermediating synaptic mediators. TRH has been postulated to have a physiological role through its action directly on axon terminals of magnocellular neurones in the posterior pituitary lobe (Lechan and Jackson 1982). However, there are no data so far whether immunoreactive-TRH fibres terminate in the posterior pituitary on endothelium, other axons, or supporting cells.

CONCLUDING REMARKS

These data suggest the involvement of TRH in the regulatory mechanisms of vasopressin and oxytocin release; the inhibitory effect of TRH on the vasopressinergic and oxytocinergic neurones cannot be excluded.

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