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THYROTROPIN-RELEASING HORMONE (TRH) MODULATES VASOPRESSIN AND OXYTOCIN RELEASE FROM THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM IN DEHYDRATED RATS*

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Rats euhydrated and dehydrated during two or four days were given intracerebroventricularly (i.c.v.) thyrotropin-releasing hormone (TRH) in a daily dose of 200 ng dissolved in 10 µl of 0.9% sodium chloride.
A single dose of TRH injected to euhydrated animals increased the hypothalamic vasopressin content but did not affect significantly the content of vasopressin in the neurohypophysis as well as that of oxytocin both in the hypothalamus and neurohypophysis.
In rats deprived of water for two days TRH completely prevented the decrease of neurohypophysial oxytocin due to stimulation of osmoreceptor origin. Similarly, TRH restrained both the hypothalamic and the neurohypophysial vasopressin and oxytocin depletion in rats dehydrated for four days.

Key words: *thyrotropin-releasing hormone (TRH), vasopressin, oxytocin, dehydration*

INTRODUCTION

Thyrotropin-releasing hormone (TRH) is a hypothalamic tripeptide (pGlu-His-Pro-NH_2) engaged in regulation of pituitary thyrotropin and prolactin synthesis and release (1, 2). TRH, localized in the brain also at several extrahypothalamic regions, is known to be involved in some regulatory events different from its TSH-releasing properties. TRH is namely thought to act as a neurotransmitter or neuromodulator (3, 4).

There are some reports confirming the effect of TRH on the hypothalamo-neurohypophysial system. TRH was shown to increase the

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vasopressin and oxytocin level in blood plasma in the rabbit (5, 6). TRH was also reported to inhibit water intake in rats (7). Nevertheless, no change in the oxytocin or vasopressin release has been noted following intravenous TRH administration in rats (8, 9).

In the present study we looked for the possible effect of TRH on the vasopressin and oxytocin content in the hypothalamo-neurohypophysial system of euhydrated and dehydrated rats.

MATERIAL AND METHODS

Animals

Male Wistar rats, weighing 248 ± 21 g (mean ± S.D.), were used. They received standard pelleted chow and were housed at room temperature of about +20°C. A 14-h light, 10-h dark cycle was provided (artificial illumination 6.00 a.m. — 8.00 p.m.). All animals had free access to tap water until the beginning of the experiment.

General experimental design

Complete experimental protocol was followed in 52 animals divided into two groups (see *Tables 1—3*) A — animals injected intracerebro-ventricularly (i.c.v.) with 0.9% sodium chloride in a daily dose of 10 µl ; B — animals injected i.c.v. with thyrotropine-releasing hormone (TRH) (synthetized by Institute of Chemistry, University of Gdańsk, Poland) in a daily dose of 200 ng dissolved in 10µl of 0.9% sodium chloride.

In each group three further experimental subgroups, 8—9 animals each, were set up: I — controls not dehydrated; II—III — animals dehydrated for two or four days, respectively. The animals of subgroup B–I were decapitated 3 hours after a single i.c.v. injection of TRH. The rats in subgroups B–II — B–III were killed after two or four days of water deprivation, respectively. In subgroups B–II — B–III the last injection of TRH was given 3 hours before killing.

The animals of corresponding subgroups in group A were sacrificed in similar manner (subgroup A–I: controls not dehydrated; subgroups A–II — A–III: animals dehydrated, untreated).

Surgical preparation

The implantation of intracerebro-ventricular cannulae for chronic injection was performed under light hexobarbital anaesthesia (i.v. injections of 7% solution of Hexobarbital Natrium: 0.15 ml/100 g b.w.). The animals were immobilized in a simple stereotaxic apparatus as recommended by Noble et al. (10) and a simple stainless steel cannula was inserted into the left lateral cerebral ventricle. After surgery, the animals were allowed to recover for up to five days before starting the experimental protocol. The i.c.v. infusions were made to previously trained conscious rats; a 50 µl Hamilton syringe with plunger pushed by a microscrew was used. The duration of the infusion was about 15—20 sec.

The effectiveness of i.c.v. infusions was verified by injecting 10—15 µl of 0.25 per cent trypan blue to similarly operated separate control animals (one rat injected with trypan blue solution for

every five injected with drug solution or 0.9% sodium chloride) and was found to be quite satisfactory, i.e., the dye was distributed in an uniform manner within all cerebral ventricles.

Experimental procedure

The rats were weighed and killed by decapitation at 10.30—11.30 a.m. Mixed arterial-venous blood from the trunk was preserved for evaluation of serum osmolality or collected in heparinized capillaries for determination of the haematocrite index. Serum osmolality was estimated using a Knauer semimicroosmometer (Halbmikro-Osmometer, Dr. Herbert Knauer, Wissenschaftliche Geräte KG, Berlin).

The brain with intact pituitary was quickly removed, the infundibular stalk cut up under a stereomicroscope and the neurohypophysis was separated. From the brain, hardened in the freezer, the hypothalamic block was dissected as follows: rostral limit — frontal plane situated about 1 mm more anteriorly than the anterior margin of the optic chiasma; caudal limit — frontal plane just behind the mammilary bodies; lateral limits — sagittal planes on both sides through the hypothalamic fissures. The depth was about 2.0—2.5 mm from the base of the brain. The net weight of such block of the brain (containing the hypothalamus and a part of thalamus) was 42.3 ± 1.9 mg (mean $\pm$ S.D.).

The neurohypophysis was homogenized in 1.0 ml of 0.25% acetic acid in 0.9% saline, the tissue suspension was transferred into a centrifuge tube and the homogenizer washed with 1.0 ml of the same solution. The pooled sample was heated for 5 min on boiling water bath, centrifuged for 30 min (room temperature; relative centrifugal force about 650 g, i.e. 6380 m/sec^2), the supernatant was removed and made up to a constant volume.

The hypothalamic extracts were prepared in a similar manner, except that the hypothalamic blocks were homogenized in 1.5 ml of 0.5 per cent acetic acid in 0.9 per cent saline. The extracts were stored at -10°C until radioimmunoassayed.

RADIOIMMUNOASSAY OF VASOPRESSIN AND OXYTOCIN

Immunization procedure.

Arginine vasopressin or oxytocin (synthesized in the Institute of Organic Chemistry, Technical University of Lodz) were conjugated with high-molecular ligands by the carbodiimide method (11).

Characteristics of antiserum.

The antibody titer to be used in the RIA was 1:24 000 for anti-AVP and 1:80 000 for anti-OT (both final dilutions). Cross reactivity with oxytocin for anti-AVP antibodies was 0.016%, with lysine vasopressin (LVP) — 2.7%; with luliberin (LH–RH), TRH, leucine enkephalin (Leu-Enk), angiotensin II (Ang II), substance P (SP), hexapeptide (pyr Glu^{6}Tyr^8)SP$_{6-11}$ and hexapeptide (Tyr^8)SP$_{6-11}$ it was $< 0.002\%$. Cross reactivity with AVP for anti-OT antibodies was 1.12%; with LH-RH, TRH, Leu-Enk and Ang II it was $< 0.002\%$. The sensitivity of anti-AVP serum was 1.73 pg AVP per tube and that of anti-OT serum — 3.56 pg OT per tube.

Iodination of arginine vasopressin and oxytocin.

Arginine vasopressin ((Arg^8)–Vasopressin, Peninsula Laboratories Europe Ltd. lot No 015907) and oxytocin (synthetized by Department of Organic Chemistry, Technical University of Łodź, Poland) were iodinated with ^{125}I using the chloramine-T method (12). 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 of Sephadex G-25 fine pre-equilibrated and eluated with 0.05 mol/l acetic acid. Labeled AVP and OT were identified in the third peak by their ability to bind to the corresponding antibodies (13). The effectiveness of the iodination procedure was 70%—90%. The top or the 1st descending portion of this peak was used as the tracer in RIA. Labeled hormones retained their antibody bindability for up to four weeks.

Statistical evaluation of the results.

The vasopressin or oxytocin content was finally expressed in nanograms for whole hypothalamus or neurohypophysis. All findings are reported as mean ± standard error of the mean (S. E. M.). For statistical evaluations Student's "t" test was used.

RESULTS

Validation of the dehydration degree (Table 1).

Under conditions of water deprivation, progressive increase of both haematocrite index and serum osmolality (on an average, up to about 51.9% and 295.3 mOsm/Kg H_2O, respectively) was noted.

A single dose of TRH administered to euhydrated animals did not result in any significant change of both parameters in question. Following two or four days of dehydration, however, serum osmolality was more marked in animals treated with TRH.

The effect of TRH on the vasopressin content in hypothalamus and neurohypophysis (Table 2).

The hypothalamic vasopressin content decreased progressively in dehydrated rats, down to about 56% of the respective control value on the fourth day (subgroup A–III).

A single dose of TRH, administered to euhydrated animals, was followed by an increase of hypothalamic vasopressin content (subgroup B–I). TRH significantly restrained the vasopressin depletion in the hypothalamus of animals dehydrated over four days (subgroup B–III).

Table 1: Serum osmolality and haematocrite index in euhydrated and dehydrated rats (mean ± S.E.M.)

Subgroups of animals	Serum osmolality		Haematocrite index		Significance as estimated by Student's "t" test	
	Group A: animals untreated (a)	Group B: animals injected i.c.v. with TRH (b)	Grup A: animals untreated (c)	Group B: animals injected i.c.v. with TRH (d)	(a) versus (b)	(c) versus (d)
I — animals not dehydrated	278.4±3.9	281.0±2.5	43.8±0.7	45.7±0.9	NS	NS
II — two days of dehydration	288.4±1.6	294.9±1.5	49.9±0.9	51.2±0.8	$p < 0.02$	NS
III — four days of dehydration	295.3±1.2	313.9±2.0	51.9±0.8	50.3±0.4	$p < 0.001$	NS
Significance as estimated by Student's "t" test						
I versus II	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$		
II versus III	$p < 0.005$	$p < 0.001$	NS	NS		
I versus III	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$		

NS — not significant

The neurohypophysial vasopressin content in animals deprived of water diminished (down to about 35% of the respective control value) on the fourth day (subgroup A–III).

Under treatment with TRH the neurohypophysial vasopressin depletion (subgroup B–III) was significantly less marked when compared with dehydrated but not TRH-treated animals.

The effect of TRH on the oxytocin content in hypothalamus and neurohypophysis (Table 3).

Under conditions of dehydration the hypothalamic oxytocin content diminished progressively, down to about 54% of the respective control value on the fourth day (subgroup A–III).

Following four days of dehydration the decrease of hypothalamic oxytocin content was significantly less marked under treatment with TRH (subgroup B–III).

Table 2: The vasopressin (VP) content in the hypothalamus and neurohypophysis of euhydrated and dehydrated rats as influenced by TRH injected intracerebroventricularly; (ng per whole hypothalamus or neurohypophysis; mean ± S.E.M.)

Subgroups of animals	Hypothalamus			Neurohypophysis		
	Group A: animals injected i.c.v. with vehicle (normal saline)	Group B: animals injected i.c.v. with TRH	Significance as estimated by Student's "t" test	Group A: animals injected i.c.v. with vehicle (normal saline)	Group B: animals injected i.c.v. with TRH	Significance as estimated by Student's "t"
I — animals not dehydrated	116.4 ± 16.3	169.4 ± 11.9	$p < 0.02$	1182.6 ± 115.9	1210.2 ± 95.7	NS
II — two days of dehydration	109.2 ± 10.5	125.6 ± 11.2	NS	1026.2 ± 97.4	1288.4 ± 135.2	NS
III — four days of dehydration	65.7 ± 8.9	103.2 ± 13.3	$p < 0.05$	412.3 ± 69.8	1017.2 ± 57.7	$p < 0.001$
Significance as estimated by Student's "t" test						
I versus II	NS	$p < 0.01$		NS	NS	
II versus III	$p < 0.01$	NS		$p < 0.001$	NS	
I versus III	$p < 0.02$	$p < 0.005$		$p < 0.001$	NS	

NS — not significant

The neurohypophysial oxytocin content diminished progressively in dehydrated animals, down to about 63% of the respective control value on the fourth day (subgroup A–III).

In dehydrated animals, the depletion of the neurohypophysial oxytocin stores (as brought about by stimulation of osmoreceptors) was distinctly less marked under treatment with TRH (subgroups B–II and B–III).

Table 3: The oxytocin (OT) content in the hypothalamus and neurohypophysis of euhydrated and dehydrated rats as influenced by TRH injected intracerebroventricularly; (ng per whole hypothalamus or neurohypophysis; mean ± S.E.M.)

Subgroups of animals	Hypothalamus			Neurohypophysis		
	Group A: animals injected i.c.v. with vehicle (normal saline)	Group B: animals injected i.c.v. with TRH	Significance as estimated by Student's "t" test	Group A: animals injected i.c.v. with vehicle (normal saline)	Group B: animals injected i.c.v. with TRH	Significance as estimated by Student's "t"
I — animals not dehydrated	81.7 ± 12.5	75.1 ± 8.7	NS	1762.7 ± 108.2	1612.4 ± 105.3	NS
II — two days of dehydration	58.9 ± 5.1	66.2 ± 6.6	NS	1573.5 ± 46.5	2412.9 ± 241.4	$p < 0.005$
III — four days of dehydration	44.1 ± 3.5	62.8 ± 4.2	$p < 0.005$	1107.2 ± 125.2	1880.2 ± 118.5	$p < 0.001$
Significance as estimated by Student's "t" test						
I versus II	NS	NS		NS	$p < 0.02$	
II versus III	$p < 0.05$	NS		$p < 0.005$	NS	
I versus III	$p < 0.02$	NS		$p < 0.005$	NS	

NS — not significant

DISCUSSION

The vasopressin and oxytocin content in the hypothalamo-neurohypophysial system of dehydrated rats.

Synthesis, axonal transport and release of vasopressin are increased under conditions of dehydration (for references see: 14, 15). Moreover, electrical activity of the supraoptic and paraventricular neurones is known to intensify under such conditions (16, 17). Similarly, the synthesis and release of oxytocin as well as the firing of oxytocinergic neurones are known to be enhanced under deprivation of water in the rat (17, 18).

Under conditions of dehydration the stores of both vasopressin and oxytocin are depleted in the hypothalamus and neurohypophysis; the

synthesis and axonal transport — although increased — being probably too poor to compensate the hormonal quantities released into the blood. In this respect, the present results are consistent with earlier findings (19, 20).

The vasopressin and oxytocin content in the hypothalamus and neurohypophysis as influenced by TRH.

As above mentioned, there are some data supporting the concept of a modulating role for TRH in the mechanisms related to release of neurohypophysial hormones (5, 6). In present experiments, a single dose of TRH, administered i.c.v. to euhydrated animals, resulted in an increase of hypothalamic vasopressin content: it may be assumed that the vasopressin transport from the hypothalamus towards neurohypophysis was then rather impaired. Similarly, the repeated treatment with TRH restrained both the hypothalamic and the neurohypophysial vasopressin depletion in rats dehydrated for four days. The present data are not consistent with earlier work of Weitzman et al. (6) as well as with that of Horita and Carino (5), both carried out on rabbits. Thus, species variability may be here of some importance. The present results seem to support previous observation by Sowers et al. (21), who reported that TRH decreased the plasma vasopressin level in man. Kasting (8) and Siren et al. (9) noted that TRH does not affect the vasopressin and oxytocin release in euhydrated rat. Under conditions of hyperosmotic dehydration, however, the depletion of vasopressin in the hypothalamus and neurohypophysis, as brought about by afferentation of osmoreceptor origin, was distinctly less marked in TRH-treated animals (this experiment).

The present data show that oxytocinergic neurones respond to TRH in the same way as the vasopressinergic do. Yet, under conditions of dehydration the depletion of hypothalamic and neurohypophysial oxytocin content was distinctly less marked in animals treated with TRH.

The present data do not allow to say which is the mechanism for the events in question. On hypothalamic level, the changes of actual neurohormonal content may be commented in terms of increased hormonal synthesis and/or impaired infundibular transport of vasopressin and oxytocin from the hypothalamus towards neurohypophysis. The actual content of both neurohypophysial hormones in the neurohypophysis is a resultant of the supply of infundibular origin as well as of their release into the blood (most probably, the latter seemed to decrease under conditions of this experiment).

It may be hypothesized that the site for TRH action on the afferents of vasopressinergic or oxytocinergic neurones is localized directly on paraventricular or supraoptic cells and/or at some TRH-susceptile junctions involved in the neural chains of respective afferents.

Concluding remarks.

It may supposed that TRH is of some importance for the function of vasopressinergic and oxytocinergic neurones, both under conditions of equilibrated water metabolism and during dehydration.

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