The aim of the present investigations was to examine the effects of the states of hypothyroidism or hyperthyroidism on vasopressin (AVP) and oxytocin (OT) release under conditions of equilibrated water metabolism as well as of osmotic stimulation, brought about by the dehydration or hypertonic saline administration. The euhydrated and simultaneously hypothyroid rats showed decreased hypothalamic AVP and OT content and somewhat higher but not significant neurohypophysial AVP content. In these animals the raised OT (but not AVP) plasma level has been observed. In hyperthyroid rats drinking tap water ad libitum the neurohypophysial AVP and OT content significantly diminished; plasma OT concentration (but not AVP) was then elevated. The state of osmotic stimulation was the reason of different response of the hypothalamo-neurohypophysial system function in hypo- or hyperthyroid rats. Significant decreases of neurohypophysial AVP and OT content were found in both hypothyroid dehydrated as well as hypothyroid hypertonic saline-treatment rats as compared with hypothyroid euhydrated ones. On the contrary, in the state of hyperthyroidism AVP content in the neurohypophysis distinctly raised in dehydrated and salt-loaded rats; in these last neurohypophysial OT content increased as well. Plasma OT (but not AVP) distinctly diminished in hyperthyroid and simultaneously dehydrated or hypertonic saline injected rats in relation to hyperthyroid control subgroup. Data from the present study suggest that: 1) altered thyroid gland function affects vasopressin and oxytocin release from the hypothalamo-neurohypophysial system in the state of equilibrated water metabolism; 2) the state of hypo- or hyperthyroidism modifies the response of AVP-ergic and OT-ergic neurons upon the osmoreceptors/osmodetectors stimulation. It may be supposed that OT-ergic neurons display greater than AVP-ergic neurons sensitivity upon the thyroid hormone influence.

Key words: vasopressin, oxytocin, hypothyroidism, hyperthyroidism, osmotic disturbances
INTRODUCTION

Vasopressin (AVP) and oxytocin (OT), synthesized in the magnocellular neurons (MCNs) of the supraoptic (SON) and paraventricular (PVN) hypothalamic nuclei, are transported to the posterior pituitary and then released into the blood.

Different stimuli of osmotic or nonosmotic character participate in the mechanisms of AVP and OT release, for example: osmotic disturbances of the extracellular fluid, fall in blood volume and arterial blood pressure, stress, parturition and suckling (1-10). Moreover, many neuropeptides and other neuroregulators of the central nervous system may modulate these neurohormones release from the hypothalamo-neurohypophysial system (HNS) (5, 6, 11, 12). Among others, thyrotropin-releasing hormone (TRH) is supposed to act at the level of HNS as a neuromodulator of AVP and OT release. Earlier studies from our laboratory have demonstrated that TRH injected intracerebroventricularly (i.c.v.) in the rat acts as an inhibitory neuromodulator of neurohypophysial neurohormones release during different states of water metabolism (4, 5, 13, 14), in the conditions of hypovolemia due to the haemorrhage (15) as well as in female rats during midlactation (16). Moreover, we noted that TRH inverts the course of daily rhythm of AVP and OT release into the blood (17). TRH, injected intravenously (i.v.), restricts of both neurohormones release during the dehydration (5). TRH suppresses AVP and OT release in vitro in the conditions of HNS incubation (18). TRH effects in this field are dependent on the dose employed (19).

The possible relationships between thyroid gland function and the pituitary neurohormones secretion are taken under consideration. Some authors reported that the change of functional thyroid state (i.e., the state of hypo- or hyperthyroidism) may influence the neurohypophysial hormones release (20-25). Waters in 1978 (24) has found increased vasopressin secretion in patients with hypothyroidism. Similarly, Macaron and Famuyiwa (25) have noted the augmentation of antidiuretic hormone level in the state of hypothyroidism. Plasma vasopressin concentration has been reported to be elevated (26) or normal (27) during experimental hypothyroidism. More recently was found that hypothyroidism did not influence AVP mRNA in parvo- and magnocellular neurons of the paraventricular nucleus (PVN) after thyroid ablation (21). Similarly, thyroidectomy did not change hypothalamic OT and AVP expression in hypernatremic rats (23). On the other hand, the treatment of rats with thyroid hormone increased hypothalamic OT mRNA, neurohypophysial OT content, as well as OT level in blood plasma (20). It is noteworthy that in humans the state of clinical hypothyroidism is known to be associated with a diminished ability to excrete free water and lower plasma osmolality and AVP level (28, 29). On the contrary, the state of hyperthyroidism manifested by polyuria and polydipsia (30, 31). However, unlike other species, including man, the rats treated with thyroxine showed a reduced urinary output (32).
The variety of mentioned above effects brought about by the changes of thyroid status was the reason for the experiments described in this paper. The main purpose of present study was to investigate the effect of the states of hypoor hyperthyroidism on vasopressin and oxytocin release from the hypothalamo-neurohypophysial system of rats under osmotic disturbances (i.e., in dehydrated or acutely hypernatremic rats).

MATERIAL AND METHODS

Animals.

Ninety adult male Wistar rats weighing 280 - 370 g were used for the experiments. All the experiments were performed with the acceptance of the Ethical Committee of Medical University of Lodz. The animals were maintained under controlled temperature (+22° C) and light (artificial illumination from 6.00 a.m. to 8.00 p.m.). All rats received standard pelleted food and tap water *ad libitum* (before experimental use).

Experimental design and procedure.

The rats were divided into three groups: group A - untreated rats served as euthyroid controls; group B - animals drinking *ad libitum* 1% 4(6)-methyl-2-thiouracyl (MTU) (Fluka, lot No 69400) solution for two weeks; group C - animals injected intraperitoneally (i.p.) with L-thyroxine (L-T₄) (Fluka, lot No 89405) solution over 30 days, in a daily dose of 10 µg/100 g body weight (b.w.). In each group three further experimental subgroups were set up: I - euthyroid controls, i.e., animals in the state of equilibrated water metabolism (drinking tap water *ad libitum*); II - animals dehydrated for two days (i.e., deprived of access to tap water); III - animals injected i.p., once daily, with 2% sodium chloride solution in a volume of 2 ml over two days.

The rats were weighed and killed by decapitation at 8.30-9.30. The decapitation of experimental animals was performed in detail as follows: subgroup AI - untreated and euthyroid rats [(EuthContr) rats] decapitated immediately after the taking of them from the cages; subgroups AII and AIII - euthyroid rats decapitated after two days of water deprivation [(EuthDeh) rats] or 2% NaCl solution i.p. injections [(EuthSalt) rats], respectively; subgroup BI - hypothyroid rats decapitated after 14 days of MTU treatment [(HypoContr) rats]; subgroups BII and BIII - immediately after 14 days of MTU treatment hypothyroid rats were dehydrated for two days [(HypoDeh) rats] or injected i.p. with 2% NaCl solution [(HypoSalt) rats] and then decapitated; subgroup CI - hyperthyroid rats decapitated after 30 days of L-T₄ treatment [(HyperContr) rats]; subgroups CII and CIII - immediately after 30 days of L-T₄ treatment hyperthyroid rats were deprived of water for two days [(HyperDeh) rats] or injected i.p. with 2% NaCl solution [(HyperSalt) rats] and then decapitated.

Mixed arterial-venous blood from the trunk was collected in heparinized tubes for AVP, OT as well as FT₃ and FT₄ estimation. Moreover, the blood 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 (Halmikro-Osmometer, Knauer, Weissenschaftliche Geräte KG, Berlin). The neurohormones were extracted from the plasma using C18 Sep-pak columns (Sep-Pak® C18 Cartridges, lot No W9224G1; Waters Corp., Milford, Massachusetts) as described by Forsling (33); the final extracts were preserved in frozen sealed vials until radioimmunoassay. The recoveries of hormones during extraction procedure were greater than 80% and therefore values were not corrected for procedural loses.
The brain with intact pituitary was quickly (i.e., not later than 2 min after decapitation) removed, the infundibular stalk cut up and the neurointermediate lobe was separated from the anterior lobe. From the brain, rapidly frozen for a few minutes at -70° C, the hypothalamic block was dissected as previously described (17). The wet weight of this block of tissue was 28.6 ± 1.3 (± S.D.). The neurointermediate lobe (respectively, the hypothalamus) was homogenized at + 4° C by sonication in Microson™ Ultrasonic Homogenizer (Labcaire, UK) in 2.0 ml of 0.25% (resp. 0.5%) acetic acid dissolved in 0.9% saline. The tissue suspension was transferred into a centrifuge tube and then the sample was heated for 5 min on boiling water bath (in order to inactivate the proteolytic enzymes contained in the homogenized tissue) and next centrifuged at 2000 rpm at +4° C for 20 min. The supernatants were removed, frozen, and stored at -70° C until radioimmunoassay.

Radioimmunoassays

The AVP and OT content of the neurohypophysial and hypothalamic extracts as well as plasma AVP and OT levels were determined by double-antibody specific radioimmunoassay as previously described by Ciosek et al. (17). Anti-AVP and anti-OT antibodies were raised in Department of Physiology and Biochemistry, Medical University of Lodz. The antibody titer was 1 : 24000 for anti-AVP and 1 : 80000 for anti-OT (both final dilutions). Cross reactivity for anti-AVP antibodies was with oxytocin 0.016%, with lysine vasopressin (LVP) 2.7%, with gonadotropin-releasing hormone (Gn-RH), TRH, leucine enkephalin (Leu-Enk), angiotensin II (Ang II) and substance P (SP) less than 0.002%. Cross reactivity for anti-OT antibodies was with vasopressin 1.12%, with Gn-RH, TRH, Leu-Enk, Ang II and SP less than 0.002%. The sensitivity of anti-AVP and anti-OT antisera was 1.25 pg AVP or OT per tube. Arginine vasopressin (Arg8-Vasopressin, Bachem AG, lot 511731) as well as oxytocin (Peninsula Lab. Ltd., lot 027179) were used for standard curves preparation as well as for iodination with 125I using the chloramine-T method. Intra-assay coefficient of variation (cv) for the AVP and OT assay was 2.0% and 3.7%, respectively.

Plasma free thyroxine (FT4) and free triiodothyronine (FT3) concentrations were determined in duplicate by RIA kits provided by POLATOM [Ośrodek Badawczo-Rozwojowy Izotopów (Research-Developmental Centre of Isotopes), Otwock-Świerk, Poland; lot No 112028 and 11027, respectively]. The intra-assay cv for FT4 and for FT3, was 5.03% and 4.88%, respectively.

Statistical evaluation of the results

The vasopressin and oxytocin content was finally expressed in nanograms per mg of the hypothalamic tissue, in nanograms for the whole neurointermediate lobe, and in picograms per 1 millilitre of plasma. The plasma FT4 and FT3 concentrations were expressed in picomols per liter. All results are reported as the mean ± standard error of the mean (S.E.M.). Data were calculated by the analysis of variance (ANOVA); if ANOVA revealed significant effects, post hoc analyses were done using the two-way Wilcoxon test (p<0.05 was used as the minimal level of significance). Statistical analysis of the experimental data was performed using "STATISTICA" Version 5.0 software, copyright StaSoft Inc., licensed to Department of Pathophysiology, Medical University of Lodz.

RESULTS

Serum osmolality in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (Table 1). Serum osmolality values significantly raised in euthyroid rats in the conditions of dehydration or hypertonic saline injections (Table 1: AI vs AII; AI vs AIII).
The state of hyperthyroidism was the reason of distinct decrease of the osmolality in rats drinking tap water *ad libitum* (*HyperContr*) in relation to euthyroid rats (*EuthContr*) (*Table 1*: CI vs AI); there was no statistical difference of this parameter value in euhydrated hypothyroid animals (*HypoContr*) (*Table 1*: BI vs AI).

In the state of hypothyroidism the level of the osmolality raised in (*HypoDeh*) and (*HypoSalt*) rats when compared with the respective control values in subgroup BI [(*HypoContr*) rats] (*Table 1*: BI vs BII; BI vs BIII) as well as with the values noted in osmotically stimulated euthyroid animals (*EuthDeh* and *EuthSalt*) (*Table 1*: BII vs AII; BIII vs AIII). In hyperthyroid animals serum osmolality significantly increased in (*HyperSalt*) rats as compared to hyperthyroid rats drinking tap water (*Table 1*: CI vs CIII). On the other hand, the plasma osmolality of (*HyperDeh*) and (*HyperSalt*) rats was significantly diminished in comparison to (*EuthDeh*) and (*EuthSalt*) rats (*Table 1*: CII vs AII; CIII vs AIII).

Haematocrite index (*Ht index*) in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (*Table 2*). Haematocrite index value distinctly increased in euthyroid and osmotically stimulated rats, i.e., in rats deprived of tap water (*EuthDeh*) (*Table 2*: AI vs AII).

The state of hyperthyroidism was the reason of distinct decrease of the osmolality in rats drinking tap water *ad libitum* (*HyperContr*) in relation to euthyroid rats (*EuthContr*) (*Table 1*: CI vs AI); there was no statistical difference of this parameter value in euhydrated hypothyroid animals (*HypoContr*) (*Table 1*: BI vs AI).

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**Table 1.** Serum osmolality in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (in mOsm/kg H$_2$O; mean ± S.E.M.)

<table>
<thead>
<tr>
<th>Subgroups of animals</th>
<th>Group A: euthyroid animals</th>
<th>Group B: hypothyroid animals</th>
<th>Group C: hyperthyroid animals</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I - animals euhydrated</td>
<td>280.75 ± 4.27</td>
<td>271.00 ± 4.93</td>
<td>260.25 ± 3.50</td>
<td>A versus B: NS, A versus C: p&lt;0.02</td>
</tr>
<tr>
<td>II - animals dehydrated for 2 days</td>
<td>294.60 ± 3.42</td>
<td>320.00 ± 4.94</td>
<td>268.33 ± 2.08</td>
<td>A versus B: p&lt;0.01, A versus C: p&lt;0.01</td>
</tr>
<tr>
<td>III - animals injected i.p. with 2% NaCl solution</td>
<td>298.75 ± 5.69</td>
<td>335.50 ± 7.51</td>
<td>283.25 ± 2.66</td>
<td>A versus B: p&lt;0.01, A versus C: p&lt;0.05</td>
</tr>
<tr>
<td>Statistical significance:</td>
<td></td>
<td></td>
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</tr>
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<td>I versus II</td>
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<td>NS</td>
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</tr>
<tr>
<td>II versus III</td>
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The state of hyperthyroidism was the reason of distinct decrease of the osmolality in rats drinking tap water *ad libitum* (*HyperContr*) in relation to euthyroid rats (*EuthContr*) (*Table 1*: CI vs AI); there was no statistical difference of this parameter value in euhydrated hypothyroid animals (*HypoContr*) (*Table 1*: BI vs AI).

In the state of hypothyroidism the level of the osmolality raised in (*HypoDeh*) and (*HypoSalt*) rats when compared with the respective control values in subgroup BI [(*HypoContr*) rats] (*Table 1*: BI vs BII; BI vs BIII) as well as with the values noted in osmotically stimulated euthyroid animals (*EuthDeh* and *EuthSalt*) (*Table 1*: BII vs AII; BIII vs AIII). In hyperthyroid animals serum osmolality significantly increased in (*HyperSalt*) rats as compared to hyperthyroid rats drinking tap water (*Table 1*: CI vs CIII). On the other hand, the plasma osmolality of (*HyperDeh*) and (*HyperSalt*) rats was significantly diminished in comparison to (*EuthDeh*) and (*EuthSalt*) rats (*Table 1*: CII vs AII; CIII vs AIII).

**Table 1.** Serum osmolality in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (in mOsm/kg H$_2$O; mean ± S.E.M.)
There were no differences in Ht index values between all subgroups of hyperthyroid and euthyroid rats.

Hypothyroid rats dehydrated (HypoDeh) or injected with 2% NaCl (HypoSalt) showed the increase of Ht index in relation to hypothyroid euhydrated animals (HypoContr) (Table 2: BII vs BI; BIII vs BI). Similarly, osmotically stimulated hyperthyroid rats (HyperDeh) had higher values of Ht index towards to respective control subgroup (HyperContr) (Table 2: CII vs CI).

Vasopressin release in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (Fig. 1, 2 and 3). In euthyroid animals the neurohypophysial vasopressin content significantly decreased during dehydration (Fig. 2: AI vs AII) as well as under i.p. treatment of 2% NaCl solution (Fig. 2: AI vs AIII). The confirmation of this observation is the increase of AVP plasma concentration during the same experimental conditions (Fig. 3: AI vs AII; AI vs AIII). Hypothalamic AVP content diminished only in (EuthSalt) rats (Fig. 1. AI vs AIII).

Some significant differences in the hypothalamo-neurohypophysial vasopressin content were found between euthyroid, hypothyroid and hyperthyroid animals receiving tap water ad libitum. Compared with euthyroid animals, (HypoContr) rats showed somewhat higher but not significant neurohypophysial AVP content (Fig. 2: AI vs BI) while hyperthyroid ones had significantly lower AVP amounts in the neurohypophysis (Fig. 2: AI vs CI). On the contrary, hypothalamic AVP stores diminished in (HypoContr) rats (Fig. 1: AI vs BI) without significant changes in (HyperContr) rats.

The state of osmotic stimulation was the reason of different response of the hypothalamo-neurohypophysial system function during hypothyroidism or

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**Table 2.** Haematocrite index in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (mean ± S.E.M.)

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<td>I – animals euhydrated</td>
<td>45.7 ± 0.56</td>
<td>40.9 ± 0.75</td>
<td>43.8 ± 0.76</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>II – animals dehydrated for 2 days</td>
<td>48.6 ± 0.64</td>
<td>47.3 ± 0.58</td>
<td>48.3 ± 0.55</td>
<td>NS</td>
</tr>
<tr>
<td>III – animals injected i.p. with 2% NaCl solution</td>
<td>45.0 ± 0.90</td>
<td>46.8 ± 0.55</td>
<td>44.0 ± 0.47</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Statistical significance:**
- I versus II: p<0.02
- II versus III: p<0.05
- I versus III: NS
- A versus B: p<0.01
- A versus C: p<0.02
- BII vs BI: p<0.01
- BIII vs BI: p<0.05

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- II versus III: p<0.05
- I versus III: NS
- A versus B: p<0.01
- A versus C: p<0.02
- BII vs BI: p<0.01
- BIII vs BI: p<0.05

There were no differences in Ht index values between all subgroups of hyperthyroid and euthyroid rats.
hyperthyroidism. Hypothalamic AVP content raised in (HypoSalt) rats when compared with the respective control values in subgroup BI (Fig. 1: BI vs BIII). On the other hand, significant decrease in neurohypophysial AVP content was found in both (HypoDeh) and (HypoSalt) rats as compared with their tap water drinking counterparts (Fig. 2: BI vs BII and BI vs BIII); vasopressin plasma level
raised in the same subgroups, however, the respective differences were not significant (Fig. 3: BI vs BII, BI vs BIII).

In the state of hyperthyroidism the neurohypophysial AVP stores distinctly increased in both (HyperDeh) and (HyperSalt) rats in relation to hyperthyroid control rats (HyperContr) (Fig. 2: CI vs CII and CI vs CIII); simultaneously, hypothalamic AVP decreased in (HyperSalt) rats (Fig. 1: CI vs CIII).

**Fig. 3.** Plasma vasopressin (AVP) concentration in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (mean+/− S.E.M.; number of animals: n = 6-10)

**Fig. 4.** The hypothalamic (Hth) oxytocin (OT) content in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (mean+/− S.E.M.; number of animals: n = 7-10)
Oxytocin release in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (Fig. 4, 5 and 6). In the euthyroid animals the state of dehydration or hypertonic saline treatment was the reason of distinct decrease of oxytocin neurohypophysial content (Fig. 5: AI vs AII and AI vs AIII) without the respective changes in the hypothalamic OT stores. OT plasma concentration increased only in the conditions of water deprivation (Fig. 6: AI vs AII).

![Fig. 5](image)

Fig. 5. The neurohypophysial (NH) oxytocin (OT) content in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (mean +/- S.E.M.; number of animals: n = 7-10)

![Fig. 6](image)

Fig. 6. Plasma oxytocin (OT) concentration in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (mean +/- S.E.M.; number of animals: n = 6-10)
The neurohypophysial OT content diminished in hyperthyroid animals drinking tap water (HyperContr) (Fig. 5: AI vs CI); plasma OT concentration distinctly raised in these animals (Fig. 6: AI vs CI). Similarly, hypothalamic OT content decreased distinctly in (HyperContr) as well as (HypoContr) rats as compared with (EuthContr) rats (Fig. 4: AI vs CI and AI vs BI).

Fig. 7. Free thyroxine (FT4) concentration in blood plasma of euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (mean +/- S.E.M.; number of animals: n = 10)

Fig. 8. Free triiodothyronine (FT3) concentration in blood plasma of euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (mean +/- S.E.M.; number of animals: n = 10)
The treatment of hypertonic saline employed in hypothyroid animals was followed by a significant decrease of neurohypophysial OT stores (Fig. 5: BI vs BIII). In (HypoDeh) animals hypothalamic OT content highly increased (Fig. 4: BI vs BII). On the contrary, in hyperthyroid rats hypothalamic and neurohypophysial OT content increased distinctly under conditions of salt loading (Fig. 4 and 5: CI vs CIII); then, OT plasma level distinctly diminished (Fig. 6: CI vs CII; CI vs CIII).

FT$_3$ and FT$_4$ blood plasma levels in euthyroid, hypothyroid and hyperthyroid rats in different states of water metabolism (Fig. 7 and 8). In euthyroid and simultaneously dehydrated (EuthDeh) salt loaded (EuthSalt) animals distinct elevation of FT$_3$ and FT$_4$ plasma concentrations has been observed (Fig. 7: AI vs AIII; Fig. 8: AI vs AII and AII vs AIII).

The state of hypothyroidism was the reason of the significant diminution of both FT$_3$ and FT$_4$ plasma levels as compared with euthyroid rats (Fig. 7 and 8: BI vs AI). Dehydration was followed by a distinct increase of FT$_3$ and FT$_4$ levels in blood in comparison with (HypoContr) rats (Fig. 7 and 8: BI vs BII).

In all subgroups of hyperthyroid animals FT3 and FT4 serum concentrations raised several times in relation to euthyroid subgroups (Fig. 7 and 8: CI vs AI, CII vs AII, CIII vs AIII). Distinct differences among these subgroups during the states of osmotic stimulation were observed: FT$_3$ and FT$_4$ plasma levels increased in (HyperDeh) rats (Fig. 7 and 8: CII vs CI).

**DISCUSSION**

Vasopressin and oxytocin release under conditions of osmotic disturbances.

Synthesis, axonal transport and release of vasopressin and oxytocin are increased in animals deprived of tap water or in salt-loaded rats (4, 5, 34). Moreover, the bioelectrical discharge of vasopressinergic and oxytocinergic neurons of magnocellular hypothalamic nuclei is known to intensify under such conditions (2, 35). In rats dehydrated, drinking hypertonic saline, injected i.c.v. or i.p. with hypertonic sodium chloride solution, both neurohypophysial hormones have been shown to deplete in the neural lobe of the pituitary and hypothalamic nuclei (1, 3, 4, 14, 36, 37). Moreover, the increase of AVP and OT concentration in blood plasma in the rat after such treatment has been observed (1, 3, 4, 14, 36, 37, 38, 39, 40). This problem has been described in detail by Bourque (39) and Ciosek (41).

In agreement with former data, also from this laboratory (4, 5, 34), the present results show that the hyperosmotic dehydration or the treatment with hypertonic sodium chloride solution diminished significantly the neurohypophysial vasopressin and oxytocin content. This diminution of neurohypophysial vasopressin and oxytocin content was observed to be accompanied by an increase of their release into the blood. It seems that the infundibular transport of both
neurohormones from the hypothalamus towards the neurohypophysis - although increased in these conditions - is probably insufficient to compensate AVP and OT quantities released into the circulation.

*Effect of thyroid status on vasopressin and oxytocin release during osmotic disturbances.* Thyroid gland status influences the hypothalamo-pituitary system function. It is known that thyroid hormones are engaged in the feedback regulation of TRH biosynthesis and secretion at the level of hypothalamic PVN region (42, 43, 44) which corresponds to the thyrotrophic hypothalamic area (42, 43, 45). A large number of TRH-synthesizing neurons are present in the medial and periventricular parvocellular subdivisions of the PVN (46, 47). Ceccatelli et al. (21) have showed the feedback effect of thyroid hormone on TRH synthesis by the TRH mRNA increase in the state of hypothyroidism and by its diminution during hyperthyroidism. In the study of Rondeel et al. (48) in thyroxin-treated rats the level of TRH in the posterior pituitary increased whereas it decreased in the methimazole-induced hypothyroid rats. It has been hypothesized that posterior pituitary TRH participates in prolactin (PRL) as well as AVP and OT secretion (48). But these interdependences may be quite different in other brain regions. For example, in the study of Pekary et al. (49) hypothyroidism reduced the content of TRH and other TRH-like peptides in the entorhinal cortex.

Thyroid hormones regulate also the secretion of other hypothalamo-pituitary hormones. The relationship between thyroid hormone and adrenocortical function is well documented. It has been reported that ACTH release is diminished in the conditions of primary hypothyroidism (50) but increased in the state of hyperthyroidism (51). Tohei et al. (52) have showed that hypothyroidism causes the hypersecretion of ACTH following by the increase in synthesis of corticotrophin-releasing hormone (CRH) in the hypothalamus. Data from the study of Dakine et al. (53) demonstrate that thyroxine raised of hypothalamic CRH gene expression in the neonatal rats. Moreover, changes in growth hormone (GH) and/or growth hormone-releasing hormone (GHRH) synthesis and release have been reported in experimentally induced thyroid dysfunction (54). Thyroid hormone exerts negative feedback influence on the basal and TRH-evoked PRL secretion in humans (55, 56). Stempieniak et al. (57) demonstrated that PRL plasma levels were increased in hypothyroid and T4-treated rats. The level of galanin mRNA (Gal mRNA) in parvo- and magnocellular PVN neurons was significantly decreased in the hypothyroid rats (21). Gal content in the pituitary and the hypothalamus was diminished in these conditions (58). Moreover, vasoactive intestinal polypeptide (VIP) is under feedback regulation by thyroid hormones; VIP mRNA expression increased in the PVN by thyroidectomy (21, 59).

Several data suggest a functional relationship between hypothalamo-pituitary-thyroid axis and hypothalamo-neurohypophysial system. Several functions for TRH in the regulation of neurohypophysial activity have been supposed. TRH has been noted to stimulate the release of vasopressin and oxytocin in the rabbit (60, 61). Similarly, the i.c.v. TRH treatment was followed by an increase of AVP
release in the rat (62). In other experiments no changes in oxytocin and
vasopressin release after i.c.v. injections of TRH were reported (63). No changes
in OT nor AVP release following the intravenous (i.v.) administration of TRH
were noted in the rat (64). Earlier studies from our laboratory have demonstrated
that TRH injected i.c.v. in the rats acts as an inhibitory neuromodulator of
neurohypophysial neurohormones release during different states of water
metabolism (4, 5, 13, 14), in conditions of hypovolemia due to haemorrhage (15),
as well as in female rats during midlactation (16). Moreover, we noted that TRH
inverts the course of daily rhythm of AVP and OT release into blood (17). TRH,
injected i.v., restricts of the two neurohormones secretion during the dehydration
(5). TRH suppresses AVP and OT release in vitro in the conditions of HNS
explants incubation (18). TRH effects in this field are dependent on a dose
employed (19).

Some reports support a modulatory role for the thyroid gland in vasopressin
and oxytocin release from the neurohypophysis. This connection may be
demonstrated experimentally especially in the conditions of the thyroid gland
dysfunction, i.e., in states of hypothyroidism or hyperthyroidism. The state of
hypothyroidism may be induced experimentally by the surgical thyroidectomy or
by administration of 4-methyl-2-thiouracyl or propyl-thiouracyl (65, 66) whereas,
the state of hyperthyroidism is usually produced by chronic thyroid hormone
administration (53, 67, 68). It cannot be excluded the direct or indirect MTU
effect on AVP and OT metabolism. Though, it has been postulated the parallely
use of the thyroidectomy or simultaneously administration of MTU and thyroxin
(as the test of suppression of MTU action), in present experiments in agreement
with most studies, the state of hypothyroidism was induced by MTU application.
In the present study plasma levels of free triiodothyronine (FT\textsubscript{3}) and free
thyroxine (FT\textsubscript{4}) as the basal indicators of the states of hypo- or hyperthyroidism
have been determined. The free T\textsubscript{3} and T\textsubscript{4} plasma concentrations represent about
0.3% or 0.03%, respectively, of total circulating hormone amounts. The state of
hypothyroidism resulted in significant decrease of FT\textsubscript{3} and FT\textsubscript{4} plasma levels; an
opposite effects (i.e., distinct increase of the two hormones level) was noted
during the hyperthyroidism. In this respect our data are quite consistent with
previous reports (48, 57, 68).

The results of the respective studies concerning possible role of the thyroid in
regulation of AVP and OT secretion are not quite consistent. In patients with
myxedema due to primary hypothyroidism plasma AVP level was raised
significantly (24, 25, 69) or, on the contrary, was undetectable or very low (27,
70). Similarly, Ali et al. (71) has found the diminution of plasma vasopressin level
in the propylthiouracil-treated rats. In primary hypothyroidism plasma
hypoosmolality and low levels of AVP have been observed (72). In a study of
Wolf et al. (67) hypo- and hyperthyroidism in rat females during gestation did not
result in a significant change in vasopressin and oxytocin content of fetal
neurohypophyses. There were no differences in plasma or pituitary vasopressin
levels during aminotriazole-induced hypothyroidism in rats (22). In addition, the hypothalamic AVP mRNA levels did not change in these conditions. Similarly, in a report of Ceccatelli et al. (21) hypothyroidism did not affect AVP mRNA expression in parvo- and magnocellular PVN neurons. On the other hand, treatment of rats with thyroid hormone ($T_3$) raised OT mRNA expression, the neurohypophysial OT content as well as OT level in blood (20). Carter et al. (73) has been noted that AVP mRNA level in the SON and PVN and in the neurointermediate lobe of the pituitary significantly raised in a state of hypothyroidism due to 6-propyl-2-thiouracil but was not changed in consequence of surgical thyroidectomy. In the study from our laboratory (57) plasma AVP and OT concentrations were reduced in rats with hypothyroidism but after $T_4$ administration AVP (but not OT) plasma level distinctly increased. In the report of Tohei et al. (65) AVP release in median eminence was distinctly intensified in hypothyroid rats; there was no alteration in hypothalamic AVP content. Hyperthyroidism observed in patients resulted in AVP plasma level increase (74) recurring to that of controls after normalization of thyroid status. On the other hand, in neonatal rats, injected with $T_4$, any modification of AVP mRNA in the parvo- and magnocellular cell bodies of the PVN neurons has been observed (53).

The osmotic stimulus employed during the thyroid gland dysfunctions resulted in a different consequences as to AVP and OT release. Vargas et al. (75) has showed that urinary excretion of AVP was increased in hyperthyroid and diminished in hypothyroid rats in response to osmotic stimuli (i.e., the water deprivation or a hypertonic saline load). Thyroidectomy did not modify hypothalamic OT and AVP mRNA in response to chronic hypernatremia in male rats (23). Experiments of Mogulkoc et al. (66, 76) have been showed that in PTU-induced hypothyroid rats or in surgically thyroidectomized rats hypertonic or hypovolemic treatment caused lower increase of AVP plasma level as compared to the respective intact animals.

Our results seem to be partly consistent with some mentioned above data. The hypothyroid state induced in rats being in equilibrated water metabolism seem to be the reason of the restrained vasopressin release from the neurohypophysis (however, without AVP plasma level change). In our opinion it cannot be excluded inhibitory influence of TRH which is released in greater amounts in a state of thyroid hypofunction. Based upon the observation of our laboratory (4, 5, 13, 14, 15, 16, 18) this hypothalamic peptide is thought to be an inhibitory neuromodulator for AVP and OT release. On the contrary, the decrease of the neurohypophysial AVP storages in a state of hyperthyroidism may testify to the intensified AVP release. These data seem to be similar to some results obtained from the studies of Howard et al. (22) and Stempniak et al. (57). As to oxytocin, it is noteworthy that both states of thyroid dysfunction (i.e., hypo- and hyperthyroidism) resulted in an increase of OT secretion into blood.

In dehydrated or hypertonic saline-injected hypothyroid rats the tendency to the enhanced AVP and OT release has been occured as compared with euhydrated
hypothyroid rats. But this degree of AVP and OT secretion was less marked in relation to euthyroid rats under osmotic stimulation. We noted reduction of the two neurohormones release in hyperthyroid and simultaneously dehydrated or hypertonic saline-treated rats in comparison with animals drinking tap water ad libitum. The state of hyperthyroidism was the reason of augmented AVP and OT secretion as compared with euthyroid and osmotically stimulated rats. In this respect, our results are consistent with those from the experiments of Vargas et al. (75) and Mogulkoc et al. (66, 76). On the other hand, it is probably that the hypermetabolism as the result of the state of hyperthyroidism could be the reason of intensified AVP and OT degradation.

In conclusion, our study suggests that:

1 - altered thyroid gland function distinctly affects vasopressin and oxytocin release from the hypothalamo-neurohypophysial system in the state of equilibrated water metabolism;
2 - the state of hypo- or hyperthyroidism modifies the response of AVP-ergic and OT-ergic neurons upon the osmoreceptors/osmodetectors stimulation. It may be supposed that OT-ergic neurons display greater than AVP-ergic neurons sensitivity upon the thyroid hormone influence.

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Author’s address: Joanna Ciosek, Ph.D., D.Sc., Department of Pathophysiology, Medical University of Lodz, ul. Narutowicza 60, 90-136 Lodz, Poland. Tel: (4842) 630-61-87, fax: (4842) 631-97-23.
E-mail: joannack@poczta.onet.pl