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Oxytocin and vasopressin secretion from the rat hypothalamo–neurohypophysial system is stimulated by triptorelin

Uwalnianie oksytocyny i wazopresyny z układu podwzgórze–część nerwowa przysadki szczura jest nasilane przez tryptorelinę

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Abstract

Introduction: Several observations have suggested that the secretion of neurohypophysial hormones could be modified by gonadotropin-releasing hormone (GnRH). Since, in medical practice, more often than GnRH itself, its analogues are used, the present study was undertaken to investigate the influence of the GnRH agonist — triptorelin on oxytocin (OT) and vasopressin (AVP) release from the rat hypothalamo-neurohypophysial (H-N) system both *in vitro* and *in vivo.*

Materials and methods: Male rats served as donors of the H-N explants, which were placed in 1 mL of Krebs-Ringer fluid (nKRF) and incubated successively in: 1 — nKRF (B1); 2 — incubation fluid as B1 enriched with an excess amount (56 mM) of K^+ (S1); 3 — incubation fluid as B1 enriched with an appropriate concentration of triptorelin, i.e., 10^{-11} — 10^{-5} M (B2); and 4 — incubation fluid as S1 enriched with the same concentrations of triptorelin (S2). After 20 minutes of incubation, each medium (B1, S1, B2, S2) was collected and frozen before OT and AVP estimation by the RIA. During *in vivo* experiment, animals were infused intracerebroventricularly (icv) with triptorelin, at a concentration of 10^{-7} M, and 20 minutes later they were decapitated. The neurohypophysis was dissected from the brain and blood plasma samples were collected and frozen for further OT and AVP RIA assays.

Results: The GnRH agonist — triptorelin stimulates both OT and AVP release from isolated H-N system at concentrations of 10^{-9}–10^{-5} M. The strongest effect was displayed by triptorelin at a concentration of 10^{-7} M. Under the conditions of K^+ stimulation, triptorelin affects neither OT, nor AVP secretion in vitro. When infused icv, triptorelin, at a concentration of 10^{-7} M, significantly stimulated both OT and AVP secretion into the blood.

Conclusions: Triptorelin may play a role as a neuromodulator contributing to the functional regulation of OT and AVP secretion in the rat. **(Pol J Endocrinol 2012; 63 (3): 176–182)**

Key words: *oxytocin, vasopressin, triptorelin*

Streszczenie

Wstęp: Wcześniejsze obserwacje sugerowały, że gonadoliberyna (GnRH) może modyfikować uwalnianie hormonów części nerwowej przysadki. W praktyce klinicznej zamiast GnRH stosuje się analogi tego hormonu, dlatego celem pracy było zbadanie, czy agonista GnRH — tryptorelina zmienia uwalnianie oksytocyny (OT) i wazopresyny (AVP) z układu podwzgórze–część nerwowa przysadki (H-N) szczura *in vitro* oraz *in vivo*.

Materiał i metody: Po wyosobnieniu z mózgu, układ H-N umieszczano w probówkach zawierających 1 ml płynu Krebsa-Ringera (nKRF). Po okresie równoważenia dodawano nKRF zawierający podstawowe (płyn B1) lub zwiększone (56 mM) stężenie jonów K^+ (płyn S1), a następnie płyny B1 lub S1 zawierające dodatkowo roztwór tryptoreliny w wybranym stężeniu, tj. 10^{-11}–10^{-5} M (płyny B2 i S2). Po inkubacji układu H-N w każdym z roztworów (kolejno: B1, S1, B2, S2) przez 20 min płyn inkubacyjny pobierano i zamrażano do czasu oznaczenia w zebranych próbkach zawartości OT i AVP metodą RIA. W doświadczeniu *in vivo* szczurom, do bocznej komory mózgu (*icv*), podawano roztwór tryptoreliny w stężeniu 10^{-7} M/L lub jej rozpuszczalnik. Po 20 min od *icv* infuzji tryptoreliny zwierzęta dekapitowano w celu pobrania od nich krwi oraz części nerwowej przysadki. Wyciągi z przysadki i osocze zamrażano do czasu oznaczenia w nich zawartości OT i AVP metodą RIA.

Wyniki: Tryptorelina zwiększa podstawowe uwalnianie OT i AVP do płynu inkubacyjnego *in vitro* w stężeniach 10^{-9}–10^{-5} M, najsilniejszy efekt wywierając w stężeniu 10^{-7} M. W warunkach pobudzania nadmiarem jonów K^+, niezależnie od badanego stężenia tryptoreliny, uwalnianie OT i AVP z układu H-N do płynu inkubacyjnego nie różni się istotnie od stwierdzanego w grupie kontrolnej. Infundowana *icv* tryptorelina, w stężeniu 10^{-7} M, istotnie zwiększa uwalnianie OT i AVP do krwi obwodowej szczura.

Wnioski: Tryptorelina może pełnić u szczura rolę neuromodulatora modyfikującego proces uwalniania OT i AVP. **(Endokrynol Pol 2012; 63 (3): 176–182)**

Słowa kluczowe: *oksytocyna, wazopresyna, tryptorelina*

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Introduction

In mammals, most neurons containing gonadotropin-releasing hormone (GnRH-I) are present in the preoptic-hypothalamic region and project to the median eminence [1], where the hormone is secreted in a pulsatile pattern to stimulate the biosynthesis and secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH) from anterior pituitary gonadotrope cells [2]. In the rat and men, neurons which contain the GnRH-I have been found to be located close to the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus [1, 3], where the neurohypophysial hormones, i.e. oxytocin (OT) and vasopressin (AVP), are synthesised [4]. The GnRH-containing fibres entering the neurohypophysis have been observed in the human pituitary [5], and anatomical synapses between GnRH- and AVP-immunoreactive neurons have been demonstrated in primates [6]. All the above observations suggest that GnRH can influence, at least in part, the synthesis and/or secretion of OT and AVP. However, very few studies have been performed, either *in vivo* or *in vitro*, to investigate the effect of GnRH on OT and/or AVP secretion from the rat hypothalamo-neurohypophysial system. It has been demonstrated that the action of GnRH on oxytocinergic and/or vasopressinergic neurons activity depends on the peptide dose, the experimental conditions (*in vitro* or *in vivo*), and the state of water-electrolyte balance [7–12].

GnRH-I is metabolised very fast. The half-life of the hormone is very short, i.e. about 2–4 min [13] and GnRH mRNA has a rapid rate of decay ($t_{1/2}$, 5–13 min) [14], while its agonistic analogues are more resistant to degradation and show stronger receptor binding and activation of the receptor than native GnRH-I [15, 16]. Therefore, in medical practice, more often than GnRH-I itself, its synthetic analogues (i.e. agonists or antagonists) are used for the treatment of a wide variety of reproductive disorders and hormone-dependent malignancies [15, 17, 18]. Such agonists, depending on the dose and method and/or duration of treatment (i.e. pulsatile versus prolonged application) may have a stimulatory or inhibitory influence on gonadotropin synthesis and release. On the other hand, GnRH antagonists are able to block GnRH receptors on the pituitary and thus inhibit LH and FSH production and/or secretion [15, 16].

Triptorelin belongs to one of the commonest GnRH agonists used in clinical practice [18–20]. It has been shown to have a much longer plasma half-life than native GnRH, with an elimination half-life (after single peripheral administration) superior to 80 min [21].

The aim of this study was, therefore, to investigate the influence of the GnRH agonist — triptorelin on OT and AVP secretion from the rat hypothalamo-neurohypophysial system *in vitro* and *in vivo*, and whether such an effect depends on the concentration of the peptide.

Materials and methods

Animals

Three-month old male Wistar rats (weighing 250–350 g) were housed under conditions of constant temperature, humidity and lighting (a 12/12 hr light/dark schedule; lights on from 6 am). The animals received standard pelleted food and had free access to tap water.

Drugs

The gonadotropin-releasing hormone agonist [i.e. (D-Trp^6)-LHRH acetate salt; Pyr-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2] — triptorelin was purchased from BACHEM AG, Bubendorf, Switzerland. The OT (oxytocin synth.) and AVP (vasopressin synth.) for standard curve preparation as well as for iodination with ^{125}I, were from Peninsula Laboratories Europe Ltd. The anti-VP and anti-OT antibodies were raised by dr hab. Monika Orłowska-Majdak (Department of Experimental Physiology, Chair of Department of Experimental and Clinical Physiology, Medical University of Lodz).

Experimental procedure in vitro

Series I. Triptorelin-dependent OT and AVP secretion under basal and K^+-stimulated conditions was assessed in vitro. All animals were decapitated between 10 am and 11.30 am. The brain and the pituitary with intact pituitary stalk were carefully removed from the skull, and a block of hypothalamic tissue was dissected to obtain a hypothalamo-neurohypophysial system as previously described [22, 23]. After dissection, the hypothalamo-neurohypophysial (H-N) explant was immediately placed in a polypropylene tube with 1 ml of normal Krebs-Ringer fluid (nKRF) containing: 120 mM NaCl, 5 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM KH_2PO_4, 0.7 mM $MgSO_4$, 22.5 mM $NaHCO_3$, 10 mM glucose, 1.0 g/L bovine serum albumin and 0.1 g/L ascorbic acid (pH = 7.4–7.5, osmolality = 285–295 mOsm/kg). Tubes were placed in a water bath at 37 °C, constantly gassed with carbogen (a mixture of 95% O_2 and 5% CO_2). At the beginning of the experiment, the H-N explants were equilibrated in nKRF, which was aspirated twice and replaced with 1 ml of fresh buffer. After 80 minutes of such preincubation, necessary for OT and AVP release stabilisation, the media were discarded and the explants were incubated for 20 minutes in 1 ml of nKRF alone (control group) or nKRF supplemented with an appropriate concentration of triptorelin. Explants were incubated successively in: 1 — normal KRF (fluid

B1); 2 — modified KRF containing the excess amount (56 mM) of K^+ (the NaCl concentration was appropriately reduced to maintain medium osmolality) (fluid S1); 3 — incubation fluid as B1 (control group) or nKRF supplemented with an appropriate concentration of triptorelin, i.e. 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} or 10^{-5} M (fluid B2); and 4 — incubation fluid as S1 enriched with the same concentration of the studied peptide (fluid S2). In between incubation periods S1 and B2, the explants were washed for 20 min in nKRF and these samples were discarded. After each 20 min incubation period, the media (i.e. B1, S1, B2, S2) were collected and samples immediately frozen and stored at –20 °C until OT and AVP estimation by radioimmunoassay (RIA).

To determine the triptorelin-induced OT and AVP secretion *in vitro*, the B2/B1 (basal secretion, i.e. without K^+ stimulation) and S2/S1 (K^+-stimulated secretion) ratios were calculated for each H-N explant. The results are expressed as a B2/B1 or S2/S1 ratio because the amount of the hormone released into the medium varies from one H-N explant to the other.

Experimental procedure in vivo

Series II. On the day of the experiment, the rats were anaesthetised by an intraperitoneal (ip) injection of 10% urethane (ethyl carbonate; 1.4 mL/100 g. b. wt) and a stainless steel cannula was inserted into the lateral cerebral ventricle (icv) as recommended by Noble et al. [24]. Rats were given an icv infusion of 5 µL 0.9% NaCl alone or triptorelin at a 10^{-7} M concentration (the concentration of the peptide was chosen on the basis of the results of the experiment *in vitro)*. The icv infusions were given via a polyethylene tube attached to a 10 µL Hamilton syringe filled with the appropriate solution. Twenty minutes later, the animals were decapitated and the neurohypophysis was dissected from the brain. Blood plasma samples were collected and frozen for further OT and AVP RIA assays, as previously described [25].

The experiments were performed with the consent (No 12/ŁB 452/2009 and No 9/ŁB 536/2011) of the Local Committee for Animal Care.

Radioimmunoassay of OT and AVP

The AVP and OT concentrations in all samples were determined in duplicate by a specific RIA method described previously [23]. Arginine vasopressin and oxytocin were iodinated with ^{125}I using the chloramine-T method. The final dilution of anti-AVP antibodies was 1:24,000. Cross reactivity with oxytocin for anti-AVP antibodies was 0.016%, with lysine vasopressin — 2.7%, with gonadotrophin-releasing hormone, thyrotrophin-releasing hormone, leucine enkephalin, angiotensin II and substance P — less than 0.002%. The lower limit of detection for the assay was 1.56 pg AVP per tube. The intra- and inter-assay coefficients of variation were 3.3% and 6.3%, respectively. The OT antibody titer was 1:80,000 (final dilution). The lower limit of detection was 3.12 pg OT per tube. The intra- and inter-assay coefficients of variation were 4.5% and 8.3%, respectively.

Statistical evaluation of the results

The data was expressed as means ± S.E.M. and the significance between means was evaluated by use of the Kruskal-Wallis analysis of variance (ANOVA) by ranks for each set of data; as the amount of samples per subgroup was small, non-parametric tests were chosen. Thereafter, the statistical significance of differences between the means of two compared subgroups was determined by Mann-Whitney 'U' test (Statistica, StatSoft, Krakow, Poland), estimating $p < 0.05$ as the minimal level of significance.

Results

Series I. The GnRH agonist — triptorelin significantly stimulated basal OT (Fig. 1A) and AVP (Fig. 2A) release from isolated rat H-N system at concentrations of 10^{-9}–10^{-5} M. The strongest effect was displayed by triptorelin at a concentration of 10^{-7} M for both OT (8.27 ± 1.98 *vs* 1.3 ± 0.26, $p < 0.05$) and AVP (4.69 ± 1.12 *vs* 1.11 ± 0.19, $p < 0.05$). Two concentrations of the peptide, i.e. 10^{-10} M (2.0 ± 0.39 *vs* 1.3 ± 0.26 for OT, $p > 0.05$ and 1.45 ± 0.4 *vs* 1.11 ± 0.19 for AVP, $p > 0.05$) and 10^{-11} M (0.96 ± 0.16 *vs* 1.3 ± 0.26 for OT, $p > 0.05$ and 1.06 ± 0.26 *vs* 1.11 ± 0.19 for AVP, $p > 0.05$), remained inactive in modifying these hormones secretion *in vitro*. Under the conditions of K^+ stimulation, triptorelin affected neither OT nor AVP secretion *in vitro* (Figs. 1B and 2B).

Series II. The GnRH agonist, at a concentration of 10^{-7} M, significantly stimulated both OT and AVP secretion from the rat neurohypophysis into the blood (Figs. 3A and 3B). Namely, triptorelin was able to diminish both OT (911.16 ± 84.75 vs 1,594.67 ± 177.9, $p < 0.01$) and AVP (689.77 ± 75.0 vs 1,360.42 ± 110.0, $p < 0.01$) content in the neurohypophysis (Fig. 3A), while blood plasma OT (25.43 ± 6.23 vs 8.3 ± 0.92, $p < 0.01$) and AVP (16.3 ± 2.5 vs 8.46 ± 0.93, $p < 0.01$) concentrations were significantly higher in rats infused icv with triptorelin than in control animals (Fig. 3B).

Discussion

The oxytocinergic and vasopressinergic neurons function is altered by several factors, e.g. parturition, suckling, hypovolaemia, hyperosmotic stimulation, angiotensin II or stress [7–10, 25–30] as well as by numerous peptides [23, 26, 31–33], which play a role as neuromediators and/or neuromodulators in the hypo-

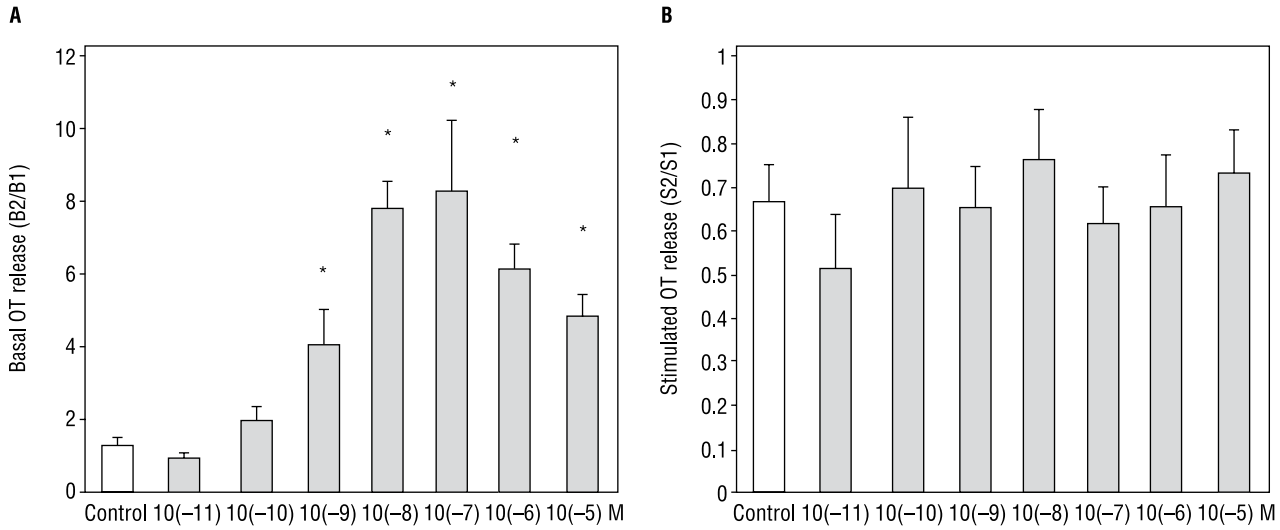

Figure 1. *The effect of gonadotropin-releasing hormone (GnRH) agonist — triptorelin, at concentrations of 10^{-11}–10^{-5} M, on basal (***A***) and K^+-stimulated (***B***) oxytocin (OT) release from the rat hypothalamo–neurohypophysial complex in vitro. Each bar represents mean ± SEM; number of samples per group (n) = 8–9, *p < 0.05 — significantly different vs control*

Rycina 1. *Wpływ agonisty gonadoliberyny — tryptoreliny, w stężeniach 10^{-11}–10^{-5} M, na podstawowe (***A***) i pobudzane nadmiarem jonów K^+ (***B***) uwalnianie oksytocyny (OT) z układu podwzgórze–część nerwowa przysadki szczura* in vitro. *Wyniki przedstawiają średnią ± SEM; liczba próbek w grupie (n) = 8–9; *p < 0,05 — różnica istotna statystycznie względem kontroli*

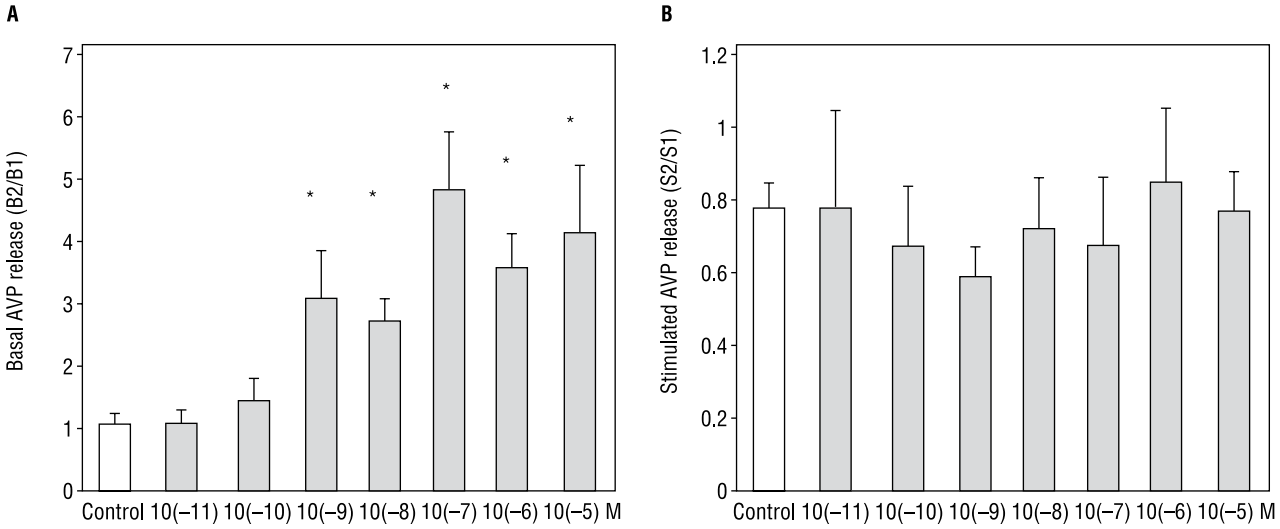

Figure 2. *The effect of gonadotropin-releasing hormone (GnRH) agonist — triptorelin, at concentrations of 10^{-11}–10^{-5} M, on basal (***A***) and K^+-stimulated (***B***) vasopressin (AVP) release from the rat hypothalamo–neurohypophysial complex in vitro. Each bar represents mean ± SEM; number of samples per group (n) = 9; *p < 0.05 — significantly different vs control*

Rycina 2. *Wpływ agonisty gonadoliberyny — tryptoreliny, w stężeniach 10^{-11}–10^{-5} M, na podstawowe (***A***) i pobudzane nadmiarem jonów K^+ (***B***) uwalnianie wazopresyny (AVP) z układu podwzgórze–część nerwowa przysadki szczura* in vitro. *Wyniki przedstawiają średnią ± SEM; liczba próbek w grupie (n) = 9; *p < 0,05 — różnica istotna statystycznie względem kontroli*

thalamus and other central nervous system regions. Results from the present study show, for the first time, that the GnRH agonist – triptorelin is a potent stimulus (at concentrations of 10^{-9}–10^{-5} M) for OT and AVP output from isolated rat hypothalamo-neurohypophysial complex. The most active in this respect concentration of the peptide is 10^{-7} M, which is in accordance with the effect of the other GnRH agonist — histrelin [11–12]. We have also shown that centrally administered triptorelin (at a concentration of 10^{-7} M) may be involved in the mechanisms controlling the activity of oxytocinergic and vasopressinergic neurons, acting probably through a specific GnRH-I receptor in the hypothalamus. Such a suggestion is based on the fact that in the rat, the

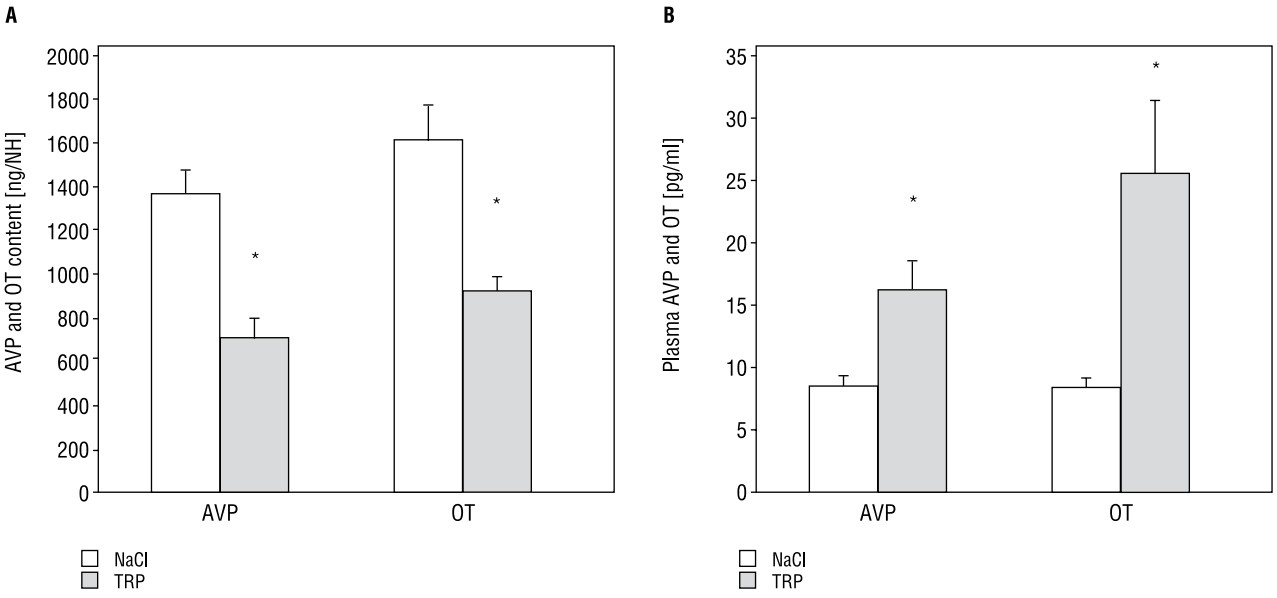

Figure 3. *The effect of icv infusion of gonadotropin-releasing hormone (GnRH) agonist — triptorelin, at a concentration of 10^{-7} M, on the neurohypophysial (NH) content (***A***) and blood plasma concentrations (***B***) of oxytocin (OT) and vasopressin (AVP). Each bar represents mean ± SEM; number of animals per group (n) = 8; *p < 0.01 — significantly different vs control (0.9% NaCl)*

Rycina 3. *Wpływ dokomorowej (*icv*) infuzji agonisty gonadoliberyny — tryptoreliny, w stężeniu 10^{-7} M, na zawartość oksytocyny (OT) i wazopresyny (AVP) w części nerwowej przysadki (***A***) oraz stężenie OT i AVP we krwi obwodowej (***B***) szczura. Wyniki przedstawiają średnią ± SEM; liczba zwierząt w grupie (n) = 8; *p < 0,01 — różnica istotna statystycznie względem kontroli (0,9% NaCl)*

GnRH-immunoreactive fibres are present in the hypothalamus and median eminence, but they do not project into the posterior pituitary [34].

The GnRH agonist employed in our present study, i.e. triptorelin, is one of the commonest GnRH agonists [17–20], and is approximately 15 to 20 times more potent than native GnRH-I [15, 35]. It has been found to stimulate LH release from the rat pituitary *in vitro* [16]. Moreover, in adult rats icv administration of 10, 100 and 1,000 ng/animal of triptorelin exerts significant actions on the central nervous system, as early as 20 minutes after the injection [36]. Under present experimental conditions, 20 min incubation *in vitro* of the hypothalamo-neurohypophysial explant in the medium containing triptorelin (and similarly *in vivo*, 20 min interval between icv infusion of triptorelin and decapitation) should imitate the effect of one pulse of natural GnRH on neurohypophysial hormones secretion at the physiological situation.

It is well established that K^+-induced depolarisation stimulates both cell bodies in the magnocellular nuclei and axon terminals in the neurohypophysis [22, 31]. Under present *in vitro* conditions, triptorelin failed to modify the K^+-evoked release of the neurohypophysial hormones. The possibility is that the increase in the hormone's release as evoked by K^+ stimulation under S1 period attenuated the responsiveness of both oxytocinergic and vasopressinergic neurones to GnRH agonist during the successive incubation period S2. However, when the effect of LHRH on K^+-evoked release of AVP and OT was studied *in vitro*, the hormone at a dose of 40 nM displayed no significant influence on OT [8], but reduced AVP [7] secretion from the hypothalamo-neurohypophysial explants obtained from euhydrated rats.

Mechanisms by which GnRH agonist could affect OT and/or AVP release are not well understood. In the rat, only one of the two receptors present in mammals, i.e. GnRH-I receptor, is active [37]. The GnRH-I receptors are localised mainly in the hypothalamus (its mediobasal and ventromedial part) and anterior pituitary [38, 39], but not in the neurohypophysis. Since for the present *in vitro* experiment we used the whole hypothalamo-neurohypophysial complex (with direct axonal projection from the SON and PVN to the neurohypophysis), instead of the posterior pituitary separated from the brain, it is very probable that GnRH agonist — triptorelin was able to increase OT and AVP release acting at the hypothalamic level through GnRH-I receptor (both *in vitro* and *in vivo*). GnRH agonist could also modify the SON and/or PVN neurons activity indirectly via suprachiasmatic nucleus (SCN); the explant employed in the present *in vitro* experiments contained, apart from SON and PVN, also SCN and other anterior hypothalamic nuclei [22]. An anatomical basis for such a hypothesis is the existence of synaptic contacts between GnRH-containing neurons

of the preoptic area (the region that contains the majority of the GnRH-synthesising neurons in the rat) and neurons in the SCN [40], which have direct neuronal projection to PVN [41] and SON [42]. Some of the SCN neurons could, therefore, integrate the afferent signals from other hypothalamic nuclei and/or different brain areas (both under *in vitro* and *in vivo* conditions) and thereafter transmit them directly to oxytocinergic and vasopressinergic neurons in the SON and/or PVN. It has been found that the SCN neurons can influence function of the PVN and SON neurons by releasing from their axonal endings either excitatory (glutamate) or inhibitory (GABA) amino acids [41, 42], which have been shown to increase or decrease plasma OT and/or AVP level, respectively [26]. Another possibility involves indirect projection from the SCN to the SON through the GnRH-ergic cells in the preoptic area [1, 43]. Such a direct neuronal pathway from the SCN to the preoptic area has been found to transmit, in the female rat, the circadian rhythm from the SCN to the GnRH neurons *via* AVP-containing fibres [44].

The influence of GnRH agonist on OT and AVP secretion can also be mediated by some neuromediators/neuromodulators and neuroactive agents (present in the hypothalamus and/or posterior pituitary) such as dopamine, arachidonic acid and its metabolites, glutamate, GABA, nitric oxide, opioid peptides, neurosteroids and oestrogens [33, 36, 45–48]. Quite recently, triptorelin was found to induce nitric oxide synthase type 1 (NOS1) expression in female rat pituitary cells, both *in vitro* and *in vivo* [49].

All the above mentioned factors are involved in modifying OT and AVP release from the rat hypothalamo-neurohypophysial system [26, 31, 33, 50, 51] and a certain combination of these agents may be crucial for the mechanisms by which oxytocinergic and vasopressinergic neurons are influenced by GnRH and/or its agonists.

Conclusions

The GnRH agonist — triptorelin, acting probably through a specific GnRH receptor in the hypothalamus, may play a role as a neuromodulator contributing to the regulation of oxytocinergic and vasopressinergic neurons secretory activity in the rat, and thus may indirectly influence water balance of the organism and several other processes, which are regulated by OT and/or AVP.

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Conflict of interest

None declared.

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