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Forskolin-stimulated vasopressin and oxytocin release from the rat hypothalamo–neurohypophysial system *in vitro* is inhibited by melatonin

Pobudzane stosowaniem forskoliny uwalnianie wazopresyny i oksytocyny z układu podwzgórze–część nerwowa przysadki szczura *in vitro* jest hamowane przez melatoninę

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Abstract

Introduction: Previous *in vivo* and *in vitro* studies have shown that melatonin changes vasopressin (AVP) and oxytocin (OT) secretion from the rat neurohypophysis. Additionally, melatonin is known to inhibit the forskolin-induced (forskolin is a strong adenylyl cyclase - AC activator) increase in cAMP accumulation in the rat pituitary. To determine whether the possible response of vasopressinergic and/or oxytocinergic neurones to melatonin could be mediated through a cAMP-dependent mechanism, the effect of different concentrations of melatonin (i.e. 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} and 10^{-3} M) on forskolin-stimulated AVP and OT release from the rat hypothalamo-neurohypophysial (H-NH) system was studied *in vitro*.

Materials and methods: Male rats served as donors of the H-NH explants, which were placed in 1 mLof normal Krebs-Ringer fluid (nKRF), heated to 37°C and constantly gassed with carbogen (95% O_2 and 5% CO_2). The H-NH explants were incubated successively in nKRF {fluid B1} and incubation fluid as B1 enriched with an appropriate concentration of melatonin, i.e. 10^{-11} – 10^{-3} M and/or forskolin (at a concentration of 10^{-5} M) or their vehicles (0.1% ethanol or DMSO) {fluid B2}. After 20 min incubation in fluid B1 and next B2, the media were collected and immediately frozen before AVP and OT estimation by the RIA. The AVP and OT secretion was determined by using B2/B1 ratio for each H-NH explant.

Results: We have demonstrated that the highly effective AC activator — forskolin significantly stimulated both AVP and OT release from isolated rat H-NH system. Such an effect of forskolin was reduced by melatonin at concentrations of 10^{-9}, 10^{-7} and 10^{-5} M. The strongest effect was exerted by this hormone at a concentration of 10^{-7} M, which inhibited not only forskolin-stimulated, but also basal, AVP and OT release. On the contrary, the highest studied concentration (i.e. 10^{-3} M) of melatonin stimulated both AVP and OT basal release, but when forskolin was present in the medium melatonin at such a concentration remained inactive in modifying these hormones release from the H-NH system *in vitro*.

Conclusions: Our present results demonstrate that in the male rat:

1. The influence of melatonin on the vasopressinergic and oxytocinergic neurones activity is mediated partly through a cAMP-dependent mechanism.
2. The effect of melatonin in this respect depends on its concentration. **(Endokrynol Pol 2014; 65 (2): 125–131)**

Key words: *vasopressin; oxytocin; forskolin; melatonin*

Streszczenie

Wstęp: Wcześniejsze badania wykazały, że melatonina modyfikuje proces uwalniania wazopresyny (AVP) i oksytocyny (OT) zarówno *in vivo*, jak i*n vitro*. Ponadto stwierdzono, że melatonina hamuje indukowaną forskoliną (forskolina jest silnym aktywatorem cyklazy adenylanowej, której pobudzenie zwiększa syntezę cAMP) akumulację cAMP w przysadce szczura. Aby określić czy cAMP pośredniczy we wpływie melatoniny na czynność wydzielniczą neuronów wazopresynergicznych i/lub oksytocynergicznych zbadano wpływ różnych stężeń tego hormonu (tj. 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5}, 10^{-3} M) na wywołane stosowaniem forskoliny uwalnianie AVP i OT z układu podwzgórze - część nerwowa przysadki (H-NH) szczura *in vitro*.

Materiał i metody: Po wyosobnieniu z mózgu, układ H-NH umieszczano w probówkach zawierających 1 mL płynu Krebsa-Ringera (K-R) ogrzanego do temperatury 37°C oraz nasycanego mieszaniną karbogen (95% O_2 i 5% CO_2). Po okresie równoważenia, do probówek dodawano normalny płyn K-R {płyn B1}, a następnie płyn B1 zawierający dodatkowo rozpuszczalnik melatoniny (0.1% etanol) lub jej roztwór w odpowiednim stężeniu, tj. 10^{-11} – 10^{-3} M i/lub forskolinę (w stężeniu 10^{-5} M), bądź jej rozpuszczalnik (0,1% DMSO) {płyn B2}. Po inkubacji układu H-NH w każdym z roztworów (B1 i B2) przez 20 min płyn inkubacyjny pobierano i natychmiast zamrażano do czasu oznaczenia w zebranych próbkach zawartości AVP i OT metodą RIA. Stopień uwalniania AVP i OT z układu H-NH *in vitro* wyrażano jako stosunek B2/B1.

Wyniki: Wykazano, że forskolina istotnie zwiększa uwalnianie AVP i OT z układu H-NH do płynu inkubacyjnego *in vitro*, natomiast melatonina (w stężeniach 10^{-9}, 10^{-7} i 10^{-5} M) efekt ten istotnie ogranicza. Najsilniejszy efekt hamujący melatonina wywiera w stężeniu 10^{-7}

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M, hamując nie tylko pobudzane forskoliną, ale także podstawowe uwalnianie obydwu neurohormonów. Przeciwnie, w stężeniu 10^{-3} M melatonina istotnie nasila wydzielanie AVP i OT do płynu inkubacyjnego, natomiast nie zmienia pobudzanego stosowaniem forskoliny ich uwalniania.

Wnioski: Wyniki tych badań sugerują, że:

1. W mechanizmie wpływu melatoniny na sekrecyjną aktywność neuronów wazopresynergicznych i oksytocynergicznych u szczura ma znaczenie cAMP.
2. Efekt ten zależy od stężenia melatoniny. **(Endokrynol Pol 2014; 65 (2): 125–131)**

Słowa kluczowe: *wazopresyna; oksytocyna; forskolina; melatonina*

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Introduction

Melatonin, discovered as a hormone produced by the pineal hormone, is now well known to be synthesised in various organs and tissues. One of the richest sources of melatonin in the organism is the gastrointestinal tract (GIT), which produces several hundred times more melatonin than the pineal gland, and where melatonin demonstrates several advantageous, but rather local, effects (e.g. gastroprotective and liver-protective actions) under the conditions of health and disease [1–3]. Produced by the pineal gland, melatonin is released into the general circulation as well as directly into the cerebrospinal fluid, and influences the function of numerous structures in the central nervous system [4–5] and the pituitary, both anterior [6–7] and posterior [8] part of the gland.

Melatonin has been shown to influence the activity of hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei and modify vasopressin (AVP) and oxytocin (OT) synthesis and release under different experimental conditions, both *in vivo* and *in vitro*. However, data is not consistent and shows that the effect of melatonin on AVP or OT release from the hypothalamus and/or neurohypophysis depends on the concentration of the hormone, the time of day, and the species of animal used as donors of a tissue for the *in vitro* studies [8]. As early as 1979, melatonin was reported to stimulate AVP secretion from the rat neurohypophysis in a dose-dependent manner [9]. Later *in vitro* studies have shown that this hormone was able to stimulate the release of both AVP and OT from isolated neurointermediate lobe of sham-operated (i.e. not pinealectomised) or pinealectomised rats when used at relatively high concentrations (10^{-6} M and 10^{-3} M), but at a concentration of 10^{-7} M it was ineffective [10]. In contrast to these observations, Yasin et al. [11] showed that melatonin had an inhibitory effect on both AVP and OT release from isolated rat hypothalamus, with maximal inhibition at 10^{-7} M. In addition, the effect of melatonin on the neurohypophysial hormones secretion was found to depend on the light:dark cycle and could be seen only during the day [12]. When Syrian hamsters were used as donors of the tissue, the inhibitory effect of melatonin on AVP and OT secretion from the neurointermediate lobe was also noted [13]. Since melatonin has been shown to have either a stimulatory or an inhibitory influence, or to be without effect on AVP or OT secretion from isolated hypothalamus or neurointermediate lobe, the primary purpose of the present study was, therefore, to investigate whether melatonin affects the *in vitro* release of AVP and OT from isolated rat hypothalamo-neurohypophysial system in a dose-dependent manner. This is the first time this has been investigated.

The intracellular mechanism of melatonin action involves the inhibition of calcium influx and calcium mobilisation from intracellular stores, as well as the inhibition of an adenylyl cyclase (AC)-dependent rise in cyclic adenosine monophosphate (cAMP) production [14–16]. Melatonin has been shown to inhibit the forskolin-induced (forskolin is a strong AC activator) increase in cAMP accumulation in the rat pituitary [14–15]. Moreover, forskolin has been found to elicit an increase in cAMP accumulation in the rat hypothalamic SON and in the neural lobe of the pituitary *in vitro* [17] and cAMP-dependent stimulation of AVP and OT release from the posterior pituitary has also been described [18].

The second purpose of the present study was, therefore, to investigate whether the forskolin-stimulated secretion of AVP and OT from the rat hypothalamo-neurohypophysial system *in vitro* could be modified by melatonin and whether such an effect depends on a concentration of the hormone in the incubatory medium.

Material and methods

Animals

Three-months old male Wistar rats (weighing about 220–350 g), maintained in a light:dark cycle 12L:12D (lights on from 6 a.m.), at a constant temperature (+22°C), with standard pelleted food and water available *ad libitum*, were used for the experiments.

Drugs

Melatonin (N-acetyl-5-methoxytryptamine), forskolin and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich Chemie GmbH. The AVP (Vasopressin synth.) and OT (Oxytocin synth.), for standard curve preparation as well as for iodination with ^{125}I, were from Peninsula Laboratories Europe Ltd. The anti-AVP and anti-OT antibodies were raised by dr hab. Monika Orłowska-Majdak (Department of Experimental Physiology, Chair of Experimental and Clinical Physiology, Medical University of Lodz).

Experimental procedure in vitro

On the day of the experiment, the animals were decapitated between 9:30 and 10:30 a.m. The brain together with the pituitary was carefully removed from the skull, and a block of tissue containing the hypothalamus was isolated as previously described [19]. After dissection, the hypothalamo-neurohypophysial (H-NH) explant was placed in a polypropylene tube with 1 mL of normal Krebs-Ringer fluid (nKRF) heated in a water bath to 37°C and constantly gassed with carbogen (95% O_2 and 5% CO_2). The nKRF contained: 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 within the range 285–295 mOsm/kg). At the beginning of the experiment, the H-NH explants were equilibrated in 1 mL of nKRF (2 × 40 min), the media were aspirated and removed. After 80 min of such preincubation, which is necessary for stabilisation of AVP and OT release [11], explants were incubated for 20 min in 1 mL of nKRF {fluid B1} and then, for the next 20 min, in 1 mL of KRF supplemented with the studied substance(s) or their vehicle {fluid B2}.

Series I

The aim of the first series was to examine the effect of different concentrations of melatonin on AVP and OT release from isolated rat hypothalamo-neurohypophysial (H-NH) system. Explants were therefore incubated successively in: (i) nKRF {fluid B1} and (ii) nKRF alone (control group) or KRF enriched with melatonin vehicle (0.1 % ethanol; VEH group), or an appropriate concentration of melatonin, i.e. 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} or 10^{-3} M (n: number of samples per group, n = 7) {fluid B2}.

Series II

In series II, the influence of melatonin on forskolin-induced AVP and OT release from isolated rat hypothalamo-neurohypophysial (H-NH) system was tested *in vitro*. The experimental protocol was similar to that of series I. Therefore, after incubation in nKRF {fluid B1}, explants were next incubated in one of the following media: KRF enriched with forskolin vehicle (0.1% DMSO) or forskolin solution at a concentration of 10^{-5} M, or KRF supplemented with forskolin and melatonin with an appropriate concentration, i.e. 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} or 10^{-3} M (n = 8) {fluid B2}.

In both series, directly after each incubation period, the media (i.e. fluids B1 and B2) were aspirated, immediately frozen and stored at –20°C until AVP and OT estimation by radioimmunoassay (RIA).

To determine basal and forskolin-induced AVP and OT secretion *in vitro*, the B2/B1 ratio was calculated for each H-NH explant. The results are expressed as B2/B1 ratio, because the amount of the neurohormone released into the medium varies from one H-NH explant to the other.

The experimental procedures were done with the consent (No. 19/ŁB 516/2010) of the Local Committee for Animal Care.

Radioimmunoassay of AVP and OT

The AVP and OT concentrations in all samples were assayed in duplicate by a specific RIA described previously [19–20]. 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 for these antibodies with oxytocin was 0.016%, with lysine vasopressin —2.7%, and with GnRH, TRH, 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 for AVP assay were less than 3.5% and 6.5%, respectively. The OT antibody titre was 1:80,000 (final dilution), and the lower limit of detection was 3.12 pg OT per tube. The intra- and inter-assay coefficients of variation for OT assay were less than 5.0% and 8.5%, respectively.

Statistical evaluation of the results

The neurohypophysial hormone release *in vitro* is finally expressed as a percentage of the control (Figs. 1A and 2A) or DMSO (Figs. 1B and 2B) value. All results are reported as mean ± standard error of the mean (S.E.M.). Significance of the differences between means was evaluated by one-way analysis of variance (ANOVA) followed by Student's *t*-test (for two means comparison); $p < 0.05$ was considered as the minimal level of significance.

Results

Series I

Melatonin at a concentration of 10^{-7} M inhibited significantly basal AVP and OT secretion from isolated rat H-NH explants. When other concentrations of the hormone, i.e. 10^{-11}, 10^{-9} or 10^{-5} M, were present in the buffer, the AVP and OT output from the H-NH explants was

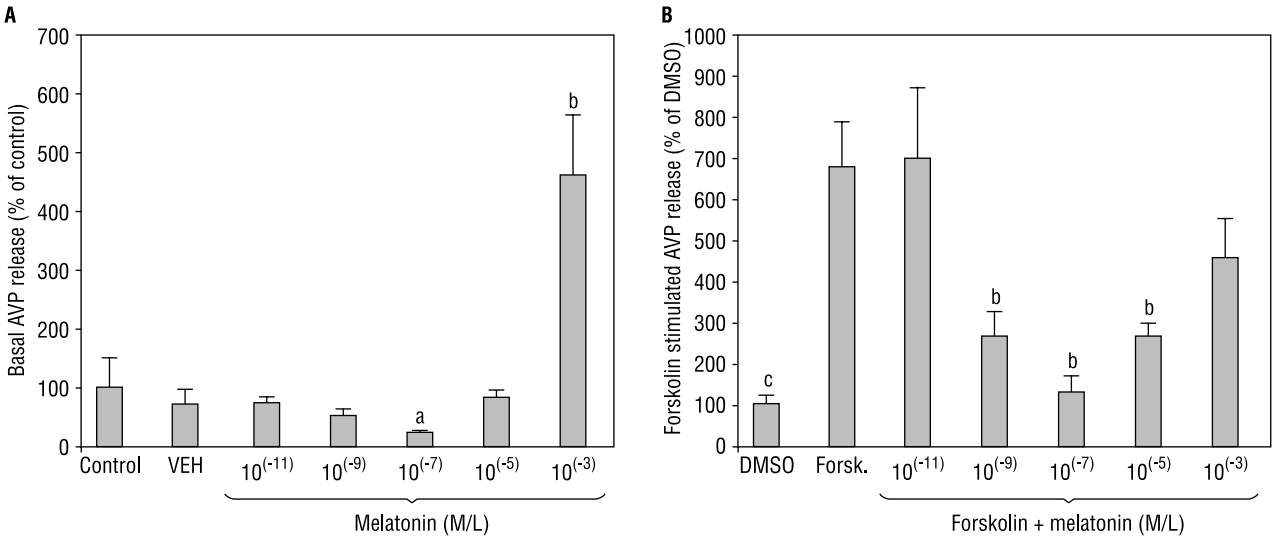

Figure 1. *The effect of melatonin, at the concentrations of 10^{-11} – 10^{-3} M, and forskolin (Forsk.), at a concentration of 10^{-5} M, on basal* **(A)** *and forskolin-stimulated* **(B)** *vasopressin (AVP) release from the rat hypothalamo–neurohypophysial complex in vitro.*
Each bar represents mean ± S.E.M.; number of samples per group (n) = 7–8; a — $p < 0.05$ — significantly different vs. melatonin vehicle (VEH) (Fig. 1A); b — $p < 0.005$ — significantly different vs. VEH (Fig. 1A) or Forsk. (Fig. 1B); c — $p < 0.0005$ — significantly different vs. Forsk. (Fig. 1B)

Rycina 1. *Wpływ melatoniny, w stężeniach 10^{-11} – 10^{-3} M, i forskoliny (Forsk.), w stężeniu 10^{-5} M, na podstawowe* **(A)** *i pobudzane forskoliną* **(B)** *uwalnianie wazopresyny (AVP) z układu podwzgórze–część nerwowa przysadki szczura in vitro.*
Wyniki przedstawiają średnią ± S.E.M.; liczba próbek w grupie (n) = 7–8; a — $p < 0.05$ — różnica istotna statystycznie względem VEH (ryc. 1A); b — $p < 0.005$ — różnica istotna względem VEH (ryc. 1A) lub Forsk. (ryc. 1B); c — $p < 0.0005$ — różnica istotna względem Forsk. (ryc. 1B)

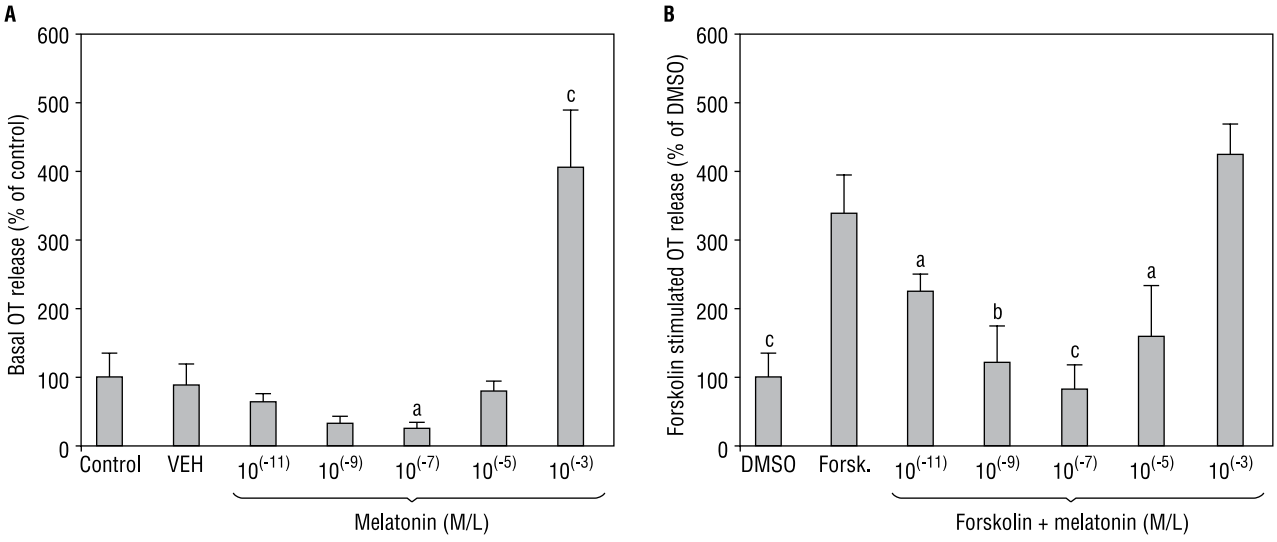

Figure 2. *The effect of melatonin, at the concentrations of 10^{-11}–10^{-3} M, and forskolin (Forsk.), at a concentration of 10-5 M, on basal* **(A)** *and forskolin-stimulated* **(B)** *oxytocin (OT) release from the rat hypothalamo–neurohypophysial complex in vitro.*

Each bar represents mean ± S.E.M.; number of samples per group (n) = 7–8; a — $p < 0.05$ — significantly different vs. melatonin vehicle (VEH) (Fig. 2A) or Forsk. (Fig. 2B); b — $p < 0.01$ — significantly different vs. Forsk. (Fig. 2B); c — $p < 0.005$ — significantly different vs. VEH (Fig. 2A) or Forsk. (Fig. 2B)

Rycina 2. *Wpływ melatoniny, w stężeniach 10^{-11}–10^{-3} M, i forskoliny (Forsk.), w stężeniu 10^{-5} M, na podstawowe* **(A)** *i pobudzane forskoliną* **(B)** *uwalnianie oksytocyny (OT) z układu podwzgórze–część nerwowa przysadki szczura in vitro.*

Wyniki przedstawiają średnią ± S.E.M.; liczba próbek w grupie (n) = 7–8; a — $p < 0.05$ — różnica istotna statystycznie względem VEH (ryc. 2A) lub Forsk. (ryc. 2B); b — $p < 0.01$ — różnica istotna względem Forsk. (ryc. 2B); c — $p < 0.005$ — różnica istotna względem VEH (ryc. 2A) lub Forsk. (ryc. 2B)

not different from the control. However, melatonin at a concentration of 10^{-3} M significantly increased both AVP and OT basal release into the medium (Figs. 1A and 2A).

Series II

Forskolin, at a concentration of 10^{-5} M, significantly stimulated both AVP (Fig. 1B) and OT (Fig. 2B) secretion into the medium. Melatonin at the concentrations of 10^{-9}, 10^{-7} and 10^{-5} M was able to reduce the forskolin-stimulated AVP and OT secretion (the strongest effect was exerted by the hormone at a concentration of 10^{-7} M). However, when melatonin was added to the medium at a concentration of 10^{-3} M, it remained inactive in modifying the forskolin-induced AVP (Fig. 1B) and OT (Fig. 2B) output from the H-NH system *in vitro*.

Discussion

Previous experiments *in vitro* have shown that the action of melatonin on AVP and OT release from the rat hypothalamic explants depends not only on a concentration of the hormone, but also on the time of day. Melatonin inhibited AVP and OT secretion when the hypothalamic tissue was obtained from animals in light conditions (i.e. about 3 h after lights on), but no effect of the hormone could be seen when tissue samples were obtained during the night, i.e. 4–5 h after lights off [12]. Our present experiments were, therefore, performed during the light period of the light/dark cycle (at a time when the hypothalamus was found to be responsive to melatonin), i.e. about 4 h after lights on, and the results confirmed partly previous observations. We have shown that melatonin, at a concentration of 10^{-7} M, is able to inhibit basal AVP (Fig. 1A) and OT (Fig. 2A) release from the H-NH system, which accords with previous results obtained when rat hypothalamus [11] or hamster neurointermediate lobe [13] were incubated *in vitro*. Melatonin, at a concentration of 10^{-11} M, remained inactive in influencing the AVP and OT output from isolated rat H-NH complex (Fig. 1A and 2A) or rat hypothalamus [11], but when hamster neurointermediate lobe was incubated *in vitro*, the three concentrations of melatonin (10^{-11}, 10^{-9} and 10^{-7} M) induced inhibitory effects of similar magnitude on OT and AVP release [13]. What is more, the highest concentration of melatonin used in our present experiments, i.e. 10^{-3} M, significantly stimulated basal AVP and OT release from the rat H-NH (Fig. 1A and 2A), which is compatible with the previous findings of Lemay et al. [9] and Juszczak et al. [10], but in conflict with the results of Yasin et al. [11], who have shown the inhibitory influence of 10^{-3} M melatonin on AVP and OT output from the rat hypothalamus *in vitro*. The observed discrepancies may result from the fact that for the present *in vitro* experiments we used the explants which contained intact neuronal projections from the hypothalamic SON and PVN nuclei to the neurohypophysis, i.e. intact axons of the oxytocinergic and vasopressinergic neurones, while in the other studies only isolated hypothalamus [11] or neurohypophysis [9, 13] were incubated *in vitro*.

The obtained data provides further evidence in favour of the idea that melatonin influences the neurohypophysial hormone secretion through a cAMP-dependent mechanism. Cyclic AMP seems to be the main intracellular second messenger for melatonin, although it can also modify intracellular concentration of cGMP and phosphoinositide signal transduction cascades [14–16, 21]. Melatonin has been found to inhibit the enhancement of cAMP concentration evoked by forskolin-induced increase of AC activity in the rat pars tuberalis of the pituitary [14]. In several studies, forskolin has been found to be able to increase intracellular cAMP accumulation at the concentrations of 10^{-4} to 10^{-6} M [14–16, 21]. Therefore, for the present experiment, forskolin was employed at a concentration of 10^{-5} M, and we found that it stimulated significantly both AVP (Fig. 1B) and OT (Fig. 2B) release into the medium. Together with the findings that forskolin increases cAMP accumulation in the rat hypothalamo-neurohypophysial system *in vitro* [17] and cAMP stimulates both AVP and OT release [18], our results suggest that, under present experimental conditions, forskolin stimulates the neurohypophysial hormone release acting via a cAMP-dependent mechanism.

Forskolin has been described as increasing the cAMP accumulation in the pituitary cells after 30 min of incubation, which was inhibited by melatonin in a dose-dependent (10^{-10} to 10^{-7} M) manner [15]. Under present experimental conditions, we incubated the H-NH system for 20 min in the presence of forskolin and/or melatonin, and the three concentrations of melatonin (10^{-9}, 10^{-7} and 10^{-5} M) significantly diminished the forskolin-induced AVP and OT release, with maximal inhibition produced by melatonin at a concentration of 10^{-7} M (Figs. 1B and 2B), which is compatible with the previous findings.

On the other hand, the addition of melatonin at a concentration of 10^{-3} M to the medium containing forskolin did not further alter the forskolin-stimulated AVP and OT release into the medium (Figs. 1B and 2B). Therefore, since such a high concentration of melatonin did not further modify significantly the stimulatory influence of forskolin on the neurohypophysial hormones release, this could imply a role for other intracellular mechanisms (e.g. calcium ions or nuclear receptors) responsible for 10^{-3} M melatonin-dependent AVP and OT secretion from the rat H-NH system. Indeed, melatonin may easily cross cellular

membranes and act directly on the genome through nuclear orphan RZR/ROR receptors [22–23]. Numerous studies on the actions of melatonin have reported opposite effects of so-called pharmacological (above 1 μM) or supraphysiological (1 nM – 1 μM) and physiological (below 1 nM) doses of the hormone [24], but it is difficult to determine which actions are mediated through G protein-coupled melatonin receptors, and which are mediated through membrane receptors-independent mechanisms.

The possible mechanism by which melatonin can modify the vasopressinergic and oxytocinergic neurones activity includes specific G protein-coupled membrane receptors, called MT_1 and MT_2, activation of which inhibits the synthesis of cAMP [21, 24]. These receptors are situated mainly in the pars tuberalis of the pituitary [25] and in the hypothalamic suprachiasmatic (SCN) [15, 26–28] as well as magnocellular SON and PVN nuclei [29]. It has been found that AVP-containing cells in the SCN express both MT_1 [27] and MT_2 [28] melatonin receptors and melatonin inhibits AVP release from cultured SCN neurones [15, 28]. Moreover, it has been found that the cAMP-dependent pathways are involved in an increase in AVP gene expression in the SCN [30]. Thanks to the presence of MT_1 and MT_2 receptors, the SCN neurones could respond to melatonin signal [5] and then transmit it to PVN [31] and/or SON [32], via excitatory (glutamate) or inhibitory (GABA) amino acids, which are known to modify AVP and OT secretion in the rat [33]. Moreover, the interaction of melatonin with its receptors present in other brain regions cannot be excluded. Namely, a small amount of MT_1 receptors has been observed in human posterior pituitary [29], which may suggest that melatonin exerts its influence on the AVP/OT release acting not only at the level of the hypothalamus, but also directly on the axonal endings located in the neurohypophysis. The anatomical basis for such a hypothesis is not only the existence of direct neuronal projection from the SCN to PVN and SON, but also the fact that the explant we used for the present *in vitro* experiments contained the whole hypothalamo-neurohypophysial system.

Melatonin may, therefore, affect the vasopressinergic and oxytocinergic neurones activity and secretion of AVP and OT by acting directly on specific membrane receptors and/or nuclear orphan receptors, or it may act indirectly via modification of the metabolism of certain neuromediators/neuromodulators in the hypothalamus and/or in the neurointermediate lobe [34–35]. Indeed, melatonin has been found to have an enhancing effect on the GABA system [36] and to affect the activity of tyrosine hydroxylase in different brain regions [37], whereas acetylcholine, dopamine and prostaglandins have been found to participate in an inhibitory influence of melatonin on neurohypophysial hormone release from the rat hypothalamus *in vitro* [38]. The above mentioned neurotransmitters (as well as various neurohormones and neuropeptides present in the central nervous system) and other numerous agents (e.g. biogenic amines, excitatory and inhibitory amino acids, nitric oxide, neurosteroids, opioids, etc.), have been shown to influence the activity of hypothalamic SON and PVN nuclei and modify the synthesis and release of both neurohypophysial hormones [19–20, 33–35, 39–43], so certain combinations of these agents may be of some importance for the mechanisms by which vasopressinergic and oxytocinergic neurones are influenced by melatonin.

In summary, this paper demonstrates that melatonin significantly reduces the *in vitro* response of vasopressinergic and oxytocinergic neurones to forskolin, suggesting that such an effect of melatonin is mediated through a cAMP-dependent mechanism. However, the intracellular mechanism of melatonin action involves other possibilities, especially when a very high concentration (i.e. 10^{-3} M) of the hormone is considered.

The present study provides further evidence that melatonin, apart from its well known action under the conditions of health and disease [44–45], also plays a role in the regulation of AVP and OT secretion from the rat hypothalamo-neurohypophysial system and, in this way, may indirectly influence water balance of the organism and brain function.

Conclusions

Our present results demonstrate that in the male rat:

1. The influence of melatonin on the vasopressinergic and oxytocinergic neurones activity is mediated partly through a cAMP-dependent mechanism.
2. the effect of melatonin in this respect depends on its concentration.

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