Endokrynologia Polska
DOI: 10.5603/EP.a2018.0051
Tom/Volume 69; Numer/Number 5/2018
ISSN 0423–104X

The role of the cAMP/PKA signalling pathway in the inhibitory influence of melatonin on oxytocin and vasopressin secretion from the rat hypothalamo–neurohypophysial system

Udział szlaku sygnałowego cAMP/PKA w hamującym wpływie melatoniny na wydzielanie oksytocyny i wazopresyny z układu podwzgórze–część nerwowa przysadki szczura

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Abstract

Introduction: Melatonin was found to inhibit forskolin-stimulated oxytocin (OT) and vasopressin (VP) release in vitro. The purpose of the present investigation was to evaluate the contribution of the cyclic 3′,5′-adenosine monophosphate/protein kinase A (cAMP/PKA) signalling pathway in melatonin-dependent inhibition of OT and VP secretion from the rat hypothalamo-neurohypophysial (H-NH) system in vitro.
Material and methods: The H-NH explants were placed in 1 ml of normal Krebs-Ringer (nK-R) buffer and first preincubated for 30 min in control buffer or in the presence of PKA inhibitor, i.e. cAMPS-Rp or H-89. Next, they were incubated in nK-R buffer {fluid F1} and then in buffer as F1 enriched with melatonin (10^{-9} M or 10^{-7} M) and/or PKA activator, i.e. cAMP analogue (8-Br-cAMP), or their vehicles {fluid F2}. After 20 min of incubation in fluid F1 and then F2, the media were collected and frozen, to be assayed for OT and VP by the RIA.
Results: 8-Br-cAMP increased OT and VP secretion when the H-NH explants were preincubated in control medium, while PKA inhibitors eliminated its stimulatory effect on OT and VP release. Melatonin (10^{-7} M) diminished basal OT and VP output from the H-NH system, and inhibited (at both concentrations studied) the cAMP analogue-stimulated release of both neurohormones under control conditions. The effect of melatonin on OT and VP release was completely blocked when cAMPS-Rp, but not H-89, was used to disrupt the cAMP/PKA pathway.
Conclusions: Melatonin employs the cAMP/PKA signalling pathway to inhibit OT and VP secretion from the rat H-NH system; nonetheless, other cAMP-mediated mechanisms are not excluded. **(Endokrynol Pol 2018; 69 (5): 560–566)**

Key words: *melatonin, oxytocin, vasopressin, 8-Br-cAMP, cAMPS-Rp, H-89, PKA*

Streszczenie

Wstęp: Melatonina hamuje pobudzane forskoliną wydzielanie oksytocyny (OT) i wazopresyny (VP) *in vitro*. Celem badań było wykazanie udziału szlaku sygnałowego 3′,5′-cykliczny adenozynomonofosforan/kinaza białkowa A (cAMP/PKA) w zależnym od melatoniny hamowaniu wydzielania OT i VP z układu podwzgórze–część nerwowa przysadki szczura (H-NH) *in vitro*.
Materiał i metody: Po wyosobnieniu z mózgu, układ H-NH umieszczano w probówkach zawierających 1 ml normalnego buforu Krebsa-Ringera (nK-R). Układ H-NH podlegał początkowo preinkubacji, przez 30 min, w buforze kontrolnym (nK-R) lub zawierającym inhibitor PKA, tj. cAMPS-Rp lub H-89. Następnie, inkubacja przebiegała w buforze nK-R {płyn F1}, a potem w buforze jak F1 wzbogaconym o melatoninę (10^{-9} M lub 10^{-7} M) i/lub aktywator PKA, tj. analog cAMP (8-Br-cAMP) lub ich rozpuszczalniki (0.1% etanol lub DMSO) {płyn F2}. Po inkubacji układu H-NH w każdym z roztworów (F1 i F2) przez 20 min płyn inkubacyjny pobierano i natychmiast zamrażano do czasu oznaczenia w zebranych próbkach zawartości OT i VP metodą RIA.
Wyniki: 8-Br-cAMP zwiększał wydzielanie OT i VP z układu H-NH gdy preinkubacja tego układu odbywała się w medium kontrolnym, podczas gdy inhibitory PKA (cAMPS-Rp oraz H-89) eliminowały wpływ analogu cAMP na wyrzut OT i VP. Melatonina (10^{-7} M) zmniejszała podstawowe wydzielanie OT i VP z układu H-NH, jak również hamowała (w obydwu badanych stężeniach) pobudzane przez 8-Br-cAMP wydzielanie obydwu neurohormonów w warunkach kontrolnych. Gdy w celu przerwania szlaku cAMP/PKA zastosowano cAMPS-Rp, ale nie H-89, hamujący wpływ melatoniny na wyrzut OT i VP do płynu inkubacyjnego był całkowicie zniesiony.
Wnioski: Melatonina wykorzystuje szlak sygnałowy cAMP/PKA do hamowania wydzielania OT i VP z układu H-NH szczura, niemniej, nie można wykluczyć udziału w tym procesie innych zależnych od cAMP mechanizmów. **(Endokrynol Pol 2018; 69 (5): 560–566)**

Słowa kluczowe: *melatonina, oksytocyna, wazopresyna, 8-Br-cAMP, cAMPS-Rp, H-89, PKA*

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Introduction

Melatonin affects the function of many organs and cells, mainly via membrane MT_1 and MT_2 receptors, which belong to the G-protein-coupled receptor (GPCRs) superfamily [1–3]. Together with ligand-gated ion channels and intracellular mechanisms, GPCRs are known to be engaged in the stimulation of magnocellular neuron activity and the secretion of neurohypophysial hormones [4, 5]. Recent studies have shown that a subtype MT_1 membrane receptor mediates the inhibitory impact exerted by melatonin on vasopressin (VP) [6] and oxytocin (OT) [7] secretion from the rat hypothalamo-neurohypophysial system.

Activation of the MT_1 receptor by melatonin results in induction of two parallel intracellular pathways [2, 8]. One of them engages adenylyl cyclase (AC) and G_i proteins that mediate the inhibition of the cyclic 3′,5′-adenosine monophosphate (cAMP)-dependent signal transduction pathway, including inhibition of protein kinase A (PKA) activity (i.e. cAMP/PKA pathway) and the nuclear factor — cAMP response element-binding protein (CREB) phosphorylation. The MT_1 receptor can also couple to $G_{q/11}$ proteins, which induces a phospholipase C (PLC)-dependent signalling pathway, with intensification of phosphoinositide turnover and stimulation of diacylglycerol (DAG) and inositol triphosphate (IP_3) formation; this results in protein kinase C (PKC) activation (i.e. DAG/PKC pathway) and greater cytosolic free calcium ion accumulation [2, 3, 8–10].

Melatonin has been shown to inhibit the elevation in cAMP accumulation induced by forskolin (forskolin strongly activates AC and increases cAMP production) in the rat pituitary [10, 11]. In mammals, cAMP exerts its effects through a few direct intracellular targets, i.e. cAMP-dependent PKA, the exchange proteins directly activated by cAMP (EPACs), and cyclic nucleotide-gated cation channels (CNGCs) [12–14]. It has been found that 8-Br-cAMP, a membrane-permeable cAMP analogue with increased hydrolytical stability, is a strong activator of cAMP-dependent PKA [15] and is able to stimulate OT and VP release in vitro [16]. Moreover, it is known that the transcription of VP gene in the rat hypothalamus is regulated by CREB: a cAMP-dependent transcription factor [17]. Previous in vitro studies have shown that melatonin significantly inhibits forskolin-stimulated OT and VP output from isolated rat hypothalamo-neurohypophysial explants when used at physiological concentration in rat blood (i.e. 10^{-9} M) and above (10^{-7} M), i.e. close to its level in the cerebrospinal fluid [18]; this observation suggests that cAMP-mediated mechanism(s) play a role in the influence of melatonin on OT and VP release.

To disrupt the cAMP/PKA signalling pathway, several pharmacological inhibitors of PKA can be employed. Two of them, i.e. cAMPS-Rp (which acts as a competitive cAMP antagonist which inhibits PKA activation) [19, 20] and H-89 (a potent inhibitor of PKA and some other kinases) [20–22], have been shown to antagonise the effects of cAMP/PKA stimulators, such as forskolin [21, 23, 24], pituitary adenylate cyclase-activating polypeptide (PACAP) [25–27], or cAMP analogue(s) [25–29]. These two PKA inhibitors have also been used frequently when studying the role of the cAMP/PKA-dependent signal transduction cascade in CNS function and behaviour [5, 22, 30–33]. Therefore, the purpose of the present investigation was to evaluate the contribution of the cAMP/PKA signalling pathway in melatonin-dependent inhibition of OT and VP secretion from an isolated rat hypothalamo-neurohypophysial system, which has not been studied yet. To achieve this aim, the study employs the PKA inhibitors (cAMPS-Rp and H-89) and the PKA activator, i.e. cAMP analogue (8-Br-cAMP).

Material and methods

Animals

Adult male Wistar rats, weighing 260–360 g, from the breeding stock of the Medical University of Lodz, Poland, were housed in quarters with controlled temperature (22 ± 1°C), constant humidity, and regulated light/dark (L:D) cycles (12L/12D; light from 06.00 to 18.00 h). The animals had free access to commercial pelleted food (LSM, Bacutil, Poland) and tap water.

Compounds and reagents

Melatonin (N-acetyl-5-methoxytryptamine) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemie GmbH. 8-Br-cAMP (8-Bromoadenosine-3′,5′-cyclic monophosphate) (cAMP analogue; activator of the cAMP-dependent PKA), H-89 (N-[2-[[(3-(4-Bromophenyl)-2-propenyl]amino] ethyl]-5-isoquinolinesulfonamide dihydrochloride) (an inhibitor of PKA and some other kinases) and cAMPS-Rp ([R]-Adenosine, cyclic 3′,5′-[hydrogen phosphorothioate] triethylammonium) (a potent cAMP antagonist that inhibits PKA activation) were obtained from Tocris Bioscience. Synthetic OT (Oxytocin synth.) and VP (Vasopressin synth.), used for iodination with ^{125}I and for preparation of the standard curve, came from Peninsula Laboratories Europe Ltd.

Experimental procedure

On each day of the experiment, the rats were decapitated between 9:00 and 10:10 a.m. To obtain the hypothalamo-neurohypophysial (H-NH) system, the

brain and the pituitary with intact pituitary stalk were thoroughly removed from the skull, and then a block of hypothalamic tissue was rapidly dissected from the brain, as described previously [34]. Such explant contained the hypothalamic suprachiasmatic (SCN), supraoptic (SON), and paraventricular (PVN) nuclei with intact neuronal projections to the neurohypophysis [34]. Each explant of the H-NH system was immediately placed in a tube containing 1 mL of normal Krebs-Ringer (nK-R) buffer heated in a water bath to 37°C; the content and parameters of nK-R buffer have been described previously [6, 7, 34]. Additionally, to each tube where one H-NH explant was incubated, a mixture of 95% O_2 and 5% CO_2 was continuously delivered. At the beginning of the experiment, to stabilise the OT and VP secretion into the buffer, the H-NH explants were equilibrated for 80 min in 1 mL of nK-R buffer and these samples were discarded. Afterwards, the explants were first preincubated for 30 min in 1 mL of nK-R buffer (control — series A), nK-R buffer enriched with cAMPS-Rp (series B), or nK-R buffer enriched with H-89 (series C). Both PKA inhibitors, i.e. cAMPS-Rp and H-89, were given at a frequently-used concentration of 10 μM for PKA inhibition.

Immediately after the preincubation period, the explants were incubated for 20 min in 1 mL of nK-R buffer {fluid F1} and then in 1 mL of nK-R buffer enriched with the appropriate substances {fluid F2} for a further 20 min. In each series (A-C), seven different groups of formulation were used for fluid F2: Group 1 — melatonin vehicle (VEH — 0.1% ethanol; n: number of samples per group, n = 7–8), Group 2 — 10^{-7} M melatonin (10^{-7} M MT; n = 6–7), Group 3 — 10^{-9} M melatonin (10^{-9} M MT; n = 6–7), Group 4 — a solvent of 8-Br-cAMP (0.1% DMSO; n = 6–8), Group 5 — 10^{-5} M 8-Br-cAMP (n = 6–8), Group 6 — 8-Br-cAMP & 10^{-7} M MT (n = 6–8), and Group 7 — 8-Br-cAMP & 10^{-9} M MT (n = 6–8). The melatonin concentrations, i.e. 10^{-7} M and 10^{-9} M, were selected on the basis of the results of previous in vitro studies. After each 20-min incubation period, the media (fluids F1 and F2) were aspirated and immediately stored at –20°C, to be later assayed for OT and VP by the radioimmunoassay (RIA). To determine the in vitro release of OT and VP, a F2/F1 ratio was calculated for each H-NH explant, because the quantity of neurohypophysial hormone secreted into the buffer differs greatly, within one experimental group, from one H-NH explant to the other.

All series of the experiment were performed between 09.00 and 11.50 a.m. This timing was necessary because the expression of MT_1 receptor mRNA and melatonin binding in the rat pars tuberalis and SCN are raised during daytime [35], and because the H-NH system is responsive to exogenous melatonin during this period [36]. The experiments were performed with the approval (No. 75/ŁB 583/2011) of the Local Commission of Ethics for Animal Care.

Radioimmunoassay of OT and VP

The concentration of OT and VP in the incubation medium was determined for each sample in duplicate, using a double-antibody RIA [37]. Iodination of OT and VP with ^{125}I was executed by the chloramine-T method. Anti-OT and anti-VP antibodies were raised in rabbits, and their specification was given earlier [6, 7]. The anti-OT antibody titre was 1:80,000 (final dilution). The sensitivity of the OT assay was 2.55 pg per tube, and intra-assay coefficient of variation (CV), determined for several points of the standard curve, was less than 5.0%; the inter-assay CV was less than 8.5%. The terminal dilution of the anti-VP antibodies was 1:24,000. The sensitivity of this assay was 1.56 pg VP per tube and intra-assay CV (determined as for OT) was 3.3%; the inter-assay CV was 6.3%.

Statistical evaluation of the results

All data are expressed as mean ± standard error of the mean (SEM). The significance of the differences between means was assessed by one-way analysis of variance (ANOVA), followed by the post-hoc Fisher test; $p = 0.05$ was considered as the minimal level of significance. STATISTICA (version 12) software (StatSoft, Poland) was used for all calculations.

Results

8-Br-cAMP (i.e. cAMP analogue) significantly increased OT and VP secretion from the rat H-NH system in vitro when the explants were preincubated in the control medium (Figures 1A and 2A). This stimulatory effect was eliminated by preincubation of the explants with the medium enriched with PKA inhibitors, i.e. cAMPS-Rp (Figures 1B and 2B) or H-89 (Figures 1C and 2C). Under control conditions, 10^{-7} M melatonin, but not 10^{-9} M melatonin, significantly diminished basal OT (Fig. 1A) and VP (Fig. 2A) output from the rat H-NH system and inhibited the cAMP analogue-stimulated secretion of OT and VP, at both concentrations studied (Figures 1A and 2A). Such effects of melatonin on the secretion of both neurohypophysial hormones could not be seen when the explants were first preincubated in the presence of cAMPS-Rp (Figures 1B and 2B). In contrast, melatonin did not influence the basal release of OT and VP after preincubation of the explants with H-89; however, treatment with both 8-Br-cAMP and melatonin, 10^{-7} M and 10^{-9} M, significantly reduced OT and VP secretion, compared to 8-Br-cAMP alone (Figures 1C and 2C).

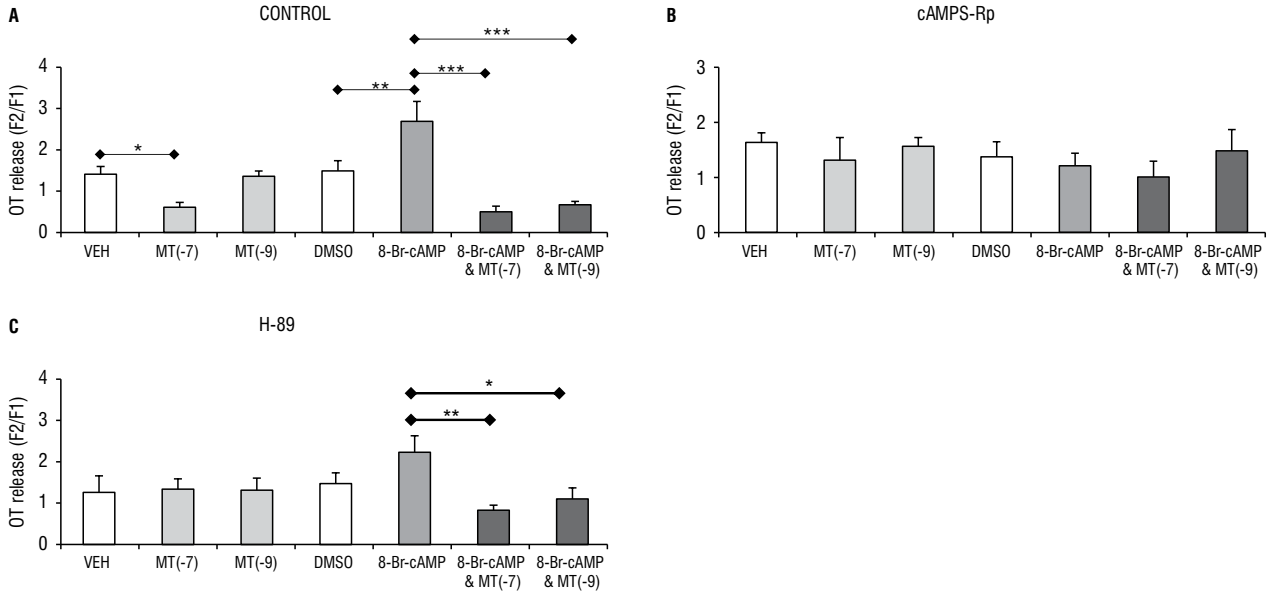

Figure 1. *The influence of melatonin (MT), at the concentrations of 10^{-9} and 10^{-7} M, on the basal and cAMP analogue (8-Br-cAMP)-dependent oxytocin (OT) release from the rat hypothalamo-neurohypophysial (H-NH) system in vitro, preincubated in normal K-R buffer (control) (**A**) or in the presence of PKA inhibitors — cAMPS-Rp (**B**) or H-89 (**C**). Each bar represents mean ± SEM; number of samples per group (n) = 6–8; $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.00005$*

Rycina 1. *Wpływ melatoniny (MT) w stężeniach 10^{-9} i 10^{-7} M na podstawowe i zależne od analogu cAMP (8-Br-cAMP) wydzielanie oksytocyny (OT) z układu podwzgórze–część nerwowa przysadki (H-NH) szczura in vitro, preinkubowanego w normalnym buforze K-R (kontrola) (**A**), bądź w obecności inhibitorów PKA — cAMPS-Rp (**B**) lub H-89 (**C**). Wyniki przedstawiają średnią ± SEM; liczba próbek w grupie (n) = 6–8; $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.00005$*

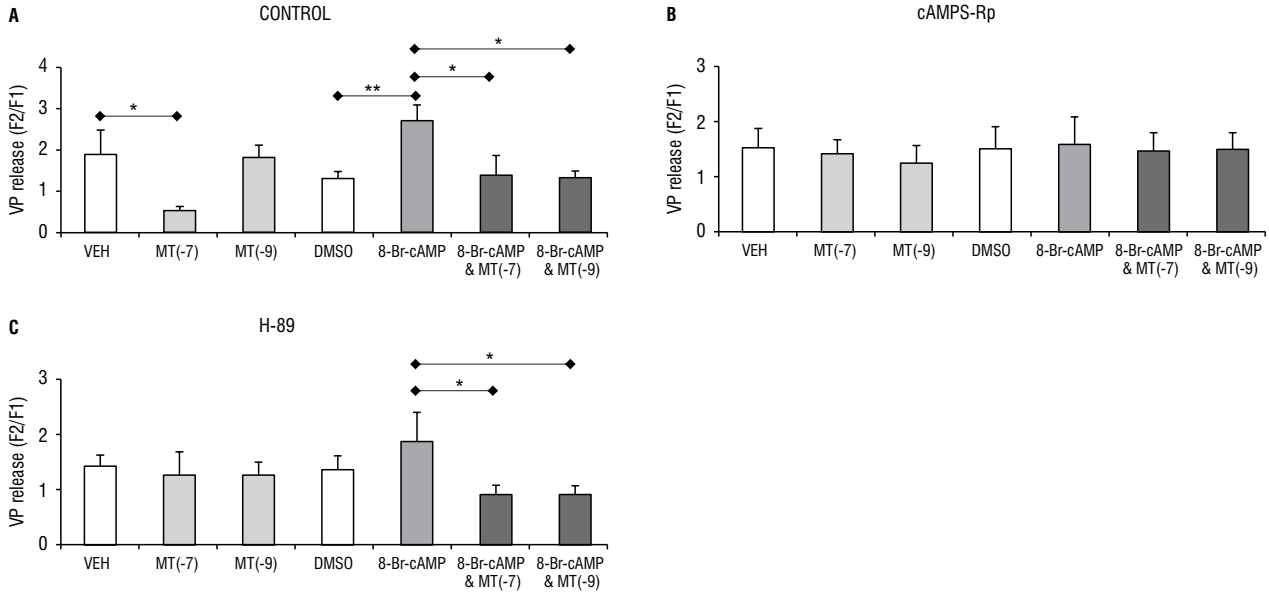

Figure 2. *The influence of melatonin (MT), at the concentrations of 10^{-9} and 10^{-7} M, on the basal and cAMP analogue (8-Br-cAMP)-dependent vasopressin (VP) release from the rat hypothalamo-neurohypophysial (H-NH) system in vitro, preincubated in normal K-R buffer (control) (**A**) or in the presence of PKA inhibitors — cAMPS-Rp (**B**) or H-89 (**C**). Each bar represents mean ± SEM; number of samples per group (n) = 6–8; $^{*}p < 0.05$, $^{**}p < 0.01$*

Rycina 2. *Wpływ melatoniny (MT), w stężeniach 10^{-9} i 10^{-7} M, na podstawowe i zależne od analogu cAMP (8-Br-cAMP) wydzielanie wazopresyny (VP) z układu podwzgórze–część nerwowa przysadki (H-NH) szczura in vitro, preinkubowanego w normalnym buforze K-R (kontrola) (**A**), bądź w obecności inhibitorów PKA — cAMPS-Rp (**B**) lub H-89 (**C**). Wyniki przedstawiają średnią ± SEM; liczba próbek w grupie (n) = 6–8; $^{*}p < 0.05$, $^{**}p < 0.01$*

Discussion

Our findings indicate that the cAMP analogue (8-Br--cAMP) has a stimulatory role on OT and VP secretion from the rat hypothalamo-neurohypophysial (H-NH) system in vitro. This observation confirms that cAMP plays a significant role in the regulation of neurohypophysial hormone release and is in line with previous observations [16]. Additionally, by demonstrating the ability of potent PKA inhibitors to negate the stimulatory effect of 8-Br-cAMP on OT and VP output from the rat H-NH system in vitro, our findings confirm that active PKA is a prerequisite for cAMP-mediated excitation of neurohypophysial hormone secretion.

Inactive PKA consists of two regulatory and two catalytic subunits. For PKA to be activated, two molecules of cAMP bind to each regulatory subunit. This binding of cAMP molecules initiates the dissociation of two catalytic subunits from the regulatory subunits; these become catalytically active by binding adenosine triphosphate (ATP) and can then phosphorylate target substrate(s) [20, 38]. Several pharmacological inhibitors of PKA, which can easily cross cell membranes, have commonly been used to investigate the physiological functions of PKA, either individually or in combination. One such inhibitor is H-89, which has been found to act as a competitive antagonist of ATP at its binding site on the PKA catalytic subunits [20], and to offer great value in discriminating between the effects of PKA and other cAMP-regulated proteins such as EPACs. As a potent PKA inhibitor, H-89 has been found to abolish various effects of cAMP/PKA stimulation by forskolin, PACAP, or 8-Br-cAMP in vitro [21, 24, 25, 28, 29]. However, H-89 has also been found to have a range of properties not associated with PKA, and to restrain the activity of several kinases other than PKA, including protein kinase C (PKC), Rho-associated kinase (ROCK-II), extracellular signal-regulated kinase 1 and 2 (ERK 1/2), mitogen-activated protein kinase (MAPK), mitogen- and stress-activated protein kinase 1 (MSK1), and ribosomal protein s6 kinase (S6K1) [20, 39]. To provide more reliable results when studying the physiological functions of PKA, H-89 is usually employed in combination with other PKA inhibitors and/or analogues. Hence, the present experiment uses a combination of two PKA inhibitors: H-89 and cAMPS-Rp, which acts as a competitive antagonist of PKA by interaction with cAMP binding sites on the regulatory subunits [19, 20]. Our findings show, for the first time, that both PKA inhibitors are capable of negating the stimulatory effect of cAMP analogue on OT and VP secretion from the rat H-NH system. This observation is in agreement with those of previous studies demonstrating the ability of H-89 and/or cAMPS-Rp to antagonise various effects of cAMP/PKA stimulators under a range of experimental conditions [25, 26, 28, 29]. For example, H-89 antagonised the effect of 8-Br-cAMP on epidermal growth factor (EGF)-induced ERK2 phosphorylation in primary cultures of rat hepatocytes [29], while in another study, 8-Br-cAMP and PACAP treatment significantly increased nitric oxide synthase type 1 (NOS1) level in rat pituitary gonadotrophs in vitro, and H-89 was able to inhibit such stimulation [25].

Previous studies strongly suggest that the inhibitory impact exerted by melatonin on OT and VP secretion from the rat H-NH system engages the cAMP-mediated mechanism(s) [18]. However, although it is likely that PKA is engaged in the intracellular cAMP-mediated signalling pathway in the melatonin-dependent inhibition of neurohypophysial hormone release, this remains to be demonstrated. Therefore, it was hypothesised in the present study that the cAMP/PKA signal transduction pathway is engaged in melatonin-dependent inhibition of OT and VP release. To demonstrate such mediation, cAMPS-Rp and H-89 were used, these being two PKA inhibitors with different mechanisms of action: cAMPS-Rp blocks the activity of both the regulatory and catalytic subunits of PKA, while H-89 mainly blocks the catalytic subunits. The obtained results confirm our hypothesis that the cAMP/PKA signal transduction pathway is engaged by melatonin to inhibit OT and VP output from the rat hypothalamo-neurohypophysial system; however, the precise mechanism of its action is more complicated and remains to be elucidated. Our present finding that cAMPS-Rp application completely blocks the effects of melatonin on OT and VP secretion suggests that the regulatory subunits of PKA need to be active to allow melatonin to exert its inhibitory effect on the basal and cAMP analogue-stimulated secretion of the neurohypophysial hormones. In mammals, an existence of two classes of regulatory subunits (RI and RII) and two subtypes (α and β) in each class determine a basis for classification of four PKA isoenzymes: RIα, RIβ, RIIα, and RIIβ [40], which play a crucial role in neuronal functions and are distributed in different brain regions [41]. In the mouse brain, the map of the intracellular distribution of PKA regulatory subunits shows that in the neuron, subunit RIβ is present in cell bodies and dendrites, whereas subunit RIIβ is more concentrated in the axons [41]. As explants containing both cell bodies, where OT and VP are synthesised, as well as the axons of oxytocinergic and vasopressinergic neurons, were used in the present in vitro experiment, it may suggest that activated by melatonin the cAMP/PKA-mediated pathway is engaged in the modification of synthesis, axonal transport, and/or secretion of the neurohypophysial hormones. In addition, because melatonin, acting via the MT_1 receptor, participates in cAMP/PKA-dependent inhibition of the nuclear factor

CREB phosphorylation [2, 3, 9], and CREB was found to play a role in cAMP-mediated regulation of VP gene transcription in the rat hypothalamus [17], it is possible that nuclear factor CREB may be involved in the melatonin-dependent cAMP/PKA-mediated inhibition of OT and VP synthesis and/or secretion from the rat H-NH system. This, however, remains to be demonstrated and does not exclude the participation of other transcription factors and/or other mechanisms.

Namely, H-89 was able to block the effect of melatonin on the basal secretion of OT and VP, but it did not interfere with the inhibitory action of melatonin on their release in the presence of the cAMP analogue in the medium. Therefore, it should be considered that melatonin may exert an influence on OT and VP release by cAMP-mediated mechanisms other than the cAMP/PKA signalling pathway. As mentioned above, H-89 inhibits the activity of several protein kinases and has various non-PKA-based effects. In addition, melatonin is known to act by modulating signal transduction cascades mediated by various kinases other than PKA, such as MAPK, ERK1/2, and PKC [42–45]. Melatonin, acting via the MT_1 receptor, and the cAMP-mediated pathways may also elicit signalling responses involving the regulation of chloride, sodium, potassium, or calcium fluxes and of specific ion channels, such as cAMP-mediated phosphorylation of calcium-activated potassium channels (BK_{Ca}), voltage-dependent calcium channels (VDCC), or inward-rectifier potassium channels (Kir), depending on the cell type [2, 46]. For example, melatonin is thought to inhibit the neuronal firing in the SCN by the activation of Kir3 channels [2]. Melatonin is also able to affect the production of reactive oxygen species, among others nitric oxide [47], while the cAMP/PKA signalling pathway has been found to induce the expression of NOS1 in rat pituitary cells in vitro [28]. In this context, it should be mentioned that together with various ions, e.g. potassium and calcium, nitric oxide [48] plays a significant role in the neurosecretory function of magnocellular neurons [5, 49, 50]. Therefore, it is possible that in the current study, under the conditions of PKA catalytic subunits blockade by H-89, melatonin might employ an alternate route to inhibit cAMP analogue-dependent OT and VP output from the rat H-NH system. Moreover, the explant used for our present in vitro study was isolated from other brain regions, which in normal conditions deliver excitatory and/or inhibitory afferent signals, through several neuromediators and neuromodulators, to the magnocellular nuclei [49–51]. The lack of such signals could hide or modify the effect of melatonin on neurohypophysial hormone release through the cAMP/PKA signalling pathway.

In conclusion, our results show, for the first time, that melatonin employs the cAMP/PKA signal transduction pathway to inhibit OT and VP secretion from the rat hypothalamo-neurohypophysial system. However, other cAMP-mediated mechanisms are not excluded from this process and future studies are needed to determine which of them, possibly including the contribution of transcription factors, are responsible for the inhibitory influence of melatonin on neurohypophysial hormone synthesis and/or secretion.

Funding

The study has been supported by Medical University of Lodz, contract No. 503/6-103-01/503-61-001.

Conflict of interest

The authors declare that they have no competing financial interests

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