EFFECT OF MELATONIN ON THE VASOPRESSIN SECRETION AS INFLUENCED BY TACHYKININ NK-1 RECEPTOR AGONIST AND ANTAGONIST: IN VIVO AND IN VITRO STUDIES

Department of Pathophysiology and Experimental Neuroendocrinology and Department of General and Experimental Pathology, Medical University of Łódź, Poland

The aim of the present study was to investigate the influence of melatonin on vasopressin (AVP) release from the rat hypothalamo-neurohypophysial (H-NH) system, both in vivo and in vitro, possibly modified by the peptide NK-1 and/or NK-2 receptor agonists and antagonists. Highly selective NK-1 receptor agonist, i.e., [Sar\(^9\),Met(O\(^{2}\))\(^{11}\)]-Substance P, has been shown to enhance the AVP release from isolated rat H-NH system in vitro, while the NK-1 receptor antagonist - (Tyr\(^6\),D-Phe\(^7\),D-His\(^9\))-Substance P (6-11) as well as the NK-2 receptor selective agonist - (\(\beta\)-Ala\(^8\))-Neurokinin A (4-10) and antagonist - (Tyr\(^5\),D-Trp\(^6,8,9\),Lys-NH\(^2\)\(^{10}\))-Neurokinin A (4-10) were essentially inactive in modifying AVP secretion. Melatonin inhibited basal release of AVP but was not able to reduce significantly the in vitro response of vasopressinergic neurones to NK-1 receptor agonist. After intracerebroventricular (icv) administration, substance P (SP), neurokinin A (NKA) and the NK-1 receptor agonist (all at the concentration of 10\(^{-7}\) M/L) significantly enhanced plasma AVP concentration. Such stimulatory effect of the latter peptide on AVP output from the neurohypophysis was reduced by an intravenous (iv) injection of melatonin, which itself (at a concentration of 5 ng/ml) caused a significant decrease in AVP release 10 min after injection. The inhibitory influence of melatonin on the AVP secretion was absent in rats injected icv with both tachykinin receptors antagonists, the NK-2 receptor agonist or NKA. The present data indicate a distinct role for NK-1 receptor in NKA/SP-mediated regulation of AVP release from the rat H-NH system. They have also shown that, under present experimental conditions, the stimulatory effect of NK-1 receptor activation on AVP secretion into the blood is sensitive to inhibitory influence of melatonin.

Key words: NK-1/NK-2 receptors, substance P, neurokinin A, melatonin, vasopressin
INTRODUCTION

The hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei, where vasopressin (AVP) is synthesized, are innervated by a great number of fibres containing tachykinins (1 - 4) – a family of peptides involved in the regulation of, among others, secretion of pituitary hormones (5 - 8). The hypothalamic SON and PVN as well as the posterior pituitary, from which AVP is released into the blood, contain the major members of this family of peptides, i.e., substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), and they co-express the respective tachykinin receptors (9 - 14). Moreover, a strong evidence for SP-immunoreactive contacts on AVP-containing neurons has been obtained by Heike et al. (15).

The SP and NKA are protein products encoded by the same pre-protachykinin A (PPT-A) gene, while the pre-protachykinin B gene (PPT-B) encodes only for NKB (5). These tachykinins exert their biological activity via specific tachykinin receptors, which are widely distributed in both central and peripheral nervous system (12, 16 - 18). The three receptors are: NK-1 (neurokinin-1), NK-2 (neurokinin-2) and NK-3 (neurokinin-3) receptors. They all belong to the family of G-protein-coupled receptors showing seven membrane-spanning regions. An amino acid sequence of these receptors is over 65 per cent homologous, but it is postulated that each tachykinin acts as an endogenous ligand of its own receptor. Indeed, SP is mainly a ligand of the NK-1 receptor, the NKA and NKB are natural ligands of the NK-2 and NK-3 tachykinin receptors, respectively. However, each of them is simultaneously an agonist of tachykinin receptors of every type (18).

Numerous studies have proved an important role of NKB in modulation of the AVP secretion in the rat and a significant participation of NK-3 receptor in the respective mechanisms is now well established (12 - 13). Much less is known, however, about the NK-1 and NK-2 receptors, especially the latter. A role of SP and/or NKA in the control of AVP secretion has already been confirmed by results of both in vitro (6 - 7, 19 - 20) and in vivo (3, 21 - 23) studies. As protein products of the same gene, SP and NKA are mostly co-localized and co-synthesized in NKA/SP-ergic neurons (24 - 25). However, there are data suggesting that SP and NKA are differentially involve in the control of hypothalamic magnocellular neurons function, which could result from stimulation of different classes of tachykinin receptors. Recently, a distinct role for NK-1 receptor in tachykinin-mediated stimulation of AVP (20) and oxytocin (26) release from the rat hypothalamo-neurohypophysial system in vitro has been described. So far, the influence of NK-1 and/or NK-2 receptors on AVP secretion from the rat posterior pituitary into the blood has not been evaluated. Therefore, the first goal of the present experiments was to study the in vivo effect of peptide NK-1 and NK-2 receptors agonists and antagonists on AVP release from the rat neurohypophysis into the blood.

There are several lines of evidence that point to pineal gland participating in the regulation of neurohypophysial hormones secretion under different
experimental conditions (27). A recently published study showed that AVP-containing neurons in the PVN and SON co-express MT1 melatonin receptor (28). Melatonin was also found to inhibit the NKA- and SP-stimulated (6 - 7) or NK-1 receptor agonist-stimulated (26) oxytocin output from the rat hypothalamo-neurohypophysial system in vitro. To date, however, the effect of melatonin on AVP release from the rat hypothalamo-neurohypophysial system, possibly modified by the NK-1 and/or NK-2 receptor agonists or antagonists, has not been studied. Therefore, the second aim of the present investigation was to evaluate, both in vivo and in vitro, whether the influence of peptide NK-1 and/or NK-2 receptor agonists (or tachykinin receptors natural ligands, i.e., SP and NKA) and antagonists on the AVP secretion could be modified by melatonin.

MATERIALS AND METHODS

Animals

Three-months old male Wistar rats (weighing 320 ± 50 g) were maintained under conditions of constant temperature, humidity and lighting (a 12/12 hr light/dark schedule; lights on from 6 a.m.). The animals received standard pelleted food and had free access to tap water.

Drugs

The [Arg8]-Vasopressin, for standard curve preparation as well as for iodination with 125I, was purchased from Peninsula Laboratories Europe Ltd., melatonin (N-acetyl-5-methoxytryptamine) from Sigma-Aldrich Chemie GmbH, and the peptides, i.e.: substance P, tachykinin NK-1 receptor agonist [(Sar9,Met(O2)11)-Substance P] and antagonist [(Tyr6,D-Phe7,D-His8)-Substance P (6-11)] as well as neurokinin A, tachykinin NK-2 receptor agonist [(β-Ala9)-Neurokinin A (4-10)] and antagonist [(Tyr5,D-Trp6,8,9,Lys-NH210)-Neurokinin A (4-10)], come from BACHEM AG, Bubendorf, Switzerland.

Experiment in vitro (series I)

On the day of experiment, animals were decapitated between 10.00 and 11.00 a.m. The brain and the pituitary with intact pituitary stalk were carefully removed from the skull and a block of hypothalamic tissue was dissected as previously described (6). Such hypothalamo-neurohypophysial (H-NH) explant was placed immediately in one polypropylene tube with 1ml of Krebs-Ringer fluid (KRF) containing: 120 mM NaCl, 5 mM KCl, 2.6 mM CaCl2, 1.2 mM KH2PO4, 0.7 mM MgSO4, 22.5 mM NaHCO3, 10 mM glucose, 1.0 g/l bovine serum albumin and 0.1 g/l ascorbic acid (pH = 7.35 - 7.45, osmolality = 285 - 295 mOsm/Kg). Tubes were placed in a water bath at 37°C and constantly gassed with carbogen (a mixture of 95% O2 and 5% CO2). At the beginning of experiment, the H-NH explants were equilibrated in KRF which was aspirated twice and replaced with 1 ml of fresh buffer. After 80 min of such preincubation, the media were discarded and explants were incubated for 20 min in 1 ml of KRF alone or comprised either melatonin vehicle (i.e., 0.1% ethanol; VEH) or melatonin solution at the concentration of 10-9 M/L (MLT) and enriched with the respective peptide. Explants were therefore incubated first in normal KRF {B1} and thereafter in one of the following media: incubation fluid as B1 containing either VEH (n - number of samples per group; group 1, n = 9) or MLT (group 2, n = 9), KRF enriched
with the NK-1 receptor agonist and VEH (group 3, n = 6) or MLT (group 4, n = 6), KRF containing the NK-1 receptor antagonist and VEH (group 5, n = 7) or MLT (group 6, n = 7), KRF containing the NK-2 receptor agonist and VEH (group 7, n = 6) or MLT (group 8, n = 6), KRF enriched with the NK-2 receptor antagonist and VEH (group 9, n = 7) or MLT (group 10, n = 7); all peptides at the concentration of 10^{-7} M/L {B2}. After each incubation period, the media were aspirated and samples immediately frozen and stored at -20°C until AVP estimation by the radioimmunoassay (RIA). To determine the AVP secretion, the B2/B1 ratio was calculated for each H-NH explant.

**Experiment in vivo**

On the day of experiment, 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. (29). Immediately after the end of icv cannulation, a polyethylene cannula (rinsed with 25 U heparin/ml saline) was introduced into the left femoral vein (iv). During experiments in vivo rats were given an icv injection of 5 µl 0.9% NaCl alone or containing the respective peptide at the concentration of 10^{-7} M/L. The icv injections were given via polyethylene tube attached to a 10 µl Hamilton syringe filled with the appropriate solution. Ten min later, animals were treated iv with 0.2 ml of melatonin solution (at a dose of 5 ng/ml) or with 0.2 ml of melatonin vehicle, i.e., 0.1% ethanol in 0.9% NaCl. Ten min after iv administration of melatonin or its vehicle, animals were decapitated and the hypothalamus, neurohypophysis and plasma samples were collected and frozen before AVP estimations by RIA, as previously described (30 - 31).

**Series II: the effect of substance P (SP), the NK-1 receptor agonist or antagonist and melatonin on AVP secretion from the rat neurohypophysis into the blood was tested.** The following experimental groups were chosen. Rats treated iv with VEH or MLT and icv with the respective solution of: a - saline (control group: n = 7 - 8), b - SP (n = 7 - 8), c - the NK-1 receptor agonist (n = 7 - 8) or d - the NK-1 receptor antagonist (n = 7 - 8).

**Series III: the effect of neuropeptide A (NKA), the NK-2 receptor agonist or antagonist and melatonin on AVP secretion was examined.** In this series, similar experimental groups were chosen, i.e., rats treated iv with VEH or MLT and icv with the solution of: a - saline (control: n = 7 - 8), b - NKA (n = 7 - 8), c - the NK-2 receptor agonist (n = 7 - 8) or d - the NK-2 receptor antagonist (n = 7 - 8).

All the experiments were done at a time when the H-NH system was found to be responsive to melatonin, i.e., from 10:00 till 12:00 a.m. (32). The experimental procedures were done with the consent (No L/BD/82 and 14/LB320/2006) of the Local Committee for Animal Care.

**Radioimmunoassay**

The concentration of AVP in all samples was determined by the RIA method described previously (30 - 31), using an antiserum prepared by Dr. Monika Orlowska-Majdak (Department of Experimental Physiology, Chair of Experimental and Clinical Physiology, Medical University of Lodz). The anti-AVP antibody titer used in the RIA was 1:50,000 (final dilution). Cross reactivity for these antibodies with oxytocin was 0.016%, with lysine vasopressin was 2.7%, and with gonadotropin releasing hormone, thyrotropin releasing hormone, leucine enkephalin, angiotensin II, substance P and hexapeptide (PyrGlu^6Tyr^8)SP^6-11 was less than 0.002% (see: 30). For iodination with \textsuperscript{125}I, the chloramine-T method, was employed. The lower limit of detection for the assay was 1.25 pg AVP/100 µl, and intra-assay coefficient of variation was less than 3.5% (all samples within the same experimental series were tested in the same RIA to avoid inter-assay variability).
Statistical evaluation of the results

Hormone release in vitro and its hypothalamic, neurohypophysial and plasma levels are expressed as a percentage of the control value. Results were calculated and expressed as means ± S.E.M. Statistical significance was determined by use of the Kruskal-Wallis analysis of variance (ANOVA) for each set of data (all subgroups) followed by Mann-Whitney “U” test (two means comparison); p<0.05 was considered as the minimal level of significance.

RESULTS

Series I. The NK-1 receptor agonist [(Sar⁹,Met(O₂)¹¹)-Substance P] stimulated the AVP secretion from isolated rat H-NH system. Melatonin failed to inhibit significantly this effect, although, it diminished by itself basal release of AVP when compared to the vehicle (control group). The NK-1 receptor antagonist [(Tyr⁶,D-Phe⁷,D-His⁹)-Substance P (6-11)] could not modify the AVP secretion into the medium, neither alone nor in the presence of melatonin (Fig. 1). Again, neither the NK-2 receptor agonist [(ß-Ala⁸)-Neurokinin A (4-10)] nor the NK-2

![Figure 1](image_url)

*Fig. 1. Effect of melatonin (MLT) on the vasopressin (AVP) release from the rat hypotalamo-neurohypophysial complex in vitro as influenced by the NK-1 receptor agonist [(Sar⁹,Met(O₂)¹¹)-Substance P] or antagonist [(Tyr⁴,D-Phe³,D-His⁸)-Substance P (6-11)] and the NK-2 receptor agonist [(ß-Ala⁸)-Neurokinin A (4-10)] or antagonist [(Tyr⁴,D-Trp⁶⁸⁹,Lys-NH₂¹⁰)-Neurokinin A (4-10)]. Each bar represents mean ± S.E.M.; a p<0.05 - significantly different versus control-VEH, b p<0.05 - significantly different versus control-MLT.*
receptor antagonist [(Tyr<sup>5</sup>,D-Trp<sup>6,8,9</sup>,Lys-NH<sub>2</sub><sup>10</sup>)-Neurokinin A (4-10)] were able to affect the AVP output from isolated rat H-NH system, irrespective of the presence of melatonin or its vehicle in the incubation medium (Fig. 1).

**Series II.** The hypothalamic levels of AVP were not modified in vivo by any of the studied substances (Fig. 2, 3). Plasma AVP concentration was diminished and its neurohypophysial level was significantly higher after iv injection of melatonin (Fig. 2). The icv administration of SP or the NK-1 receptor agonist resulted in the rise of plasma AVP concentration. In rats treated icv with the NK-1 receptor agonist, the neurohypophysial content of AVP was significantly increased and its plasma concentration was decreased by iv injection of melatonin, when compared to VEH-injected animals. On the other hand, the icv injection of NK-1 receptor antagonist resulted in diminution of plasma AVP concentration and augmentation the neurohypophysial AVP content in VEH-treated rats, which were not further modified by melatonin (Fig. 2).

**Series III.** The inhibitory effect of melatonin on AVP secretion into the blood (found in the control group) could not bee seen in animals icv-administered by NKA, the NK-2 receptor agonist or antagonist. Although, the NK-2 receptor antagonist significantly increased the neurohypophysial AVP content in vehicle-injected rats, this peptide was not able to modify plasma AVP concentration, irrespective of the iv treatment with melatonin or its vehicle (Fig. 3).

**DISCUSSION**

*Tachykinins receptors and vasopressin release*

The neurohypophysial hormones synthesis and secretion is known to be stimulated by several factors, e.g., haemorrhage (33 - 34), dehydration (30), hiperosmotic stimulation (35), angiotensin II (33 - 34) or stress (36). The response of magnocellular vasopressinergic (AVP-ergic) and/or oxytocinergic (OT-ergic) neurones to such stimuli depends on the presence in the central nervous system of numerous agents (3 - 4), among which NKB has been shown to play an important role, especially in mediating (through activation of NK-3 receptor) the effect of plasma hiperosmolality on AVP secretion (12 - 13). Chronic osmotic stimulation was also found to be associated with increased PPT-A mRNA synthesis in the hypothalamus (10) and high levels of SP and NKA in the SON (9). Results of the present experiments clearly indicate that the NK-1 receptor is also involved in tachykinin-stimulated AVP secretion from the rat H-NH system. They are in concordance with previous in vitro data showing the stimulatory effect of SP on the release of neurohypophysial hormones secretion (6 - 7, 19) and indicating a distinct role for NK-1 receptor in tachykinin-mediated stimulation of OT secretion from the H-NH system (26).

Intracerebroventricular (icv) administration of SP was described to be associated with an increase in firing rate of AVP-ergic neurones of the SON (37) and plasma
Fig. 2. Hypothalamic [Hth; top panel], neurohypophysial [NH; middle panel] and plasma [low panel] vasopressin (AVP) levels in control or injected with: substance P {SP}, the NK-1 receptor agonist or antagonist as well as vehicle (VEH)- or melatonin (MLT)-treated male rats. Each bar represents mean ± S.E.M.; a p<0.05 - significantly different versus control-VEH, b p<0.05 - significantly different versus control-MLT, c p<0.05 - significantly different versus VEH.
Fig. 3. Hypothalamic [Hth; top panel], neurohypophysial [NH; middle panel] and plasma [low panel] vasopressin (AVP) levels in control or injected with: neurokinin A {NKA}, the NK-2 receptor agonist or antagonist as well as vehicle (VEH)- or melatonin (MLT)-treated male rats. Each bar represents mean ± S.E.M.; a p<0.05 - significantly different versus control-VEH, b p<0.05 - significantly different versus control-MLT.
AVP concentration (22 - 23). Therefore, all studied peptides (i.e., natural ligands of
the NK-1 and NK-2 receptors and their agonists and antagonists) were injected into
the lateral ventricle of the brain. The peptides concentration we used for the studies
was $10^{-7}$ M/L and was chosen on the base of the previous (6 - 7, 20) data. The
stimulatory effect of icv injected SP on AVP output from the rat neurohypophysis
into the blood (present in vivo studies; Fig. 2), is in agreement with other in vivo
observations (22 - 23). Additionally, we have demonstrated, for the first time, that
centrally injected highly selective NK-1 receptor agonist enhanced significantly the
release of AVP into the blood, while icv injection of highly selective antagonist of
this receptor has exerted quite opposite effect on plasma hormone concentration
(Fig. 2). On the other hand, the icv injected highly selective NK-2 receptor agonist
and antagonist were essentially inactive in modifying the AVP release, while NKA
significantly enhanced the hormone secretion from the rat neurohypophysis (Fig.
3). Because NKA is known to be efficient as endogenous agonist of not only the
NK-2, but also the NK-1 and NK-3 receptors (16, 18), the stimulation of AVP
release by NKA may reflect activation of these two receptors. Such a mode of NKA
action is consistent with the results of binding studies showing abundance of central
NK-1 and NK-3 receptors (10, 12, 13, 16, 18) and only a small number of NK-2
binding sites, detected in some specific nuclei of the rat brain (17, 18).

Several lines of evidence have shown the presence of a variety of
neurotransmitters in SP- and/or NKA/SP-ergic neurons reaching the hypothalamus
and close interactions between tachykinins and such neuroactive substances as
acetylcholine, catecholamines, serotonin, $\gamma$-aminobutyric acid (GABA), glutamate
or ATP exist in different areas of the brain (1, 3, 19, 25, 38). SP-immonoreactive
(SP-IR) inputs to the magnocellular SON and PVN arise from a number of
hypothalamic nuclei, the laterodorsal and peduculopontine tegmental nuclei as
well as the ventrolateral medulla; some of these SP-IR neurones coexists with
tyrosine hydroxylase (TH-IR) or choline acetyltransferase (1, 10, 19). Both SP-IR
and TH-IR perikarya were found mainly within the noradrenergic $A_1$ but also
within $A_2$ and adrenergic ($C_1$, $C_2$) areas of the ventrolateral medulla (1, 10). The
above mentioned neurotransmitters and/or neuromodulators play an important role
in modifying AVP release from the rat posterior pituitary (3 - 4) and certain
combination of these neuroactive substances could be of some importance for the
mechanisms by which AVP-ergic neurones are influenced by tachykinins. The
results of present in vivo and in vitro experiments strongly suggest that central
endogenous SP and/or NKA contained in brain regions projecting to the
magnocellular AVP-ergic neurones influence the AVP secretion from the rat
neurohypophysis, acting mainly via NK-1 tachykinin receptor.

Influence of melatonin on tachykinin(s)-dependent vasopressin release

After systemic administration, melatonin crosses the blood-brain barrier and
in the cerebrospinal fluid (CSF) reaches a maximum level at 5 min; at 2 min the
level of $^{14}$C-melatonin in the brain was higher than in the CSF and 30 min later it was still detected in most of the brain regions analyzed (among others the hypothalamic PVN) (39). The influence of exogenous melatonin on AVP secretion from the rat neurohypophysis into the blood was found to be stimulatory or inhibitory, depending on a dose. Namely, the in vivo release of AVP was inhibited ten min after iv injection of melatonin at a single dose of 5 ng/ml, while higher (25 ng/ml) or lower (2.5 ng/ml) doses were ineffective in this respect. Moreover, higher dose of melatonin stimulated the AVP secretion twenty min after iv injection (40). After icv injection of melatonin in a dose range 1.0-10,000 ng/ml, it was found that a single dose of 10 ng/ml produced a significant fall in circulating plasma AVP concentration at 5 min after the injection, while the higher dose of 10 µg/ml stimulated hormone release (41); the authors conclude that melatonin concentration within the physiological range appears to be the most effective in inhibition of AVP release in vivo. The comparison of daily AVP rhythm in the neurohypophysis (the highest content in the morning) and blood plasma (the lowest concentration in the morning increasing over the light phase and falling during the night) in the rat (42) with daily fluctuation of melatonin synthesis, with peak and nadir levels during dark and light hours, respectively (43) strongly suggests the inhibitory influence of melatonin on AVP secretion into the blood under physiological conditions. The concentration of melatonin employed in the present experiments is at the range of physiological level of the hormone and has previously been found to inhibit the in vitro AVP release from isolated rat hypothalamus (32, 44), hypothalamo-neurohypophysial explants (6-7) or neurohypophysis (31, 36). Therefore, the inhibitory effect of melatonin on the AVP release that we observed (Fig. 1 - 3) is in concordance with previous in vitro (6-7) and in vivo (40) data.

Under present experimental conditions, we have also found that in melatonin-injected rats low plasma AVP level was accompanied by increased neurohypophysial hormone content but without respective changes in the hypothalamus (Fig. 2, 3). Such an observation could suggest that exogenous melatonin does not affect the AVP synthesis, which is consistent with our previous results showing no influence of melatonin on AVP content or biosynthesis rate in the rat hypothalamus (31). It is also possibly, that ten min is enough time for melatonin to inhibit AVP secretion, but it is not enough to influence a genome and significantly change the hormone synthesis, although melatonin is known to act directly on a genome (45) by activation in target tissues the nuclear RZR receptors, both α- and β-subtype (46).

The question as to the possible mechanisms underlying modulation of the AVP secretion by melatonin has not been adequately answered, yet. Since both melatonin and tachykinins might serve as neuromodulators of the AVP-ergic neurones activity, we assumed that possible functional interaction between melatonin and NKA/SP-ergic neurones in the hypothalamus and/or other brain regions may contribute to the process under discussion. Moreover, a recent
discovery of melatonin receptors in human magnocellular SON and PVN (28) suggested that also in the rat melatonin is able to affect directly the AVP-ergic neurones activity, acting on the level of these nuclei, which could refer to both in vivo and in vitro experimental conditions. Results of the present studies partly confirm such a suggestion, since the stimulatory influence of icv administered tachykinin NK-1 receptor agonist on AVP secretion was inhibited by iv injection of melatonin (Fig. 2).

Melatonin could, therefore, modify the NKA/SP-dependent AVP secretion in the rat acting via specific melatonin membrane receptors located in the pars tuberalis of the pituitary (47) or in the hypothalamus, especially those located in the SON, PVN (28) and/or suprachiasmatic (SCN) nuclei (48 - 49). It has been found that AVP-containing cells in the SCN express both MT1 (48) and MT2 (49) melatonin receptors, what allows them to respond to melatonin signal; melatonin inhibits the AVP release in the SCN-slice culture (49). The SCN neurons contain not only AVP, but also SP and pre-protachykinin-A mRNA (50), the specific tachykinin receptors (16) as well as the SP-immunoreactive fibres and nerve terminals (1, 50). Some of the SCN neurons could, therefore, integrate the afferent signals derived from SP-ergic neurones and/or melatonin (via their receptors) and thereafter transmit them to AVP-ergic neurones in the PVN and/or SON. The SCN neurons can influence function of the PVN (51) and SON (52) neurones by releasing from their axonal endings either excitatory (glutamate) or inhibitory (GABA) amino acids (51) which, on the other hand, are known to modify the AVP secretion (3).

The small amount of MT1 receptor expression was also observed in human posterior pituitary (28), which may suggest that melatonin exerts its influence on the AVP release acting not only at the level of hypothalamus, but also directly on the axonal endings located in the neurohypophysis. Moreover, the interaction of melatonin with its receptors present in other brain regions could not be excluded, especially in experiment in vivo. Melatonin alters the metabolism of some biogenic amines (53 - 54) and prostaglandins (55) in the hypothalamus. It could, therefore, influence the tachykinin-dependent AVP secretion also through indirect action, i.e., by affecting in the hypothalamus and/or neurohypophysis the metabolism of neuromediators and/or neuromodulators (such as acetylcholine, dopamine or prostaglandins), which have been described to contribute to melatonin-dependent inhibition of AVP secretion (40 - 44). The present data might, therefore, suggest that melatonin is implicated in the central control of water and electrolyte homeostasis through the influence on the NK-1 receptor-mediated AVP secretion; centrally administered SP induces antidiuresis and inhibits water and salt intake (56).

In summary: The present study provides an evidence, for the first time, that centrally injected NKA and SP stimulate the AVP release which is mediated mainly by the tachykinin NK-1 receptor. We have also revealed that the stimulatory effect of the NK-1 receptor on AVP secretion into the blood is
sensitive to inhibitory influence of melatonin. The physiological meaning of these data may concern the possible indirect role of melatonin in the central control of water metabolism in the rat.

Acknowledgements: This work has been supported by Medical University of Łódź, contracts: No. 502-16-140 and No. 503-6103-1.

REFERENCES


50. Mikkelsen JD, Larsen PJ. Substance P in the suprachiasmatic nucleus of the rat: an immunohistochemical and in situ hybridization study. Histochemistry 1993; 100: 3-16.


Received: June 4, 2007
Accepted: November 5, 2007

Author’s address: Marlena Juszczyk, Ph.D., D.Sc., Department of Pathophysiology and Experimental Neuroendocrinology, Medical University of Łódź, ul. Narutowicza 60, 90-136 Łódź, Poland. Tel/Fax: (+48) 42 630 61 87