Vasopressin release from the rat hypothalamo-neurohypophysial system: Effects of tachykinin NK–1 and NK–2 receptors agonists and antagonists

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Abstract

OBJECTIVES: Present experiments were undertaken to study the influence of peptide NK–1 and NK–2 receptor agonists and antagonists as well as substance P and neurokinin A (the natural ligands for these tachykinin receptors) on vasopressin (AVP) secretion from the rat hypothalamo-neurohypophysial (HN) system in vitro.

RESULTS: The results showed that both substance P and highly selective tachykinin NK–1 receptor agonist, i.e., [Sar9,Met(O2)11]-Substance P, enhanced significantly AVP secretion, while the NK–1 receptor antagonist (Tyr6,D–Phe7,D–His9)-Substance P (6–11) – sendide – was found to antagonize the substance P–induced hormone release from isolated rat HN system (all peptides at the concentration of 10–7 M/L). The NK–2 receptor selective agonist (β–Ala 8)–Neurokinin A (4–10) was essentially inactive in modifying AVP release from the rat HN system in vitro, while neurokinin A (the natural ligand for this tachykinin receptor) was found to stimulate the AVP release; this effect of neurokinin A has been diminished by the NK–2 receptor antagonist (Tyr5,D–Trp6,8,9,Lys–NH210)–Neurokinin A (4–10).

CONCLUSION: The present data indicate a role for tachykinin NK–1 (and possibly for NK–2) receptors in tachykinin-mediated stimulation of AVP secretion from the rat HN system in vitro.

Abbreviations:

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<th>Abbreviation</th>
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<tr>
<td>AVP</td>
<td>vasopressin</td>
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<td>HN</td>
<td>hypothalamo-neurohypophysial system</td>
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<td>KRF</td>
<td>Krebs–Ringer fluid</td>
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<td>NKA</td>
<td>neurokinin A</td>
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<td>PPT–A</td>
<td>preprotachykinin–A</td>
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<td>PVN</td>
<td>paraventricular nucleus</td>
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<td>SON</td>
<td>supraoptic nucleus</td>
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<td>SP</td>
<td>substance P</td>
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Introduction

Tachykinin NK–1 and NK–2 receptors are widely distributed in both central and peripheral nervous system [1–3] where they play a role in behavioural responses, autonomic reflexes, pain transmission and regulation the neuroendocrine function of the pituitary [2–3]. Natural ligands for these tachykinin receptors, i.e., substance P (SP)
and neurokinin A (NKA), are protein products encoded by the same preprotachykinin-A (PPT-A) gene and they are members of a family of peptides known as tachykinins [4]. Substance P has preferential affinity for the tachykinin NK-1 receptor, while NKA acts preferentially on tachykinin NK-2 receptor. However, the NKA is also efficient substitute of SP as endogenous agonist at NK-1 receptor and, in turn, SP could act as an agonist at NK-2 receptor [2, 5]. Moreover, these two tachykinins are mostly colocalized and cosynthesized in the same SP/NKA-ergic neurons [6, 7], which project to several brain areas including hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei [8, 9, 10]. Also the posterior pituitary contain SP-immunoreactive fibres and terminals close to the blood vessels [10, 11].

Magnocellular neurons of the hypothalamic PVN and SON nuclei produce vasopressin (AVP), a neurohormone stored in the neurohypophysis and secreted into general circulation in response to several stimuli, e.g., hypovolaemia [12], plasma hypertonicity [13] or stress [14]. The release of AVP depends on the presence of numerous neuromediators and neuromodulators, among which neuropeptides play a very important role [15, 16]. The SON and PVN and the posterior pituitary contain both SP and NKA [9, 11, 17–19] as well as the SP binding sites [18]. By direct stimulation of PVN magnocellular neurons tachykinins were shown to cause release of AVP [20]. Centrally administered SP has been found to increase the firing rate of vasopressinergic neurons of the SON [21], induce antidiuresis and inhibit water and salt intake [22]. A role for SP and/or NKA in the central control of AVP secretion is confirmed by results of both in vivo [15, 23–24] and in vitro [25–27] studies. To date, however, the functional importance of the tachykinin NK-1 and/or NK-2 receptors in AVP secretion from the rat hypothalmo-neurohypophysial system has not been evaluated. The goal of the present experiments was, therefore, to study the effect of peptide NK-1 and NK-2 tachykinin receptor agonists and antagonists on AVP secretion from the rat hypothalmo-neurohypophysial system in vitro.

**Materials and methods**

**Animals**

Three-months old male Wistar rats (weighing about 250–350 g) were housed under a 12/12 hr light/dark schedule (lights on from 06:00 hr) and at room temperature. They received standard pelleted food and had free access to tap water.

**Drugs**

All peptides, i.e.: substance P, tachykinin NK-1 receptor agonist [(Sar9, MetO2)11]-Substance P] and antagonist [(Tyr6, D-Phe2, D-His9]-Substance P (6–11)] (Sendide) as well as neuropepin A, tachykinin NK-2 receptor agonist [(β-Ala8]-Neurokinin A (4–10)] and antagonist [(Tyr6, D-Trp6, 8, 9]-Neurokinin A (4–10)], were purchased from BACHEM AG, Bubendorf, Switzerland.

**Experimental procedure**

The experimental procedures were done with the consent (No L/BD/82) of the Local Committee for the Animal Care.

On the day of experiment, animals were decapitated between 10.30 and 11.30 hr. 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 [26]. Such hypothalomo-neurohypophysial (HN) explant was placed immediately in one polypropylene tube with 1 ml 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 thereafter 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 HN explants were equilibrated in KRF which was aspirated twice and replaced with 1 ml of fresh buffer. After 80 minutes of such preincubation, the KRF was discarded and explants were incubated for 20 minutes in 1 ml of KRF alone or containing the respective peptide.

**Series I.** In the first series of experiments the effect of NK-1 or NK-2 receptor specific agonists on AVP secretion from the HN explant was tested. Explants were incubated successively in: (1) normal KRF {B1}; (2) the incubation fluid as B1 alone (control group) or containing either the NK-1 or NK-2 receptor agonist at the concentration of 10–7 or 10–9 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).

**Series II.** In the second series of experiments the effect of specific NK-1 (or NK-2) receptor antagonist on basal and SP- (or NKA)-stimulated AVP secretion was tested. After incubation in normal KRF {B1}, explants were consequently incubated in one of the following media:

- a – the incubation fluid as B1 (control),
- b – the KRF containing either NK-1 or NK-2 receptor antagonist at the concentration of 10–9 M/L,
- c – the KRF containing either NK-1 or NK-2 receptor antagonist at the concentration of 10–7 M/L,
- d – the KRF containing either SP or NKA alone,
- e – the KRF containing either SP together with the NK-1 receptor antagonist or NKA together with the NK-2 receptor antagonist, all 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 RIA. To determine the hormone secretion, the B2/B1 ratio was calculated for each HN explant in both series.
Radioimmunoassay

The concentration of AVP in all medium samples was determined by the RIA method described previously [13], using an antiserum prepared by Dr. Monika Orłowska-Majdak (Department of Experimental and Clinical Physiology, Institute of Physiology and Biochemistry, Medical University of Lodz). The anti-AVP antibody titer used in the RIA was 1:40,000 (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 (PyGlu6Tyr8)SP6–11 was less than 0.002%. 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% (all samples within the experiment were tested in the same RIA to avoid inter-assay variability). For standard curve preparation as well as for iodination with 125I, using the chloramine-T method, the AVP ([Arg8]-Vasopressin) from Peninsula Laboratories Europe Ltd. was used.

Statistical evaluation of the results

All data are expressed as means ± S.E.M. Significance of the differences between means was evaluated by use of the Kruskal-Wallis analysis of variance (ANOVA) by ranks for each set of data (all subgroups). Thereafter, the statistical significance of differences between means of two subgroups compared was determined by Mann-Whitney “U” test, using p<0.05 as the minimal level of significance.

Results

Series I. The secretion of AVP from isolated HN explants (ANOVA, p<0.005) was enhanced markedly when the NK-1 receptor agonist [(Sar9,Met(O2)11)-Substance P] was added to the buffer at the concentration of 10−7 M/L; this peptide failed, however, to affect the hormone release at the concentration of 10−9 M/L (Fig. 1). On the other hand, both concentrations (i.e., 10−9 and 10−7 M/L) of the NK-2 receptor agonist ([β-Ala8]-Neurokinin A (4–10)) were ineffective in modifying AVP output from isolated rat HN explants (Fig. 1).

Series II. Substance P significantly enhanced the AVP output from isolated rat HN system and the NK-1 receptor antagonist [(Tyr6,D-Phe7,D-His9)-Substance P (6–11) – sendide] completely blocked this stimulatory effect of SP (ANOVA, p<0.0001). When, however, sendide (at both concentrations) was added to the medium alone, the release of AVP was not different from the control (Fig. 2A). The NK-2 receptor antagonist [(Tyr5,D-Trp6,8,9,Lys-NH210)-Neurokinin A (4–10)], at the concentration of 10−9 M/L, significantly inhibited the AVP secretion into the medium (ANOVA, p<0.0001). When used at the concentration of 10−7 M/L, this peptide did not affect the basal release of AVP, but it significantly diminished the NKA-enhanced AVP secretion from isolated HN system (Fig. 2B).

Discussion

Results of this study show that SP – the natural ligand for tachykinin NK-1 receptor – and highly selective NK-1 receptor agonist [(Sar9,Met(O2)11)-Substance P] stimulate the AVP secretion from isolated rat HN complex. The stimulatory role of SP on AVP release is in accordance with earlier in vitro [25, 27] and in vivo [15, 23, 24] reports. Additionally, we have demonstrated, for the first time, that highly selective and extremely potent antagonist of the NK-1 receptor – sendide [28] antagonized the SP-induced AVP output from the rat HN system in vitro. Therefore, the present data show that SP stimulates the AVP secretion acting via tachykinin NK-1 receptor.

![Figure 1: Effect of the NK–1 receptor agonist [Sar8,Met(O2)11]–Substance P and the NK–2 receptor agonist ( –Ala8)–Neurokinin A (4–10), both at the concentration of 10−9 or 10−7 M/L, on vasopressin (AVP) release from the rat hypothalamo-neurohypophysial complex in vitro. Each bar represents mean ± S.E.M.; figures in bars indicate the number of samples in each subgroup; *p<0.05 – significantly different versus control](image-url)
Whereas NK-1 receptors are widely distributed in the central nervous system, including hypothalamic PVN and SON [2], the NK-2 receptors were found to be present only in some specific nuclei of the brain [1, 3]. However, in spite of the low level of NK-2 receptors in the hypothalamus, a high content of NKA in the PVN neurones were reported and, what is more, the level of NKA in the hypothalamus was even higher than that of SP [17, 18]. Moreover, the NKA has been described as an efficient agonist not only at the NK-2, but also at the NK-1 tachykinin receptor [5], which suggests that both SP and NKA affect the vasopressinergic neurones activity acting as physiological ligands for the NK-1 tachykinin receptor. The above facts could, therefore, support the hypothesis that in stimulatory effect of NKA on AVP release, found under present experimental conditions, the NK-1 (rather than NK-2) tachykinin receptors are involve, which is confirmed by the observation that the NK-2 receptor selective antagonist [(β-Ala¹⁰)-Neurokinin A (4–10)] was essentially inactive in modifying the AVP secretion from isolated rat HN system. On the other hand, however, the NK-2 receptor selective antagonist [(Tyr⁵,D-Trp³⁸,⁹,Lys–NH₂¹⁰)-Neurokinin A (4–10)] [29] diminished the stimulatory effect of NKA on AVP release, suggesting a certain role for this tachykinin receptor in the process under discussion.

By using very potent agonists and/or antagonists, the tachykinin NK-1 and/or NK-2 receptors were found to regulate function of the cardiovascular, respiratory, gastrointestinal and genitourinary systems [2, 29, 30]. Present data show that peptide agonists and antagonists of these two tachykinin receptors are involve also in regulation of the posterior pituitary neuroendocrine function. Some data indicate that hypothalamic SP may play a role in mediating the release of AVP in response to changes in plasma osmolality. Namely, chronic osmotic stimulation, which strongly increases AVP secre-
tion [12, 13], was found to be associated with increased levels of SP and NKA in the SON [18] as well as with increased PPT-A mRNA synthesis in the hypothalamus [9]. Moreover, centrally administered SP has been shown to inhibit water intake; such an inhibitory effect of tachykinins on drinking induced by hyperosmotic NaCl administration was found to be mediated by the NK-2 receptors, while the NK-1 receptors are probably involved in the inhibition of angiotensin II-induced drinking [22].

Hypothalamic magnocellular neurons contain a number of peptides coexisting with AVP and coreleased with this hormone under certain conditions. The functional role of these peptides is not fully understood, yet, but they may modify the hormone secretion through local paracrine action. Moreover, coexistence of a variety of putative neurotransmitters and/or neuroactive agents in SP- and/or SP/NKA-ergic neurones, such as serotonin, acetylcholine, catecholamines, neuropeptide Y or ATP [7, 8, 25], have been demonstrated. The above mentioned neurotransmitters and/or neuromodulators are involved in modifying AVP release [15, 16] and certain combination of these agents may be of some importance for the mechanisms by which vasopressinergic neurones are influenced by tachykinins.

In summary: On the basis of the present results, it could be concluded that tachykinin NK-1 receptors are involved in tachykinin-mediated stimulation of AVP release. However, a role of tachykinin NK-2 receptors in regulation of AVP secretion from the rat HN system in vitro, can not also be excluded. Since several potent tachykinin NK-1 and/or NK-2 receptor antagonists are now under evaluation in the clinical setting, the present results could be of some importance for the assessment of possible usefulness of these peptides in treatment of human diseases.

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