Significance of galanin and the monoaminergic system in the regulation of vasopressin secretion

Ph.D. thesis

Andor Molnár

Supervisors:
Dr. Ferenc A. László, M.D., D.Sc.
Dr. Ferenc László, M.D., D.Sc.
Dr. Csaba Varga, Ph.D.

UNIVERSITY OF SZEGED
DEPARTMENT OF COMPARATIVE PHYSIOLOGY

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Introduction

Central regulation of vasopressin release

It is generally accepted that vasopressin (VP) present in the neurohypophysis (NH) is synthesized in the hypothalamic neurons, located in the supraoptical nuclei and paraventricular nuclei and other accessory cell groups. After its synthesis, VP is packed in neurosecretory granules and transported by axons to the NH, where it is secreted into the circulation. In accordance with this hypothesis, the NH plays a role only as a VP storage site.

In contrast with this opinion, another hypothesis suggests that dispersed cell cultures of isolated NH obtained from adult rats are capable of synthesizing and releasing VP. This observation on NH cultures is consistent with reports that the pituicytes of NH could be the sites of VP gene expression. The supernatant of the cell cultures contains VP that can be identified not only immunologically, but also mass spectrometrically.

The role of the monoaminergic system

Different neurotransmitters and neuropeptides are localized in the magnocellular portion of the hypothalamus. These compounds, secreted from the nerve terminals into the posterior lobe of the hypophysis, may play a role in the regulation of VP release.

Of the above-mentioned neuroactive compounds, the monoaminergic compounds of the brain, histamine (HA), dopamine (DA), and serotonin (5-HT), and the adrenergic system also play important roles in the regulation of VP secretion.

Interactions between galanin and monoaminergic compounds

Galanin (GAL), as a peptide modulator, is physiologically involved in the regulation of the NH function and VP release, and related to the monoaminergic systems of the brain.

It is generally believed that GAL and monoaminergic modulation occurs in both hypothalamic (somatodentritic) and NH (nerve terminal) regions and involves neuron-neuron and neuron-glial interactions. A majority of the literature data relating to this subject support the involvement of GAL and the monoaminergic system, via actions mainly at the somatodentritic level of the hypothalamus. The level of regulation of VP synthesis and release by GAL, monoaminergic compounds and their interactions that occur specifically at NH nerve terminals is less clear. Our experiments were designed to study the effects of these compounds on the synthesis and release of VP *in vivo* and in isolated NH tissue cultures.

Aims

Our experiments were designed to study the physiological effects of GAL, and to examine the interactions of GAL and the monoaminergic system relating to VP secretion.

In our *in vivo* studies, the following questions were examined:

1. Are there any differences between the effects of rat, porcine and human GAL, and various human GAL fragments on VP secretion following their intracerebroventricular (i.c.v.) or intravenous (i.v.) administration to rats?
2. How can the different GAL compounds and fragments modify the increase in VP release in rats after an osmotic stimulus (2.5% NaCl solution)?
3. How can the GAL treatment modify the VP concentration changes induced in rats by a non-osmotic stimulus (administration of HA)?
4. Can the GAL antagonist galantid (M15) prevent the VP secretion changes induced by GAL?

In our *in vitro* studies, the following questions were examined:

1. Is there any direct effect of the GAL-ergic system on VP release in isolated rat NH tissue cultures?
2. How can the GAL treatment modify the VP concentration changes induced in the supernatant medium of NH tissue cultures by an aspecific osmotic stimulus (administration of K^+)?
3. What are the effects of monoaminergic compounds on the VP concentration of the supernatant medium of NH tissue cultures?
4. Can the different receptor-specific antagonists modify the effects of monoaminergic compounds?
5. What are the effects of the interactions between GAL and the monoaminergic compounds on the VP secretion in rat NH tissue cultures?

Methods

In vivo methods

The experiments were performed on male rats ranging in weight from 180 to 250 g. The animal care and research protocols were in accordance with the guidelines of our university and had been approved in advance by the appropriate ethical committee. The animals were subjected to ether anaesthesia during the operations. The rats had been cannulated for 7 days before the experiment. A stainless cannula was inserted into the right lateral ventricle.

The following compounds were used:

1. GAL compounds
   (i.c.v. – 10 µl (22 µg)/animal; i.v. – 0.2 ml (220 µg)/animal)
2. 2.5% NaCl solution
   (i.p. 2 ml/100 g b.w., immediately before GAL administration)
3. HA (i.p. 0.01 mg/100 g b.w., 15 min after GAL administration)
4. Galantid (M15, a GAL antagonist)
   (i.c.v. – 10 µl (0.22 µg)/animal, 15 before GAL administration)

30 min following GAL administration, the rats were decapitated, and blood samples were collected. The plasma VP levels were measured by radioimmunoassay (RIA). Statistical analysis was performed by using the Tukey-Kramer multiple comparison test.

In vitro methods

Tissue culture method

After decapitation, the pituitary of male Wistar rats was removed. The tissue was digested enzymatically (trypsin, collagenase, DN-ase I and II). The viability was 99-100%, and the cell count per ml was determined to be 2 x 10^6. The dispersed cells were placed into 24-well plastic plates coated with collagen. The VP contents of the cell culture supernatants were determined by RIA. Statistical analysis of VP concentrations was performed with the Kruskal-Wallis test.

The following compounds were used:

1. Rat GAL (10^{-6} M)
2. Galantid (M15, a GAL antagonist, (10^{-13}-10^{-7} M)
3. Monoaminergic compounds: HA (10^{-6} M)
   DA (10^{-6} M)
   5-HT (10^{-6} M)
   Adrenaline (ADR, 10^{-6} M)
   Noradrenaline (NADR, 10^{-6} M)
4. HA antagonists: Mepyramine (MEP, H_1-receptor antagonist, 10^{-6} M)
   Cimetidine (CIM, H_2-receptor antagonist, 10^{-6} M)
   Thioperamide (TPE, $H_{3,4}$-receptor antagonist, 10^{-6} M)
4. 5-HT antagonists: WAY-100635 (WAY, 5-HT_1-receptor antagonist, 10^{-6} M)
   Ketanserin (KTS, 5-HT_2-receptor antagonist, 10^{-6} M)
   Metergoline (MTG, 5-$HT_{1,2}$-receptor antagonist, 10^{-6} M)
5. ADR antagonists: Phentolamine (PTA, α_1+α_2-receptor antagonist, 10^{-6} M)
   Corynanthine (CAT, α_1-receptor antagonist, 10^{-6} M)
   Yohimbine (YOB, α_2-receptor antagonist, 10^{-6} M)
6. NADR antagonists: Propranolol (PNL, β_1+β_2-receptor antagonist, 10^{-6} M)
   Atenolol (ATL, β_1-receptor antagonist, 10^{-6} M)
   Pindolol (PDL, β_1+β_2-receptor antagonist, 10^{-6} M)

Results and discussion

1. Effects of galanin on vasopressin secretion

There were no changes in the basal VP secretion after i.v. or i.c.v. GAL administration. I.c.v. GAL treatment partially or totally blocked the VP level enhancement induced by 2.5% NaCl or HA administration. There was no significant difference in the inhibitory effects of rat, porcine or human GAL. The human GAL 1-16 N-terminal fragment decreased the level of VP secretion. The GAL 16-30 C-terminal fragment was ineffective. This observation suggested that the active centre is localized in the N-terminal part of the GAL molecule. The GAL antagonist galantid (M15) administered before the GAL i.c.v. injection totally prevented the inhibitory effect of GAL on the increased plasma VP level following the administration of 2.5% NaCl solution or HA.

Significantly and dose-dependently decreased content of VP was detected in NH tissue cultures media following the administration of 10^{-9}–10^{-6} M doses of GAL.

Significantly elevated VP levels were measured after K^+ administration. The enhancement of VP secretion could not be prevented by previous GAL treatment. The enhancing effects of K^+ on hormone secretion is not dependent on the receptors, and we therefore concluded that there are GAL receptors on the membrane of the pituicytes of the isolated tissue cultures, and specific receptors are involved in the interactions between GAL and the monoaminergic system.

2. Effects of histamine on vasopressin secretion

Significantly increased levels of VP production were detected in the tissue culture media following HA administration, depending on the HA dose. The VP secretion elevation could be partially blocked by previous administration of the H_1-receptor antagonist MEP or the H_2-receptor

antagonist CIM. The H_3–H_4 receptor antagonist TPE did not influence the VP secretion increase induced by HA. MEP or CIM application after HA administration proved ineffective. We concluded that mainly the H_1-and H_2-receptors are involved in the HA-induced increase of VP secretion, while the H_3–H_4-receptor antagonist TPE proved ineffective in isolated NH tissue cultures. The HA-induced elevation of the VP level was partially blocked by GAL administration before HA treatment. GAL administration after HA treatment did not decrease the enhancement of VP secretion. This phenomenon can be explained in that the 20-min preincubation period is probably long enough for the VP level-increasing action of HA, and thus GAL is ineffective. The results indicate that the HA-ergic control and GAL-HA interaction relating to VP secretion from the NH tissue in rats can occur independently of the hypothalamus, at the level of the posterior pituitary.

3. Effects of dopamine on vasopressin secretion

DA increased the VP level in the medium of isolated NH tissue cultures. The VP level elevations induced by DA were totally blocked by the previous administration of GAL. The DA-blocking effect of GAL was prevented by addition of the GAL receptor antagonist galantid (M15). Our results indicate that there are DA receptors in the cells of the NH (pituicytes), and that the DA-ergic regulation and the GAL-DA interaction can occur independently of the hypothalamus, at the level of the posterior pituitary.

4. Effects of serotonin on vasopressin secretion

Significantly increased levels of VP production were detected in the tissue culture media following 5-HT administration, depending on the 5-HT dose. The VP secretion elevation could be partially blocked by previous administration of the 5-HT antagonist KTS or MTG. WAY did not influence

the VP secretion increase induced by 5-HT. Accordingly, we conclude that mainly the 5-HT_2 receptors are involved in the 5-HT-induced increase of VP secretion in isolated NH tissue cultures. GAL administration before 5-HT treatment partially blocked the 5-HT-induced elevation of the VP level. GAL administration after 5-HT treatment proved ineffective. These results reveale that VP release from the NH is influenced by the 5-HT-ergic system directly at the level of the posterior pituitary.

5***. Effects of the adrenergic system on vasopressin secretion***

Significantly increased VP levels were detected in the tissue culture media following the administration of ADR or NADR, depending on the dose. The VP secretion elevation was totally blocked by the previous administration of PTA (an α_1+α_2-receptor antagonist) or CAT (an α_1-receptor antagonist). YOB (an α_2-receptor antagonist) did not influence the VP secretion increase induced by ADR. PNL (a β_1+β_2-receptor antagonist) before NADR administration prevented the VP secretion increase. ATL (a β_1-receptor antagonist) did not block the VP secretion elevation induced by NADR. Surprisingly, the administration of PDL (a β_1+β_2-receptor antagonist) enhanced VP secretion. This contradictory effect can be explained in that PDL not only acts as a β-receptor blocker, but also exerts “intrinsic sympathomimetic action (ISA)” and a strong adrenergic agonist effect. GAL administration before ADR or NADR treatment prevented the VP level elevation induced by ADR or NADR. We concluded that mainly the α_1- and β_2-adrenergic receptors are involved in the ADR- or NADR-induced increase of VP secretion in isolated NH tissue cultures. The adrenergic control of VP secretion and the interactions between GAL and the adrenergic system can occur at the level of the posterior pituitary.

Summary

The *in vivo* results suggest that GAL, as a peptide modulator, plays an important role in the regulation of VP secretion.

Our *in vitro* experiments suggest that the GAL-ergic control of VP secretion and the interaction between GAL and the monoaminergic system in the NH tissue in rats can occur independently of the hypothalamus, at the level of the posterior pituitary.

With the results of our basic researches, we would like to contribute to better understanding of the function of hypothalamo-neurohypophyseal and GAL-ergic systems.

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