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Inhibitory effects of different galanin compounds and fragments on osmotically and histamine-induced enhanced vasopressin secretion in rats

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Abstract

The effects of rat, porcine and human galanin, and the human 1–16 and human 16–30 terminal galanin fragments on vasopressin secretion were studied in rat. The plasma vasopressin level was determined by radioimmunoassay (RIA). There were no changes in the basal vasopressin secretion after galanin administration. A significant increase in vasopressin concentration was detected following 2.5% NaCl or histamine administration. I.c.v. injected rat, porcine or human galanin or the 1–16 N-terminal galanin fragment prevented the plasma vasopressin level enhancement. Following the i.v. administration of rat galanin or the i.c.v. injected 16–30 C-terminal galanin fragment, the vasopressin concentration did not return to the normal level. Administration of the galanin antagonist galantid (M15) i.c.v. before the rat galanin i.c.v. injection prevented the inhibitory effect on the increased plasma vasopressin level following 2.5% NaCl solution or histamine administration. The results indicate that there is no significant difference in the inhibitory effect of rat, porcine or human galanin or the 1–16 galanin fragment on the enhanced plasma vasopressin secretion induced by hyperosmosis or histamine administration.

Our findings suggest that galanin, as a peptide modulator, is physiologically involved in the regulation of vasopressin release following different forms of stimulation: an osmotic response or histamine administration.

Keywords: Galanin; Galanin fragment; Galanin antagonist; Vasopressin secretion; Hyperosmosis; Histamine

1. Introduction

Following the discovery of galanin in the porcine intestine (Tatemoto et al., 1983), many data have revealed that galanin is widely distributed in the rat hypothalamus (Melander et al., 1986a; Palkovits et al., 1987; Skofitsch and Jacobowitz, 1985), and galanin coexists in the magnocellular neurons with neurohypophyseal hormones, including vasopressin (Melander et al., 1986b; Rokaeus et al., 1988; Skofitsch et al., 1986, 1989). Moreover, the hypothalamic magnocellular nuclei contain a high density of galanin-binding sites (Melander et al., 1988; Merchenthaler et al., 1993; Skofitsch et al., 1986). The hyperosmolarity induced by dehydration or salt loading reduces galanin immunoreactivity (Meister et al., 1990; Skofitsch et al., 1989) and increases galanin mRNA levels (Meister et al., 1990) in the magnocellular neurons. Vasopressin release and synthesis display changes that parallel those of galanin after osmotic stimulation (Meister et al., 1990). These results suggest that galanin, as a peptide modulator, plays an important role in the regulation of vasopressin secretion.

In our present study, the following questions were examined:

1. What are the effects of rat, porcine and human galanin on vasopressin secretion following i.c.v. or i.v. administration to rats?

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2. How can the different galanin compounds modify the increase in vasopressin release in rats after osmotic or non-osmotic stimuli?
3. Which is the biologically active sequence in the galanin molecule? Is there a difference between the effects of the human 1–16 N-terminal and 16–30 C-terminal fragments on the plasma vasopressin secretion after osmotic or non-osmotic stimuli?
4. Can the galanin antagonist galantid (M15) prevent the changes in vasopressin secretion induced by galanin?

2. Materials and methods

2.1. Experimental protocol

The experiments were performed on 3- to 4-month-old male Wistar rats, ranging in weight from 180 to 250 g (bred in our animal house; breeding stock from the Laboratory Animal Institute, Gödöllö, Hungary). The animal care and research protocols were in accordance with the guidelines of our university. The animals were subjected to ether anaesthesia during operations.

The different galanin compounds were administered i.v. into the lateral tail vein, or i.c.v. into the right lateral cerebral ventricle. The rats had been cannulated 7 days before the experiment. The animals were fixed in a stereotaxic instrument, a hole was drilled into the right parietal bone and a stainless cannula (diameter 0.4 mm) was inserted into the right lateral ventricle 0.7 mm anterior and 1.0 mm lateral to the bregma, according to the stereotaxic atlas of Paxinos and Watson (1996). The length of the cannula was 3.5–4.0 mm (depending on the body weight), starting from the cranial bone. The cannula was fixed to the surface of the bone with rapidly hardening dental cement.

The following galanin compounds were used [synthetized by Dr. L. Baláspiri (Balaspiri et al., 1998)]:

1. Galanin (rat, 1–29)
2. Galanin (porcine, 1–29)
3. Galanin (human, 1–30)
4. Galanin N-terminal fragment (human, 1–16)
5. Galanin C-terminal fragment (human, 16–30)
6. Galantid (M15)—galanin antagonist

For i.v. administration, the galanin dose was 1.0 nmol (3.2 μg) in 200 μl saline. For the i.c.v. injection of rat, porcine and human galanin, the dose was 100 pmol (0.32 μg) in 10 μl saline in 1 min, via a Hamilton syringe. Both galanin fragments (1–16 and 16–30) were administered in a dose of 100 pmol (0.17 μg) in 10 μl in 1 min. The galanin antagonist M15 was used in a dose of 10.0 nmol (22 μg) in 10 μl saline in 1 min, 15 min before the rat galanin administration. The galanin and M15 doses were determined according to Landry et al. (1995). In the control rats, we used 10 μl physiological NaCl solution i.c.v. The osmotic stimulus was induced by 2.5% NaCl solution i.p. in a dose of 2 ml/100 g b.w. immediately before the administration of different galanin compounds. As a non-osmotic stimulus, histamine (Histaminum-bihydrochloricum, Chinoin, Budapest, Hungary) was administered i.p. in a dose of 0.01 mg/100 g b.w. 15 min after the galanin administration. Thirty minutes following galanin administration, the rats were decapitated, and the blood was collected in cooled polystyrene tubes. The position of the i.c.v. cannula was controlled by the injection of 1% methylene blue (Reanal, Budapest, Hungary) following coronal section of the brain. If the position of the cannula was not correct, these cases (8.5%) were excluded from the assessment of the results.

2.2. Plasma vasopressin determination

The blood was obtained following decapitation, and 1 ml blood samples were placed in polystyrene tubes (Laczi et al., 1983) containing 1.4 mg Na_2EDTA in 30 μl isotonic NaCl, and centrifuged (1000×g, 10 min) at 4 °C within 10 min. Plasma samples were stored at −20 °C until assaying. The plasma vasopressin levels were measured by RIA, based on a technique described by Dogterom et al. (1978) with some modifications, as reported in detail earlier (Laczi et al., 1986). Synthetic arginine-8-vasopressin (Organon, Oss, The Netherlands, antidiuretic activity 408 IU/mg) was used as reference preparation for antibody production and radiolabelling. Vasopressin antibody was generated against the vasopressin-(ε-aminocaprionic acid)-thyroglobulin conjugate in sheep. The immunization regimen consisted of injection every 2 weeks for 12 weeks. The final antibody dilution used in the assay tube was 1:350,000. The cross-reactions were 23.3% with lysine-8-vasopressin, < 0.01% with oxytocin, and < 0.03% with vasotocin. ^{125}I-labelling of vasopressin was performed by the chloramine T method of Hunter and Greenwood (1962). Reverse-phase chromatography was used for purification of the labelled hormone (Janáky et al., 1982). The standard curves covered the range 1.0–128 pg per assay tube.

RIA was performed within 72 h after sampling. Vasopressin extraction was carried out with an Amprep C8 minicolumn (code RPN 1902, Amersham, Buckinghamshire, UK), with a recovery of more than 95%. Each dilution of the reference preparation was extracted from 1 ml vasopressin-free plasma from homozygous diabetes insipidus rats (Brattleboro strain, CPB-TNO, Zeist, The Netherlands). The extraction was carried out in duplicate. The RIA procedure was the same as that of Dogterom et al. (1978). The sensitivity of the RIA was 1 pg per assay tube. Vasopressin levels are given in pg/ml plasma. The intra- and interassay coefficients of variation proved to be 13.3% and 16.3%, respectively.

2.3. Statistical analysis

The data are expressed as means±S.E.M. of the results for the total number of rats per experimental group. Statistical analysis was performed by using the Tukey–Kramer multiple comparison test. P values less than 0.05 were considered significantly different.

3. Results

The effects of rat, porcine and human galanin, and the human 1–16 and human 16–30 galanin fragments on the vasopressin levels following i.c.v. or i.v. administration are demonstrated in Fig. 1. Significant changes in the plasma vasopressin concen-

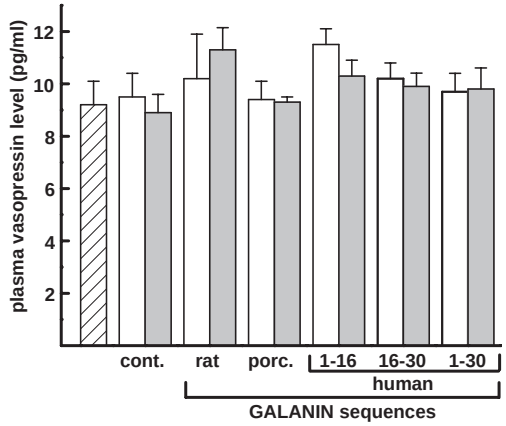

Fig. 1. Effects of rat, porcine and human galanin (GAL), and the human 1–16 and human 16–30 galanin fragments on the vasopressin levels following i.c.v. (□), or i.v. (▣) administration, in comparison with the vasopressin concentration of the untreated non-operated group (▨) and control rats (cont.) treated with 10 μl physiological NaCl solution i.c.v. After the i.c.v. or i.v. administration of rat, porcine (porc.) or human galanin, or the human 1–16 N-terminal or human 16–30 C-terminal galanin fragment, significant changes were not detected in the plasma vasopressin level. Results are shown as mean values ± S.E.M. for 8–12 animals in each group.

tration were not detected. After 2.5% NaCl solution administration, a significant increase was observed in the plasma vasopressin level (Fig. 2). I.c.v. injected rat galanin prevented the 2.5% NaCl-induced plasma vasopressin level enhancement. Following the i.v. administration of rat galanin, the vasopressin concentration did not return to the normal level. Galanin antagonist galantid (M15) administration i.c.v. before the rat galanin i.c.v. injection prevented the inhibitory effect on the increased plasma vasopressin level following 2.5% NaCl solution. After histamine injection i.p., the plasma vasopressin level increased significantly. Following the i.v. administration of rat galanin, the enhancement remained unchanged. Rat galanin administered i.c.v. reduced the histamine-induced increase in plasma vasopressin concentration, but the vasopressin levels remained higher as compared with the untreated control. Galantid (M15) injection i.c.v. before the rat galanin i.c.v. administration prevented the decreasing effect on the enhanced plasma vasopressin level after histamine injection. A comparison of the effects of porcine and human galanin and the human 1–16 N-terminal and 16–30 C-terminal galanin fragments on vasopressin release is demonstrated in Fig. 3. Following 2.5% NaCl administration, a significant enhancement was detected in the plasma vasopressin level. I.c.v. injected porcine or human galanin, or the human 1–16 galanin fragment prevented the 2.5% NaCl solution-induced plasma vasopressin increase. The 16–30 galanin fragment did not reduce the enhanced plasma vasopressin concentration after the 2.5% NaCl administration. Following histamine injection, the plasma vasopressin increased to a much higher level than that following the hyperosmotic stimulus. Porcine and human galanin and the human 1–16

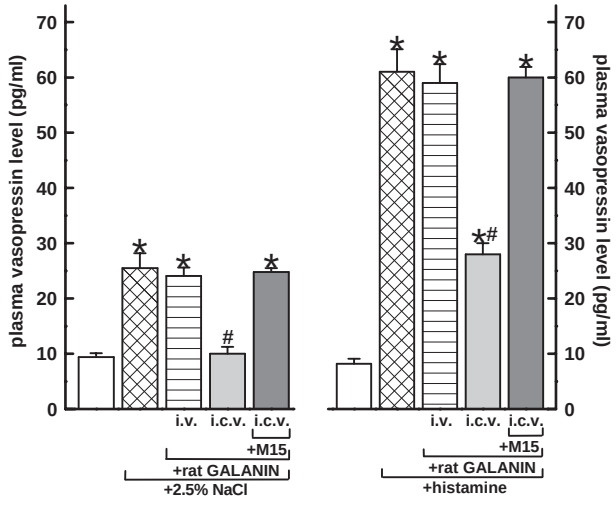

Fig. 2. Effects of rat galanin and galanin antagonist galantid (M15) on the changes in plasma vasopressin levels induced by 2.5% NaCl solution (left panel) or histamine administration (right panel). A significant increase was observed in the plasma vasopressin concentration following 2.5% NaCl solution or histamine administration. I.c.v. injected galanin prevented the 2.5% NaCl-induced plasma vasopressin level enhancement, and reduced the histamine-induced increase in plasma vasopressin concentration, but the vasopressin levels remained higher as compared with the untreated control (□). Galantid (M15) administration before the i.c.v. injection prevented the inhibitory effects on the increased plasma vasopressin level following 2.5% NaCl solution or histamine administration. Results are shown as mean values ± S.E.M. for 8–12 animals in each group. Statistical significance: (*) $P<0.05$ compared to the control group, and (#) $P<0.05$ compared to the 2.5% NaCl solution-, or histamine-treated groups.

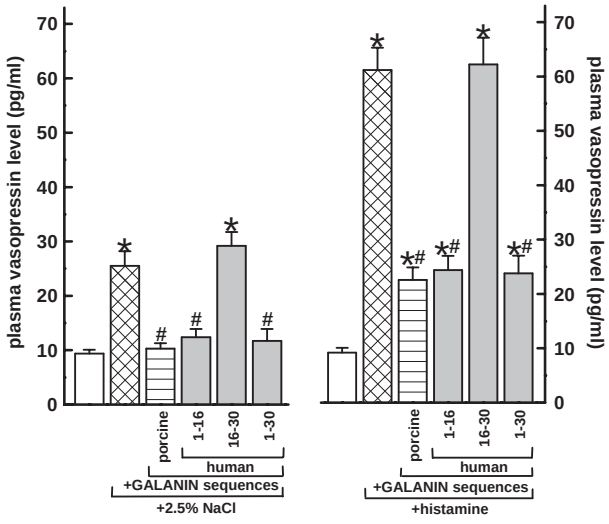

Fig. 3. Effects of porcine and human galanin and the human 1–16 N-terminal or 16–30 C-terminal galanin fragments on the changes in plasma vasopressin levels induced by 2.5% NaCl solution (left panel) or histamine administration (right panel). Following 2.5% NaCl solution or histamine administration, a significant increase was detected in the plasma vasopressin concentration. The histamine-induced plasma vasopressin enhancement was more pronounced than that after 2.5% NaCl solution administration. The porcine and human galanin and the 1–16 N-terminal galanin fragment prevented the increase in plasma vasopressin level following 2.5% NaCl administration, and the histamine-induced enhancement in plasma vasopressin concentration was also reduced, but the vasopressin levels remained higher as compared with the untreated control (□). The human 16–30 C-terminal fragment proved to be ineffective after the administration of 2.5% NaCl solution or histamine injection, the high plasma vasopressin level remaining unchanged. Results are shown as mean values ± S.E.M. for 8–12 animals in each group. Statistical significance: (*) $P<0.05$ compared to the control group, and (#) $P<0.05$ compared to the 2.5% NaCl solution-, or histamine-treated groups.

galanin fragment administered i.c.v. each reduced the increased plasma vasopressin concentration induced by histamine, but the vasopressin concentration proved to be higher as compared with the untreated control.

4. Discussion

A great number of data have been published on the effects of galanin on the hormone release from the pituitary. Following i.v. administration of synthetic human galanin, a significant increase in plasma human growth hormone level was found, but the arginine vasopressin concentration did not change significantly (Murakami et al., 1993). Bauer et al. (1986) earlier reported that the i.v. injection of porcine galanin into healthy men induced increases in the plasma human growth hormone and prolactin levels. Systemically administered galanin caused a brief small increase in blood pressure and mild diuresis, but the i.v. injection of galanin had no effect on the vasopressin-induced antidiuretic effects (Skofitsch et al., 1989).

In our present experimental series, the i.v. administration of galanin did not influence either the plasma basal vasopressin level, or the increased vasopressin concentration induced by hyperosmotic or histamine administration. The i.c.v. injection of galanin did not influence the basal vasopressin level. This finding is fully in accordance with the results of Balment and al Barazanji (1992). The enhancement in plasma vasopressin level following hypertonic NaCl solution or histamine administration, however, was totally or partially prevented by i.c.v. galanin administration. Similar observations were presented by Kondo et al. (1991, 1993). A strong relationship was proved between vasopressin production and the galaninergic system in these experiments, which showed parallel increases in the vasopressin and galanin mRNA contents in the supraoptic and paraventricular nuclei after osmotic stimuli (Meister et al., 1990; Yagita et al., 1994). A newly identified compound, galanin-like peptide, was recently discovered (Ohtaki et al., 1999), which contains 60 amino acids. Shen et al. (2001) described a decrease in galanin-like peptide mRNA in the neurohypophysis following hyperosmosis.

Initially, porcine galanin was isolated (Tatemoto et al., 1983). Later, rat (Vrontakis et al., 1987) and bovine (Rokaeus and Carlquist, 1988) galanin were cloned. All three mature peptides comprise 29 amino acids and possess an amidated C-terminal. The N-terminal regions (1–15) are identical for all three species, but differences are apparent in the C-terminal domains. Human galanin was cloned 3 years later (Bersani et al., 1991). Surprisingly, human galanin comprises 30 amino acids and its C-terminal is not amidated; the N-terminal region is identical to that of the galanins from the other three species (Bartfai et al., 1992; Hokfelt et al., 1992). The effects of the human 1–16 N-terminal and 16–30 C-terminal galanin fragments on the plasma vasopressin secretion after osmotic or non-osmotic stimuli were studied. We found that the 1–16 N-terminal fragment can moderate the increase in plasma arginine vasopressin level. This effect was the same as we detected following full-length galanin administration. Our observations are in accordance with the findings of Landry et al. (1995), who measured the hypothalamic arginine vasopressin mRNA content. However, there was no significant change when the 16–30 C-terminal galanin fragment was used. Our experiments lead us to conclude that the 1–16 N-terminal galanin fragment contains the biologically active centre of the galanin molecule. As the N-terminal 1–15 active centre is identical for rat, porcine and human galanin, it was not surprising that the effects of these three galanin compounds on arginine vasopressin secretion proved to be the same.

Galanin acts via at least 3 receptor subtypes, which differ in amino acid sequence, distribution, pharmacology and signal transduction (Branchek et al., 2000). Magnocellular vasopressin neurons in the paraventricular and supraoptic nuclei express galanin GAL_1 receptors (Burazin et al., 2001; Gundlach and Burazin, 1998; Landry et al., 1998; Mitchell et al., 1997). The low levels of the galanin GAL_3 receptor were expressed in the neurohypophyseal cells (pituicytes) (Ohtaki et al., 1999). The present findings indicate that vasopressin production can be influenced by the galaninergic system, but the potentially involved galanin receptor subtypes are as yet unknown.

During the last 10 years, many galanin receptor antagonists have been discovered which have higher affinity for the galanin receptors than that of galanin itself. These antagonists antagonize the actions of galanin in each galanin-receptive system (Bartfai et al., 1991; Lindskog et al., 1992). Among these galanin antagonists, galantid (M15) was the first to be widely used in studies on the physiological action of endogenous galanin (Bartfai et al., 1991; Lindskog et al., 1992). Landry et al. (1995) reported that M15 administration suppressed the galanin-induced decrease in vasopressin mRNA in the magnocellular region of the hypothalamus in dehydrated rats, and increased the vasopressin mRNA content in control rats. The effectiveness of M15 in antagonizing the action of galanin on vasopressin mRNA suggests the involvement of galanin receptors in the regulation of the vasopressinergic cell bodies at a hypothalamic level. To evaluate the specificity of our galanin treatment, we also examined the effects of M15 on the elevated plasma vasopressin level induced by 2.5% NaCl solution or histamine before galanin administration. We demonstrated that M15, as a full galanin receptor antagonist (Ogren et al., 1993), is able to prevent the inhibitory effect of galanin on vasopressin secretion.

A majority of the literature data relating to this subject support the involvement of galanin in the body fluid balance, via actions mainly at the hypothalamic level

(Gaymann and Martin, 1989; Koenig et al., 1989; Meister et al., 1990; Skofitsch et al., 1989). The level of regulation of vasopressin release by galanin that occurs specifically at neurohypophyseal nerve terminals is less clear (Shen et al., 2001). Further studies were needed to clarify the exact site of action involved in this galaninergic regulation of vasopressin release (Kondo et al., 1993). Earlier, we studied the effect of galanin on vasopressin secretion in isolated rat neurohypophysis tissue culture. A significantly decreased content of vasopressin was detected following galanin administration. The vasopressin concentration elevation induced by dopamine or dopamine-active drugs could be blocked by the previous administration of galanin. We concluded that the galaninergic control of vasopressin secretion in rats can also occur independently of the hypothalamus, at the level of the posterior pituitary (Galfi et al., 2002). The hypothesis of the active role of neurohypophyseal cells is supported by Boersma and Van Leeuwen (1994), who reported the existence of neuropeptide receptors on the membranes of the pituicytes. Shen et al. (2001) described that osmotic stimulation by dehydration or salt loading produced parallel increases in galanin-like peptide and vasopressin mRNA levels in the posterior pituitary.

In conclusion, our results suggest that galanin is physiologically involved in the regulation of vasopressin release following different forms of stimulation: an osmotic response or histamine administration. There is no significant difference in the inhibitory effect of i.c.v. administered rat, porcine or human galanin on the enhanced plasma vasopressin secretion following stimulation. In this respect, the 1–16 N-terminal fragment proved to contain the biologically active portion of the galanin molecule. With these experiments we should like to contribute to a better understanding of the central regulation of vasopressin release.

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