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Galanin and vasopressin response to hyperosmotic stimulation: *in vitro* study

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Galanin (Gal) – a neuropeptide present in the nervous system and peripheral tissues – may be involved in the regulation of hypothalamo-neurohypophysial system function. It was shown that centrally injected galanin inhibits osmotically stimulated vasopressin (VP) secretion into the blood and reduces VP mRNA level in the supraoptic (SON) and paraventricular (PVN) hypothalamic nuclei. The aim of the present study *in vitro* was to investigate the influence of Gal on vasopressin release from isolated rat hypothalamus (Hth), neurohypophysis (NH) or hypothalamo-neurohypophysial explants (Hth-NH). The effect of Gal on VP secretion was studied under conditions of direct osmotic (i.e., Na^+-evoked) (series 1) as well as non-osmotic (i.e., K^+-evoked) (series 2) stimulation. In series 3, vasopressin response to Gal was studied using the neural tissues obtained from animals drinking 2% NaCl solution for eight days (indirect osmotic stimulation). Gal in a concentration of 10^{-10} M and 10^{-8} M inhibited basal VP release from Hth, NH and Hth-NH explants isolated from euhydrated rats as well as from Hth-NH complex of osmotically challenged animals. When the neural tissues obtained from previously salt-loaded rats were incubated in K^+-enriched medium the inhibitory effect of Gal was completely blocked. It may be concluded that the effect of Gal is depending on the current functional status of the hypothalamo-neurohypophysial system.

Key words: galanin, vasopressin, rat, osmotic stimulus, *in vitro* explants

INTRODUCTION

Vasopressin (VP) release from the neurohypophysis into the general circulation depends on many different stimuli/factors of different character and origin. The states of hypernatraemia and hyperosmolality of plasma and cerebrospinal fluid (CSF) are both known to be main stimuli for activation of magnocellular VP-ergic neurons and VP release from the posterior pituitary (Morita et al. 2001, Cisowska-Maciejewska and Ciosek 2005).

It is known that numerous mediators are involved in the regulation of the hypothalamo-neurohypophysial system (Ciosek and Guzek 1992, Bojanowska et al. 1999, Juszczak et al. 2007, Ciosek and Izdebska 2009). Many data prove that galanin (Gal) and others members of Gal-neuropeptide family can also modulate the release of neurohypophysial hormones (Kondo et al. 1991, Björkstrand et al. 1993, Pieribone et al. 1998, Iismaa and Shine 1999, Molnár et al. 2005).

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Galanin is widely distributed in the rat central nervous system (CNS) (Gundlach et al. 2001) and peripheral tissues (Branchek et al. 2000, Waters and Krause 2000). In the rat central nervous system the highest expression of galanin-like immunoreactivity (Gal-LI) was observed in the hypothalamus, amygdaloid complex, brainstem and spinal cord as well as in neurohypophysis (Arai et al. 1990, Gundlach 2002). In the rat hypothalamus, neurons containing Gal were found to be located mainly in the preoptic area, supraoptic nuclei (SON) and paraventricular nuclei (PVN) [in magnocellular (mPVN) as well as parvocellular (pPVN) subdivision of PVN], in arcuate nucleus and median eminence (Melander et al. 1986, Gundlach et al. 2001). Galanin has been shown to coexist with VP in neurons of SON and PVN under normal conditions as well as in the states of dehydration or salt-loading (Meister et al. 1990, Landry et al. 1997, Sanchez et al. 2001). SON and PVN appear a high density of Gal binding sites of all three types (Melander et al. 1988, Mitchell et al. 1999). Gal receptor type 1 (GalR1) is present mainly in the CNS, whereas large amounts of GalR2 and GalR3 at low level are expressed in both the CNS and peripheral tissues (Branchek et al. 1998).

Some early studies on the effect of central Gal administration revealed that this neuropeptide is involved in the processes of osmotic regulation at the hypothalamic level including the PVN and SON. Centrally injections of Gal reduced water intake (Brewer et al. 2005) and inhibited osmotically evoked the increased expression of VP mRNA in the hypothalamic nuclei SON and PVN (Landry et al. 2000). Moreover, in salt-loaded rats the higher levels of Gal mRNA and Gal-R1 mRNA in the PVN and SON of the rat has been observed (Burazin et al. 2001, Meister et al. 1990). Early reports, also from our laboratory, confirm Gal participation in VP release *in vivo* from the posterior lobe of the pituitary in euhydrated as well as osmotically challenged rats (Kondo et al. 1991, Kondo et al. 1993, Landry et al. 1995, Ciosek et al. 2003, Cisowska-Maciejewska and Ciosek 2005). It was shown that centrally injections of Gal inhibited osmotically stimulated VP release in the rat (Kondo et al. 1991, Ciosek et al. 2003, Cisowska-Maciejewska and Ciosek 2005). In our latest study *in vitro* (Izdebska and Ciosek 2010) we have demonstrated distinct inhibitory Gal influence on basal VP release from the hypothalamus, neurohypophysis as well as hypothalamo-neurohypohysial explants. However, this effect of Gal was less marked during incubation of all parts of Hth-NH system in the medium enriched in K^+ ions. So far, there are no observations related to the direct influence of Gal added into the incubative fluid on osmotic-stimulated VP release from the hypothalamo-neurohypophysial system *in vitro*. Therefore, the present experiments *in vitro* were undertaken to investigate the effects of Gal in the concentrations of 10^{-10} M and 10^{-8} M on vasopressin release from the hypothalamus, neurohypophysis and hypothalamo-neurohypophysial explant in the states of direct or indirect hyperosmotic stimulation.

METHODS

Subjects

Three-month old male Wistar rats (weighing 220–300 g) were used for experiments. They were housed (4 animals per cage) under conditions of constant room temperature (20–22ºC), humidity and lighting (a 12/12 light-dark cycle; lights on from 6 AM). The animals received standard pelleted food and tap water *ad libitum* (in the third series of experiments 2% NaCl solution was provided instead of tap water for 8 days before decapitation). The experimental procedures were done with the consent (No L/BD/185/A/Dz) of the Local Commission for the Animal Care at the Medical University of Lodz. The team tried to use only the number of animals necessary to produce reliable results.

Procedures

On the day of the experiment, animals were decapitated between 9.00 and 10.00 AM. The procedure of decapitation was carried out very quickly with the using of the guillotine for experimental animals. Each rat was decapitated in the laboratory room separately from others animals. The brain and the pituitary with intact pituitary stalk were carefully removed from the skull (the disruption of the cranial bones has been started from the superior area) and then three types of neuronal tissue were prepared: (1) the block of tissue containing the hypothalamus (Hth), or (2) the neurohypophysis (NH), or (3) intact hypothalamo-neurohypophysial system (Hth-NH). A block of hypothalamic

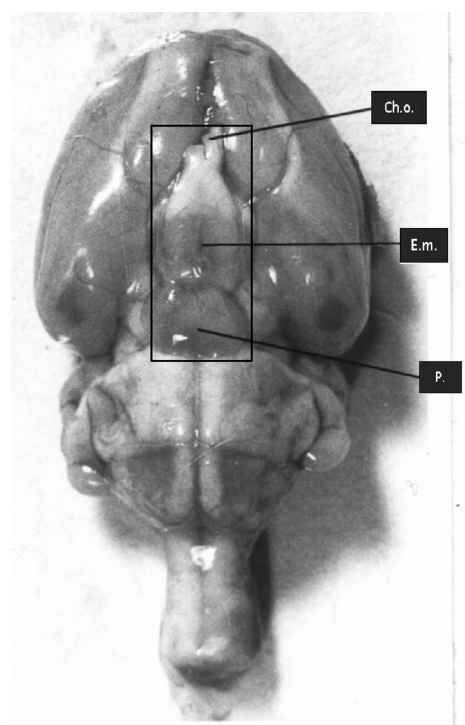

Fig. 1. The rat brain (ventral bottom) with the marked: (Ch.o.) optic chiasm; (E.m.) median eminence; (P) pituitary (the adenohypohysis is visible; the neurohypophysis is shaded). The dirk lines indicate the places of the dissection of the block tissue containing hypothalamo-neurohypophysial system.

Table I

Body weight, plasma osmolality and haematocrit in rats drinking tap water or 2% saline

Rats	Initial body weight (g)	Final body weight (g)	Haematocrit (%)	Plasma osmolality (mOsmol/kg H_2O)
Euhydrated (*n*=48)	272 ± 2	288 ± 3	46 ± 1	281 ± 2
Salt-loaded (*n*=48)	272 ± 3	233 ± 3	51 ± 1	329 ± 2

Values are mean ± SEM

tissue was dissected as follows: rostral limit – frontal plane situated about 1.0 mm more anteriorily than the anterior margin of the optic chiasm; caudal limit – frontal plane just behind the mammilary bodies; lateral limits – sagittal planes passing, on both sides, just through the hypothalamic fissures (Yasin et al. 1996) (see below). The depth of dissection was approximately 2.5–3.0 mm from the base of the brain. The single explant was approximately 5 mm wide and weighed approximately 35–40 mg. The total dissection time was about 3 min from the decapitation. Such hypothalamo-neurohypophysial explant (Hth-NH) contained suprachiasmatic nucleus (SCN) as well as the SON and PVN hypothalamic nuclei with intact axonal projections to the neurohypophysis (anterior lobe of the pituitary has been excised) (Bojanowska et al. 1999, Juszczak 2002, Cisowska-Maciejewska and Ciosek 2005). Hypothalamic tissue has been divided in half before the incubation (Yasin and Forsling 1998).

Each type of the isolated neural tissue was placed immediately in polypropylene tube with 1 ml of Krebs-Ringer fluid [termed as normal KRF (nKRF)] containing: 120 mM NaCl, 5 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM KH_2PO_4, 0.7 mM $MgSO_4$, 22.5 mM $NaHCO_3$, 10 mM glucose, 1.0 g/l bovine serum albumin and 0.1 g/l ascorbic acid (pH = 7.37–7.46; osmolality 280–290 mOsm/Kg H_2O). Tubes were placed in a water bath at 37ºC and constantly gassed with carbogen (a mixture of 95% O_2 and 5% CO_2). At the beginning of the experiment, the appropriate kind of the prepared tissue was equilibrated in nKRF which was aspirated twice (2 × 40 min) and replaced with 1 ml of fresh medium. After 80 minutes of such preincubation, the nKRF was discarded and the tissues were incubated for 20 minutes in 1 ml of nKRF alone or containing the appropriate concentrations of Gal.

In the first series of experiments the effect of Gal [Galanin (rat) lot 0560209, Bachem] on the basal and Na^+-stimulated (i.e., acute osmotic stimulation) VP release was studied. The appropriate type of prepared tissue (Hth, NH, Hth-NH) isolated from rats drinking tap water *ad libitum* (euhydrated animals) were incubated successively in: (1) normal KRF (B1); (2) modified KRF containing the excess of sodium chloride (medium osmolality in the range of 320-330 mOsm/Kg H_2O) (S1); (3) the incubation fluid as (1) alone or with Gal at the concentrations of 10^{-10} or 10^{-8} M (B2); and (4) the KRF as (2) alone or with Gal in the concentrations as (3) (S2). Incubation in each medium proceeded for 20 min. In between incubation periods (2) and (3), the tissues were washed in the normal medium and these samples were discarded.

In the second series of experiments the effect of Gal on non-osmotically stimulated (i.e., potassium-evoked) VP release was studied using neural tissues (as in first series) isolated from euhydrated animals. The experimental protocol was similar to that described above. To stimulate VP release, the incubation fluid containing the excess (56 mM) of potassium was administered instead of hyperosmotic medium. NaCl concentration in the medium was appropriately reduced to maintain the osmolality.

In the third series of experiments the effect of Gal on basal and K^+-evoked VP release from the neural tissues obtained from rats drinking 2% NaCl for 8 days (osmotically challenged animals) was studied. The experimental protocol was the same as in the second series.

In euhydrated rats (series 2) as well as in salt-loaded rats (series 3) the plasma osmolality and haematocrit

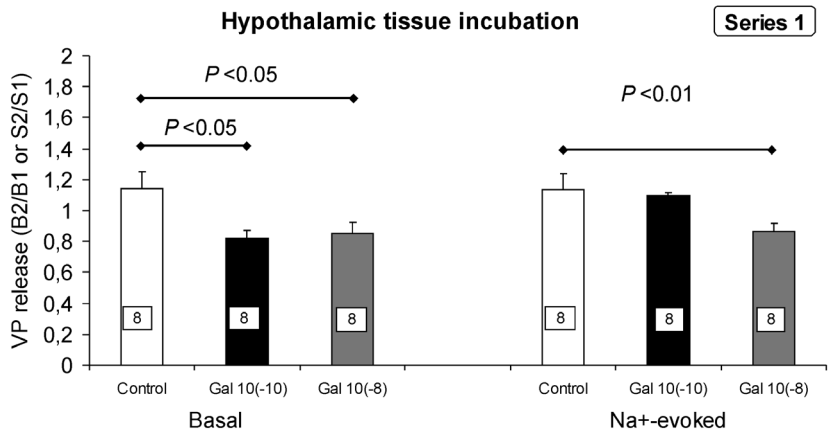

Fig. 2. The effect of galanin (Gal) on the basal and Na^+-evoked vasopressin release (as estimated on the basis of the ratio between two incubation periods) from the hypothalamus obtained from euhydrated rats (series 1). Each bar represents mean ± SEM and figures in bars indicate the number of animals in each group.

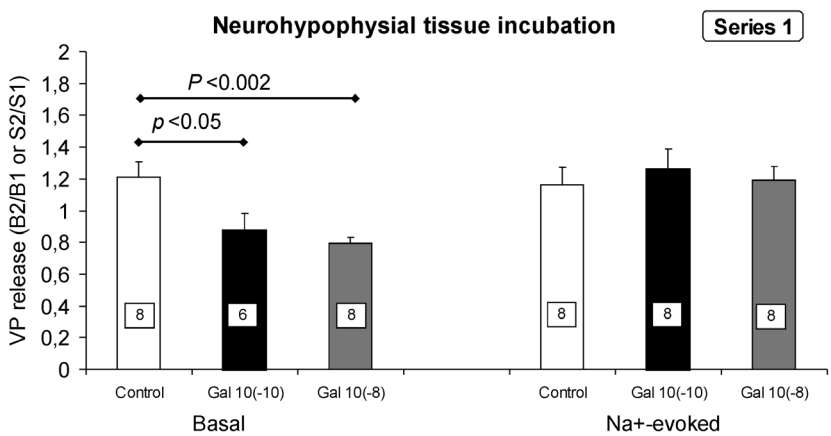

Fig. 3. The effect of galanin (Gal) on the basal and Na^+-evoked vasopressin release (as estimated on the basis of the ratio between two incubation periods) from the neurohypophysis obtained from euhydrated rats (series 1). Each bar represents mean ± SEM and figures in bars indicate the number of animals in each group.

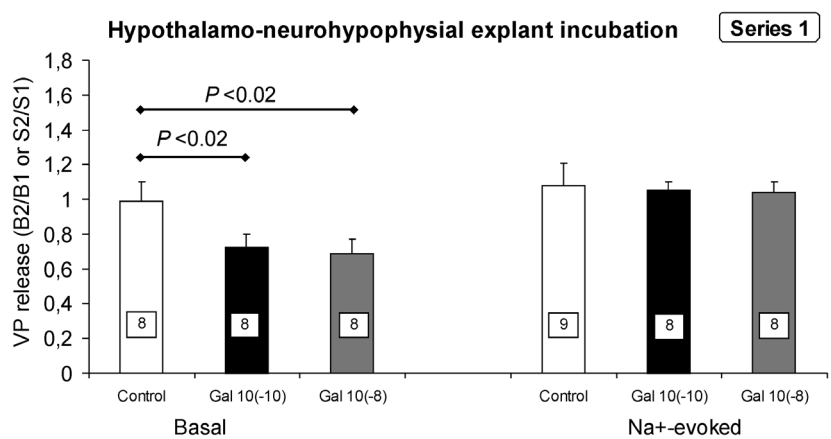

Fig. 4. The effect of galanin (Gal) on the basal and Na^+-evoked vasopressin release (as estimated on the basis of the ratio between two incubation periods) from the hypothalamo-neurohypophysial explants obtained from euhydrated rats (series 1). Each bar represents mean ± SEM and figures in bars indicate the number of animals in each group.

index were estimated. Plasma osmolality was measured in duplicate by freezing point depression using semimicroosmometer (Knauer & Co GMBH, Berlin, Germany). The body weight of rats from series 3 was determined at the beginning and at the end of the 8-day period of salt loading. The initial and final body mass of the rats in second series of the experiments was similarly estimated before and after 8 days of maintenance of animals in standard conditions. After each incubation the media were aspirated and samples immediately frozen and stored at −20ºC until VP estimation by the radioimmunoassay.

Radioimmunoassay (RIA)

The concentrations of vasopressin in the medium samples were determined by double-antibody specific RIA. Anti-VP antibodies were raised in Department of Experimental Physiology, Chair of Experimental and Clinical Physiology, Medical University of Lodz. Detailed characteristic of antibodies has been presented earlier by Cisowska-Maciejewska and Ciosek (2005). Cross reactivity with arginine vasopressin for these antibodies was 1.1%. For iodination with ^{125}I and for standard curve preparation the chloramine-T method with using of standard VP ([Arg^8]-Vasopressin; Peninsula Laboratories Europe Ltd.) was used. The lower limit of detection for the assay was 1.25 pg VP/100 µl; an intra-assay coefficient of variation for VP was less than 3.5% (all samples within each experimental series were tested in the same RIA to avoid inter-assay variability).

Statistical evaluation of the results

Vasopressin release into incubative media was estimated by using the ratios between two incubation periods B2/B1 (basal release) and S2/S1 (stimulated release) for each kind of incubated tissue (separately for the hypothalamus, neurohypophysis or hypothalamo-neurohypophysial system). In every experimental group the results were calculated and expressed as means ± SEM. Significance of the differences between the means was determined by the use of the Kruskal-Wallis analysis of variance (ANOVA) followed by Mann-Whitney "U" test (two means comparison); $P<0.05$ was considered as the minimal level of significance.

RESULTS

The values of body weight, plasma osmolality and haematocrit in euhydrated and salt-loaded rats are shown in Table I. The body weight of rats drinking 2% saline for 8 days (series 3) was reduced by 14% and their plasma osmolality and haematocrit were markedly increased (about 17% and 11%, respectively) as compared with control values obtained from euhydrated rats (series 2).

Series 1

Gal in concentrations of 10^{-10} and 10^{-8} M markedly decreased basal VP release from all tested rat tissues i.e., Hth (Fig. 2: $P<0.05$), NH (Fig. 3: $P<0.05$ and $P<0.002$) as well as Hth-NH explants (Fig. 4: $P<0.02$). Na^+-stimulated VP release from hypothalamic tissue was distinctly diminished after using of Gal only in a concentration of 10^{-8} M (Fig. 2: $P<0.01$). During incubation of remaining neural tissues (i.e., NH or Hth-NH explants) Gal did not influence Na^+-evoked VP release (Figs 3,4).

Series 2

Gal added into incubative media in concentrations of 10^{-10} or 10^{-8} M inhibited significantly the basal VP output from all isolated neural tissues i.e., Hth (Fig. 5: $P<0.005$ and $P<0.001$), NH (Fig. 6: $P<0.002$) as well as Hth-NH system (Fig. 7: $P<0.002$ and $P<0.005$). Similarly, this peptide in concentration of 10^{-10} M reduced K^+-evoked VP release from the NH (Fig. 6: $P<0.05$). The same effect of 10^{-8} M Gal has been observed as related to potassium-stimulated VP release from Hth-NH explants (Fig. 7: $P<0.05$). Gal did not modify the VP secretion from the Hth into the medium enriched with potassium ions (Fig. 5).

Series 3

When all parts of the hypothalamo-neurohypophysial system were isolated from the brain of salt-loaded rats and incubated, Gal in a concentration of 10^{-8} M markedly reduced basal VP secretion from Hth (Fig. 8: $P<0.02$) as well as Hth-NH system (Fig. 10: $P<0.05$). Similarly, 10^{-10} M Gal inhibited basal VP release from Hth-NH explants (Fig. 10: $P<0.05$). However, Gal did not influence VP release from the NH during basal

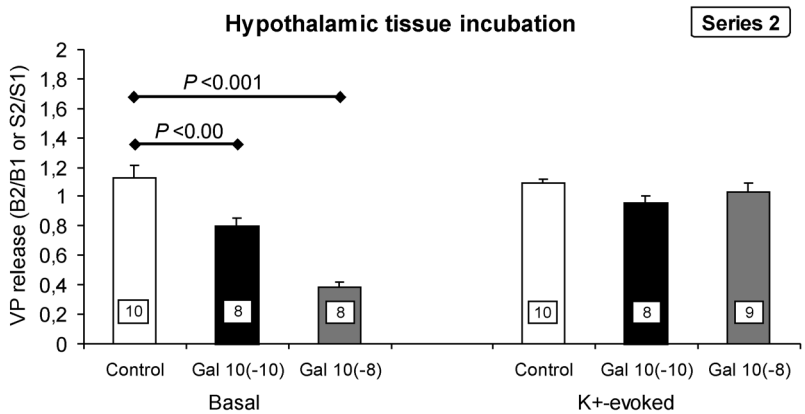

Fig. 5. The effect of galanin (Gal) on the basal and K^+-evoked vasopressin release (as estimated on the basis of the ratio between two incubation periods) from the hypothalamus obtained from euhydrated rats (series 2). Each bar represents mean ± SEM and figures in bars indicate the number of animals in each group.

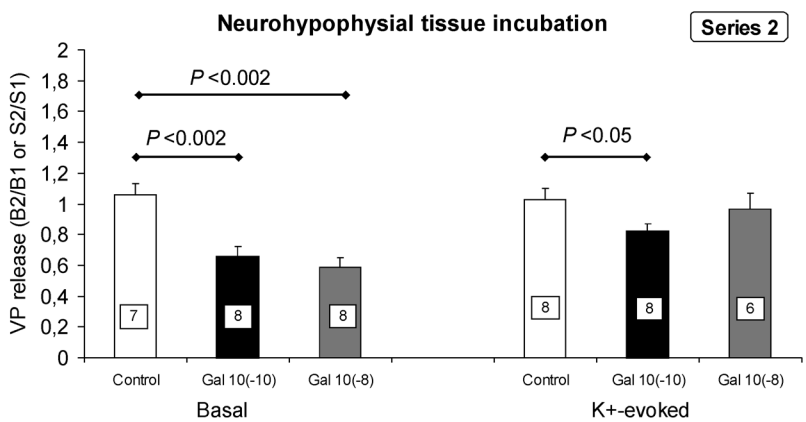

Fig. 6. The effect of galanin (Gal) on the basal and K^+-evoked vasopressin release (as estimated on the basis of the ratio between two incubation periods) from the neurohypophysis obtained from euhydrated rats (series 2). Each bar represents mean ± SEM and figures in bars indicate the number of animals in each group.

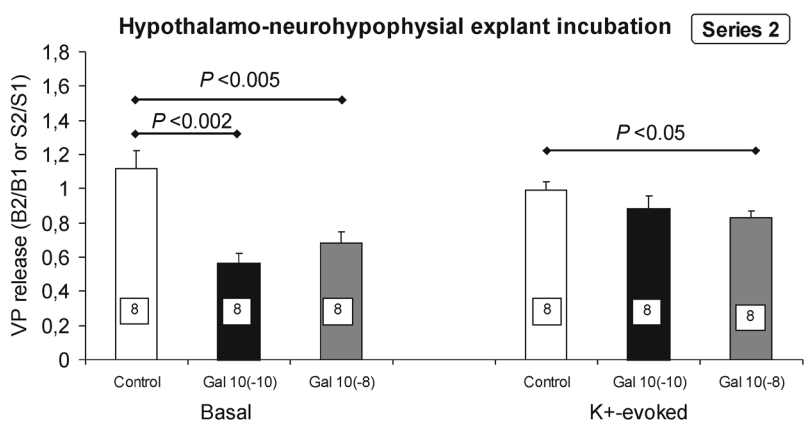

Fig. 7. The effect of galanin (Gal) on the basal and K^+-evoked vasopressin release (as estimated on the basis of the ratio between two incubation periods) from the hypothalamo-neurohypophysial explants obtained from euhydrated rats (series 2). Each bar represents mean ± SEM and figures in bars indicate the number of animals in each group.

period of the incubation (Fig. 9). Any effect Gal exerted on VP release from Hth, NH as well as Hth-NH system of osmotically challenged animals during K^+-stimulated phase of the incubation (Figs 8, 9, 10).

Some differences in basal VP release under influence of both Gal concentrations observed in series 1 and 2 are difficult to the interpretation but may result from differentiated sensitivity of VP-ergic neurons in the incubated tissues.

DISCUSSION

The results of present experiments clearly show that Gal modulates the vasopressin release from the hypothalamo-neurohypophysial system in the conditions *in vitro*, however, its effect depends on electrolyte composition of the incubation fluid. In these experiments two ways of VP-ergic neurons activation have been employed, i.e. osmotic and non-osmotic (potassium-evoked) stimuli. Experimental-evoked osmotic stimulation of VP-ergic neurons may have direct (brought about the excess of sodium ions added into incubative fluid) or indirect character (related to afferent impulsation originated from systemic and central osmoreceptors) (Leng et al. 1982, Neuman et al. 1993, Ludwig et al. 1995, Bojanowska et al. 2000). In this second case the respective information is sending to some brain structures involved in osmoregulatory mechanisms related to VP-ergic neurons excitation (Leng et al. 1982, Bourque et al. 1994, 2007).

The excess of sodium ions present in incubative media is the reason of direct osmotic excitation of magnocellular neurons. It leads to membrane depolarization of VP-ergic neurons which increases their firing rate and VP release (Leng et al. 1982). In the conditions *in vitro* only the perikarya of VP-ergic neurons can react to the direct osmotic stimulation (Gregg and Sladek 1984) whereas potassium-evoked depolarization stimulates cell bodies of these neurons and their neurohypophysial axons terminals.

We showed that in euhydrated rats (series 1 and 2) being the donors of neural tissue for the incubation procedure both concentrations of Gal (10^{-10} and 10^{-8} M) distinctly inhibited basal vasopressin release from the neurons of isolated Hth-NH explants. The interesting observation is that both tested Gal concentrations diminished VP release to the same level. It could be mean that Gal in a concentration of 10^{-10} M exerted already maximal inhibitory influence on VP release. Gal did not modify the process of osmotic-evoked VP release from the neurohypophysis as well hypothalamo-neurohypophysial system but was the reason of the restricted VP release from the hypothalamic tissue.

These data may suggest that Gal exerts its action probably at the every level of hypothalamo-neurohypophysial system modifying the hypothalamic perikarya and/or axonal endings in the neurohypophysis. It is known that Gal-ergic neurons project to supraoptic and paraventricular nuclei of the hypothalamus (Melander et al. 1986). In fact, VP-ergic neurons in the hypothalamic magnocellular nuclei as well as their terminals in posterior pituitary receive rich Gal-ergic afferentation (Landry and Hökfelt 1998, Gundlach et al. 2001, Landry et al. 2003). The coexistence of Gal with VP in the rat hypothalamic magnocellular neurons in the SON and PVN (mPVN) and/or parvocellular part of the PVN (pPVN) have shown by immunohistochemical as well as *in vitro* studies (Gai et al. 1990, Meister et al. 1990, Bartfai 1995, Gundlach and Burazin 1998, Sanchez et al. 2001). Moreover, *in situ* hybridization studies have demonstrated the high density of GalR1 mRNA in SON, mPVN and pPVN (Iismaa and Shine 1999, Gundlach et al. 2001). According to the review of Gundlach and others (2001) GalR2 is mainly present in the PVN but has been not observed in SON. Galanin may be involved in the regulation of body fluids osmolality and vasopressin release. Koenig and colleagues (1989) observed the reduced galanin content in the posterior lobe of the pituitary and in the median eminence of salt-loaded rats as well as in vasopressin-deficient rats. Moreover, salt-loading increased Gal mRNA and GalR1 mRNA expression in the PVN and SON (Meister et al. 1990, Burazin et al. 2001). Experimental induced water deprivation or salt-loading lead to the increase of GalR1 density in these neurons and higher binding of galanin (Burazin et al. 2001). Central injections of galanin inhibited VP mRNA in the SON and PVN (Landry et al. 2000) and osmotically stimulated vasopressin release (Kondo et al. 1991). On the other hand, after administration of the galanin antagonist M15 the increase of VP mRNA level has been observed (Landry et al. 2000). According to Mechentaler (2008) galanin acts as a negative feedback modulator of vasopressin release *via* GalR1 activation. The inhibitory effect of Gal on Na^+-stimulated VP release from the hypothalamic tissue observed in present experiments seem to be agreed with the findings of Kondo et al. (1991).

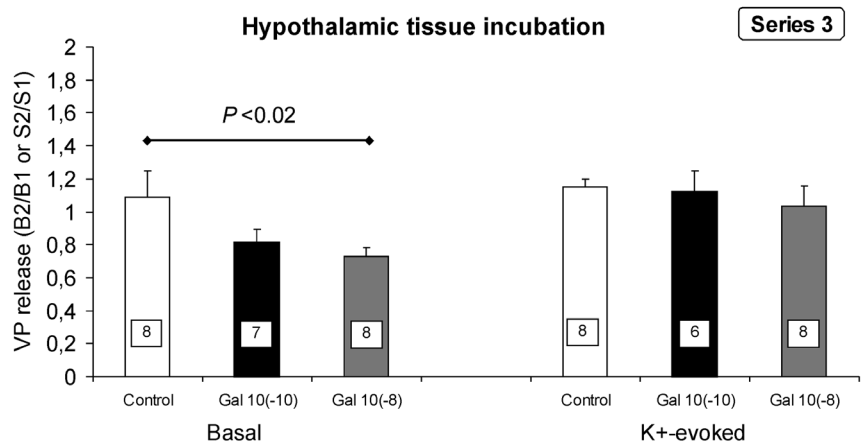

Fig. 8. The effect of galanin (Gal) on the basal and K^+-evoked vasopressin release (as estimated on the basis of the ratio between two incubation periods) from the hypothalamus obtained from rats drinking 2% saline for 8 days (series 3). Each bar represents mean ± SEM and figures in bars indicate the number of animals in each group.

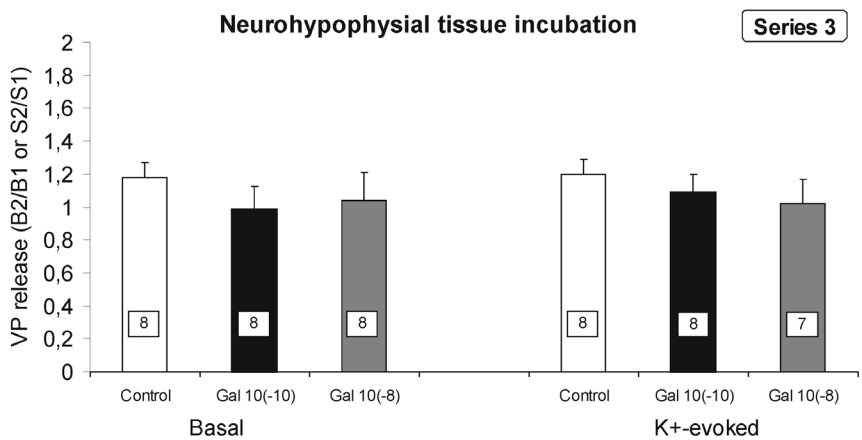

Fig. 9. The effect of galanin (Gal) on the basal and K^+-evoked vasopressin release (as estimated on the basis of the ratio between two incubation periods) from the neurohypophysis obtained from rats drinking 2% saline for 8 days (series 3). Each bar represents mean ± SEM and figures in bars indicate the number of animals in each group.

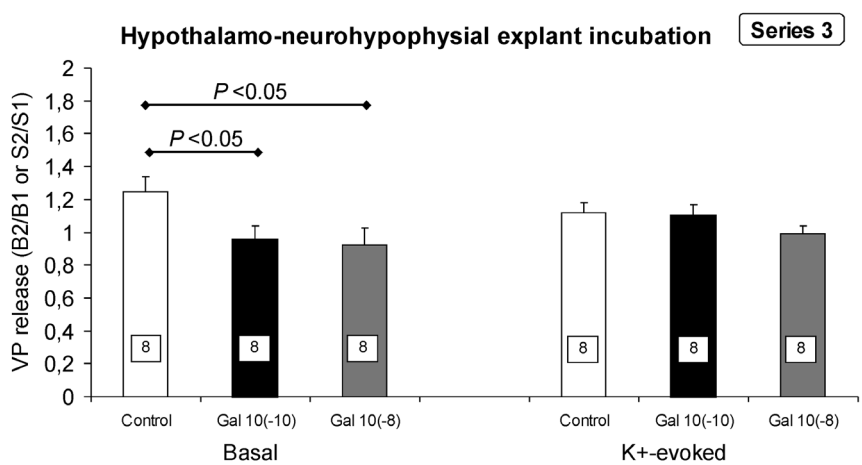

Fig. 10. The effect of galanin (Gal) on the basal and K^+-evoked vasopressin release (as estimated on the basis of the ratio between two incubation periods) from the hypothalamo-neurohypophysial explants obtained from rats drinking 2% saline for 8 days (series 3). Each bar represents mean ± SEM and figures in bars indicate the number of animals in each group.

In second series of present experiments K^+-evoked VP release from NH or Hth-NH explant was restricted when Gal was added into incubative media in the concentrations of 10^{-10} M or 10^{-8} M, respectively. This result is in agreement with earlier *in vitro* experiments of Gálfi and coauthors (2002, 2003) who observed an inhibitory influence of Gal in the concentrations of a range from 10^{-6} to 10^{-9} M on basal VP release from the 13–14 days neurohypophysial cells culture. In the same experimental model Nagyéri and others (2009) has noted the similar Gal action on VP secretion from the incubated neurohypophysial cells. This paper also presents that Gal did not change K^+-induced VP release. What is more, our team has demonstrated recently (Izdebska and Ciosek 2010) distinct inhibitory Gal influence on basal VP release from the hypothalamus, neurohypophysis as well as hypothalamo-neurohypophysial system. However, this effect of Gal was less marked during incubation of all parts of Hth-NH system in the medium enriched with K^+ ions excess. On the other hand, present findings do not confirm the results of our *in vivo* studies where Gal decreased the hypothalamic VP content in euhydrated rats without the influence on this neurohormone content in the neurohypophysis; however, plasma VP was then diminished (Ciosek et al. 2003, Cisowska-Maciejewska and Ciosek 2005). Hence, it seems that Gal may play a role of inhibitory neuromodulator of vasopressin secretion acting on perikarya of VP-ergic neurons as well as their axon terminals in the neurohypophysis. Moreover, it seems that Gal influence did not depend on the tested concentration in the medium. Such distinct diminution of VP release induced by Gal may lead to the disturbances of water-electrolyte balance being the symptoms of primary diabetes insipidus.

In third series of present experiments we have used salt-loaded rats as the donor animals of neural tissues to incubation *in vitro*. It is known that the state of hyperosmolality of extracellular fluid, including plasma and CSF, is the main stimulus for VP release from the neurohypophysis into the blood. Experimental animals drinking for at least 2–3 days a hypertonic solution of natrium chloride (mostly, 2% solution) show a prompt increase of plasma osmolality as well as VP release into the blood and the activation of the mechanisms of the thirst (Kadekaro et al. 1995, Morita et al. 2001, Cisowska-Maciejewska and Ciosek 2005). The presence of sodium ions in ECF determine, in more than 85–90%, the normal osmolality of blood plasma about 295 mosm kg^{-1} in rats (Bourque et al. 2007). In present experiments the rats of third series received 2% solution of NaCl *ad libitum* for 8 days before the decapitation. Saline in such a concentration is known to provoke hypertonic overhydration and has been previously used in the appropriate experiments (Morita et al. 2001, Cisowska-Maciejewska and Ciosek 2005). We now observed that Gal added into incubative media containing the Hth-NH complex taken from chronic salt-loading animals exerted distinct effect on basal VP release. Namely, Gal restricted basal VP release from the Hth-NH system when was tested in both concentrations (10^{-10} and 10^{-8} M) and from Hth (concentration of 10^{-8} M) but did not change basal VP release from the neurohypophysis. It seems, therefore, that Gal acts mainly as a neuromodulator of cell bodies of VP-ergic neurons. Next, K^+-stimulated VP response to both concentrations of Gal during incubation of all parts of Hth-NH tissue taken from salt-loaded rat was completely blocked as compared to the results obtained during basal conditions. Similarly, Ludwig and coworkers (1996) observed that salt loading abolished the intranuclear VP response to such stimuli as intraperitoneal hypertonic saline or direct osmotic stimulation of the SON. Next observation related to potassium-stimulated VP release from isolated NH of chronic salt-loading rats, which was completely blocked in response on neuropeptide Y, was noted earlier by Larsen and others (1994). What is more, the neurohypophysial VP stores may be completely depleted during prolonged state of hyperosmolality. In experiments of Morita and colleagues (2001) chronic hyperosmotic stimulation *in vivo* induced constant release of VP from the neurohypophysis into the blood, however, the synthesis and transport of AVP from hypothalamus to pituitary gland was then intensified. Molnár and others (2005) have noted that intravenously (iv) or intracerebroventricularly (icv) administered galanin did not influence the plasma basal VP level in the rat. These authors also observed that icv injected Gal prevented the 2.5% NaCl-induced plasma VP enhancement, but the VP levels remained higher as compared with the untreated control rats (Molnár et al. 2005). Our team has noted earlier that Gal restricted secretion of VP into systemic circulation in salt-loaded rats (Cisowska-Maciejewska and Ciosek 2005). Therefore, it is not excluded that persistent osmotic stimulation of hypothalamo-neurohypophysial system decreases the susceptibility threshold of VP-ergic neurons to different

factors/stimuli, in this experiments galanin, in the conditions *in vivo* as well as *in vitro*. So, the lack of VP-ergic neurons response to Gal during K^+-stimulated phase *in vitro* observed in salt-loaded rats needs next studies.

Galanin influences on VP-ergic neurons may be exerting by binding with three types of galanin receptors presented on these neurons. Gal can modulate the bioelectrical activity of VP-ergic hypothalamic neurons acting by GalR1 (Pieribone et al. 1998, Burazin et al. 2001). Gal present in the axonal terminals of neurohypophysis is released after depolarization of these terminals (Pieribone et al. 1998). By binding with GalR3, Gal can acts directly on VP secretion from neurohypophysis into the circulation. It has been postulated that Gal hyperpolarizes neurohypophysial axonal terminals resulting to restrict the process of VP release (Depczyński et al. 1998). Somewhat earlier Papas and Bourque (1997) demonstrated that centrally injected Gal or Gal fragment (1–16) caused the hyperpolarization of magnocellular neurosecretory cells membrane. The consequence is the suppression of firing rate of these neurons. Study of Kozoriz and coworkers (2006) demonstrated that Gal presynaptically reduced eEPSCs (excitatory postsynaptic currents) of SON magnocellular neurons (MCNs) in non dehydrated and dehydrated rats as well as that Gal increased the postsynaptic hyperpolarization of MCNs after two days of dehydration. So, the effect of Gal is to reduce depolarization of MCNs followed by restricted VP release. Our present results especially related to restricted basal VP release under influence of Gal seem to confirm these above data.

CONCLUSION

It may be therefore concluded that the effect of Gal is depending on the current functional status of the hypothalamo-neurohypophysial system; Gal may be supposed to act as the inhibitory neuromediator of vasopressin release.

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