Available online at www.sciencedirect.com
ScienceDirect
General and Comparative Endocrinology 149 (2006) 269–277
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Galanin-like immunoreactivity in the brain of the snake *Bothrops jararaca*

R.F. Alponti [a], J.M. Mancera [b], M.P. Martín-del-Río [b], P.F. Silveira [a,*]

[a] *Laboratory of Pharmacology, Instituto Butantan, Av. Vital Brazil, 1500, 05503-900 São Paulo, SP, Brazil*
[b] *Department of Animal Biology, Facultad de Ciencias del Mar, Universidad de Cádiz, Puerto Real 11510, Spain*

Received 27 March 2006; revised 8 June 2006; accepted 13 June 2006
Available online 24 July 2006

Abstract

The distribution of galanin-like immunoreactive perikarya and nerve fibers in the brain of the snake *Bothrops jararaca* was studied by means of immunohistochemistry using an antiserum against porcine galanin. Immunoreactive neurons were only detected in the infundibular recess nucleus. Immunoreactive fibers were found in the telencephalic, diencephalic and mesencephalic areas such as the dorsal cortex, nucleus accumbens, lamina terminalis, preoptic area, mediodorsal region of the supraoptic nucleus, subfornical organ, nucleus of the paraventricular organ, subcommisural organ and periventricular grey region. The habenula, paraventricular nucleus, infundibular recess nucleus and hypothalamo-hypophyseal tract presented denser innervations. The outer layer of the median eminence displayed numerous fibers located close to the portal system, while scarce fibers were seen in the inner median eminence and neural lobe of the hypophysis. The distribution of labelled neurons in the brain of this snake was more restricted than that described in a turtle. The wide hypothalamic and extrahypothalamic distribution of labelled fibers suggests that galanin peptides may have hypophysiotropic, neuromodulator and neurotransmitter roles in the snake *B. jararaca*.

Keywords: Neuropeptides; Intestinal peptide; Brain; Immunohistochemistry; Reptiles

1. Introduction

Galanin (GAL) is a 29-residue peptide first isolated from porcine intestine (Tatemoto et al., 1983). One of the first evidences showing the endocrinological function of GAL is that the peptide has exerted a mild but sustained inhibition of insulin secretion in dogs (McDonald et al., 1985). In mammals, GAL has been identified in neurons of the central and peripheral nervous system by means of immunohistochemical techniques (Rökaeus et al., 1984; Ekblad et al., 1985; Skofitsch and Jacobowitz, 1985; Melander et al., 1986; Palkovits et al., 1987; Beal et al., 1988; Gentleman et al., 1989; Merchenthaler, 1991; Elmquist et al., 1992; Kordower et al., 1992; Perez et al., 2001). In the central nervous system (CNS), GAL has been shown to act on the anterior and posterior pituitary lobe by regulating the release of prolactin (Koshiyama et al., 1987), growth hormone (Ottlecz et al., 1986; Murakami et al., 1987), luteinizing hormone (Estienne and Barb, 2005) and vasopressin (Kondo et al., 1995). The projections of corticotropin-releasing hormone (CRF)- and GAL-containing neurons to the median eminence have suggested that GAL and CRF are simultaneously involved in regulation of adrenocorticotropic hormone secretion from the anterior pituitary of rats (Niimi et al., 1992). GAL has also been reported to stimulate somatostatin (SRIF) release from rat median eminence fragments in vitro (Aguila et al., 1992). In addition, GAL has been shown to be associated with many other metabolic and neural functions. It has been demonstrated in acting as a lactotroph growth factor and as a mitogenic factor for injured neurons. Also, there have been evidences of its involvement in Alzheimer's disease, nociceptive reception,

* Corresponding author. Fax: +55 11 3726 7222.
E-mail address: pefesil@butantan.gov.br (P.F. Silveira).

0016-6480/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2006.06.007

and the processes of learning and memory (Bartfai, 1995; Hokfelt et al., 1999; Gundlach et al., 2001). In mammals, GAL-expressing neurons and GAL receptors are anatomically positioned in the hypothalamic circuitry to influence energy balance (Gundlach, 2002). In 1999, GAL-like peptide (GALP) was isolated from the porcine hypothalamus as an endogenous ligand of GalR2 (Ohtaki et al., 1999). GALP is composed of 60 amino acid residues and the amino acid sequence of GALP- (9–21) is identical to that of the biologically active N-terminal (1–13) portion of GAL (Ohtaki et al., 1999). In mammals, the biological function of GALP is considered to differ from that of GAL. GALP presents specific effect on gonadotrophin-releasing hormone secretion (Matsumoto et al., 2001). Similar to proopiomelanocortin and unlike GAL, GALP expression appears to be negatively regulated by fasting in the leptin-deficient *ob*/*ob* mouse, which can be rescued with leptin treatment (Jureus et al., 2001). GALP is currently the only known GAL-(1–29) homologue. However, comparatively low affinity of GalR3 receptor for GAL (1–29) and molecular heterogeneity of immunoreactive GAL, as well as recent data from immunohistochemical mapping of rat brain with antiserum raised against rat GAL(1–16) (Hilke et al., 2006) suggest the presence of other endogenous GAL homologues. A GAL-like molecule has already been biochemically characterized and GAL-ir has been detected in the CNS of invertebrates (Lundquist et al., 1991; Díaz-Miranda et al., 1996).

In non-mammalian vertebrates, GAL-like-immunoreactivity (GAL-ir) has been found in the CNS of lamprey (Jiménez et al., 1996), dogfish (Vallarino et al., 1991), teleosts (Batten et al., 1990a,b; Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991; Anglade et al., 1994; Rodríguez-Gómez et al., 2000), amphibians (Wolfbauer and Skofitsch, 1989; McKeon et al., 1990; Lázár et al., 1991; Olivereau and Olivereau, 1992; Pieribone et al., 1994; González-Nicolini et al., 1998), turtle (Jiménez et al., 1994) and birds (Józsa and Mess, 1993). In the oviduct of the lizard *Podarcis s.sicula* GAL seemed to interact with estrogen, vasoactive intestinal polypeptide and oviposition (Lamanna et al., 1999). GAL caused vasoconstriction and occasionally activated the gut wall of the estuarine crocodile, *Crocodylus porosus* (Kagstrom et al., 1998). There were no changes in the GAL innervation of the gut or on GAL-induced intestinal motility between fasting and digesting Burmese python, *Python molurus bivittatus* (Holmberg et al., 2003). However, there is no knowledge of the physiological role played by GAL in the CNS of reptiles. Central injection of GAL stimulates food intake in goldfish *Carassius auratus* (DePedro et al., 1995), while it does not affect feeding in the neonatal chick (Ando et al., 2000). At our knowledge the biological effects of GALP have not yet been studied in non-mammalian species.

Species specificity has been shown for not only many GAL actions but also for its distribution pattern in the CNS (Vrontakis et al., 1991; Merchenthaler et al., 1993; Hokfelt et al., 1999; Gundlach et al., 2001). Despite their crucial position among vertebrate phylogeny, there has only been one study on the distribution of GAL-ir in a reptilian species, the turtle *Mauremys caspica* (Jiménez et al., 1994). The present immunohistochemical study maps the distribution of GAL peptides in the brain of a species, the terrestrial Brazilian pit viper *Bothrops jararaca*, belonging to another reptilian radiation. This study is undertaken to compare the distribution of GAL-ir perikarya and fibers in the brain of the snake *B. jararaca* in relation to the galaninergic system of other vertebrate groups. In this snake, the knowledge about the central location of peptides physiologically known to be related to GAL peptides in mammals, such as CRF (Silveira et al., 2001), SRIF (Alponti et al., 2006) and neurohypophyseal peptides (Silveira et al., 2002), is unique among the reptiles. Thus, the presence of GAL-ir is also analyzed in order to infer whether GAL peptides could exert hormonal and/or transmitter and/or modulatory effects, as well as could interact with CRF, SRIF and the reptilian neurohypophyseal peptides vasotocin (AVT) and mesotocin (MST) in the brain of *B. jararaca*.

2. Materials and methods

2.1. Animal

Adult male ($n = 4$) and female ($n = 4$) snakes *B. jararaca* (Serpentes, Viperidae, Crotalinae, about 180 g in weight and 103 cm in length) were collected from the wild in southern and south-eastern Brazil and acclimated for controlled environmental conditions (12:12 light/dark photoperiod—lights on at 6:00 a.m., relative humidity of $65.3 \pm 0.9\%$ and temperature between 25 and 26 °C). Male snakes were identified by the exposure of one or both hemipenises, after gently pressing on the base of the tail. The macroscopic examination of the oviduct through a ventral incision on anesthetized females permitted to exclude the pregnant snakes from the experimental procedures. They were provided with adequate food (one Swiss mouse to each snake every 15 days) and freely accessible tap water. The animal and research protocols used in this study are in agreement with the Brazilian Council Directive (COBEA-Brazil) and were approved by the Ethics Committee of the Instituto Butantan.

Animals were anesthetized with sodium pentobarbital (3 mg/100 g body wt) (Silveira et al., 1992) and subsequently injected transcardially with 0.1 ml of sodium heparin solution (1000 IU/ml of Ringer's solution for *B. jararaca*) (Silveira and Mimura, 1999). Specimens were then perfused with Bouin's fluid for 40–50 min at a flow rate of 2.4–4.8 ml/min. The brains were removed and fixed for 48 h in Bouin's fluid. Afterwards, the fixed brains were dehydrated and embedded in paraffin.

2.2. Histology and immunohistochemistry

Serial sagittal and transverse sections stained with hematoxylin–eosin and cresyl violet were used for anatomical examinations. The identification and the nomenclature of the brain regions and nuclei were respectively based on or adopted from Donkelaar and Nieuwenhuys (1979), Prasada-Rao et al. (1981), Fernández-Llebrez et al. (1988), Smeets et al. (1990), Mancera et al. (1991), Jiménez et al. (1994), Silveira et al. (2001, 2002) and Alponti et al. (2006).

Serial sagittal and transverse 8-μm thick sections were hydrated and immunostained according to the peroxidase–antiperoxidase (PAP) method (Sternberger, 1986) using an antiserum against porcine GAL raised in rabbit (AB 1985; Chemicon, New York). The sections were first incubated in Tris buffer containing 0.3% H_2O_2 for 15 min at 22 °C in order to avoid endogenous peroxidase activity and then incubated for 18 h at 22 °C in the primary antiserum (1:1000). Sections were subsequently incubated in the secondary antiserum (anti-rabbit IgG raised in goats, kindly

provided by Dr. P. Fernández-Llebrez, Málaga, Spain) at a dilution of 1:40 for 45 min at 22 °C, and then in the rabbit-PAP complex (Dakopatts, Copenhagen, Denmark) at a dilution of 1:75 for 45 min at 22 °C. All antisera and the PAP complex were diluted in Tris buffer, pH 7.8, containing 0.7% non-gelling seaweed gelatin (Sigma), lambda carrageenan (Sigma), 0.5% Triton X-100 (Sigma) and 0.02% sodium azide (Merck). As an electron donor, 0.04% 3.3′-diamonibenzidine tetrahydrochloride (Sigma) in Tris buffer, pH 7.8 containing 0.007% H_2O_2 (Merck) and 0.04% ammonium nickel sulfate hexahydrate (Fluka) was used for incubation and colour development in darkness for 15 min at 22 °C.

The specificity of the employed anti-porcine GAL serum was qualitatively tested by immunoadsorption, according to Jiménez et al. (1994). Briefly, two aliquots of the diluted antiserum (1:1500) were separately incubated, for 18 h at 22 °C, with two preparations (10 and 20 μg/ml) of GAL (Sigma), GALP (Phoenix Pharmaceuticals, California) and the related peptides (Rökaeus, 1987) substance P (Sigma) and physalaemin (Sigma). Immunoadsorbed GAL antiserum with GAL or GALP stained no structures, while the distribution of immunoreactivity in snake brain sections was not changed by previous immunoadsorption of GAL antiserum with substance P or physalaemin. In order to verify the immunohistochemical procedure, some control sections were not treated with the primary antiserum. No positive structures were found in any of these sections.

The areas showing GAL-ir perikarya were determined by examining one-tenth of every immunostained section using a Nikon E600 microscope equipped with a CoolSNAP-PRO digital camera coupled to a microcomputer system. In the schematic drawings taken from a camera lucida, circles represent GAL-ir perikarya. The number of circles is a relative estimation of the number of GAL-ir perikarya observed in the immunostained section. The schematic drawings are identical to those shown in our earlier studies (Silveira et al., 2001, 2002; Alponti et al., 2006). The size of cell bodies was measured using the image processor Image-Pro-Plus 4.0 (Media Cybernetics, USA). Histometric data were presented as mean ± SD. Diameter was measured across the long axis of cell bodies. The total number of cells measured for each nucleus was 15–20.

3. Results

The pattern of GAL-like immunostaining showed bilateral symmetry, but sex-related differences were not detected. Since all animals were killed at the same time of the year and at approximate hour in the day, seasonal and/or circadian differences could not be evaluated. The anatomical distribution of perikarya and fibers in the brain of *B. jararaca* is schematically represented in transverse sections (Figs. 1a–h).

3.1. Neuronal perikarya

Numerous GAL-ir perikarya, showing strong immunoreactivity, were detected in the infundibular recess nucleus (RIN).[1] They were small in size and round or pear shaped (6.6 ± 0.3 μm in diameter). Some of them displayed an apical process toward the ventricle (Fig. 2D).

3.2. Nerve fibers

In general, GAL-ir fibers were distributed throughout several brain regions in *B. jararaca* (Fig. 1). In the telencephalon, GAL-ir fibers were seen in the dorsal cortex (DC), extending from the olfactory bulb rostrocaudally to the anterior commissure (AC) (Figs. 1a–d), and in the nucleus accumbens (NA) (Fig. 2A). In the lamina terminalis (LT), a bundle of GAL-ir fibers was observed (Fig. 2B). The diencephalon contained the majority of the GAL-ir fibers in the brain of *B. jararaca*. In the hypothalamus, GAL-ir fibers were present in the preoptic area underneath the AC, in the mediodorsal region of the supraoptic nuclei (SON) and in the subfornical organ (SFO) (Figs. 1c–e). The SON, paraventricular nucleus (PVN) (Fig. 2C), and RIN (Fig. 2D) were moderately innervated by GAL-ir fibers. The diencephalic floor and the hypothalamo-hypophyseal tract (HHT) showed numerous GAL-ir fibers. Other diencephalic areas such as the habenula (H), the nucleus of the paraventricular organ (NPO) and the basal portion of the ependymal cells of the subcommissural organ (SCO) were also innervated by GAL-ir fibers. The outer layer of the median eminence (ME) displayed numerous GAL-ir fibers close to the portal capillaries (Fig. 2E), whereas few fibers were seen in the inner ME and in the neural lobe of the hypophysis (NL). In the mesencephalon, GAL-ir fibers were observed in the periventricular gray (PG) region.

4. Discussion

In the present study, an antiserum against porcine GAL was used to localize GAL peptides in the brain of the snake *B. jararaca*. The amino acid compositions and sequences of mammalian GAL (Rökaeus, 1987) and GALP (Ohtaki et al., 1999) are known but not those of this snake. The amino acid composition and sequences of GAL but not of GALP are known in bird (Kohchi and Tsutsui, 2000), frog (Chartrel et al., 1995) and fish (Anglade et al., 1994). GAL structures are also identified in alligator and tortoise (Wang and Conlon, 1994; Wang et al., 1999). The structure of GAL in alligator is more similar to that of sheep (three amino acid substitutions) than that of chicken (four amino acid substitutions) (Wang and Conlon, 1994), while it differs from that of tortoise by five amino acid residues (Wang et al., 1999). The residues 1–22 of GAL in alligator and tortoise are identical to the counterpart in mammals (pig, sheep and rat) (Wang and Conlon, 1994; Wang et al., 1999). The anti-GAL serum used in the present study has identified GAL-ir perikarya and fibers in mammalian and non-mammalian vertebrates, including the turtle *M. caspica* (Jiménez et al., 1994). Neither the use of immunoadsorbed anti-GAL serum with porcine GAL or GALP nor immunohistochemistry omitting the primary antiserum

[1] *Abbreviations used:* AC, anterior commissure; AM, amygdaloid complex; DC, dorsal cortex; H, habenula; HHT, hypothalamo-hypophyseal tract; IL, intermediate lobe of the hypophysis; LC, lateral cortex; LT, lamina terminalis; ME, median eminence; NA, nucleus accumbens; NPO, nucleus of the paraventricular organ; NL, neural lobe of the hypophysis; NIII, nucleus of the oculomotor nerve; OC, optic chiasm; OT, optic tract; PO, paraventricular organ; PG, periventricular gray; PVN, paraventricular nucleus; RCN, retrochiasmatic nucleus; RIN, infundibular recess nucleus; S, septum; SCO, subcommisural organ; SFO, subfornical organ; SON, supraoptic nucleus.

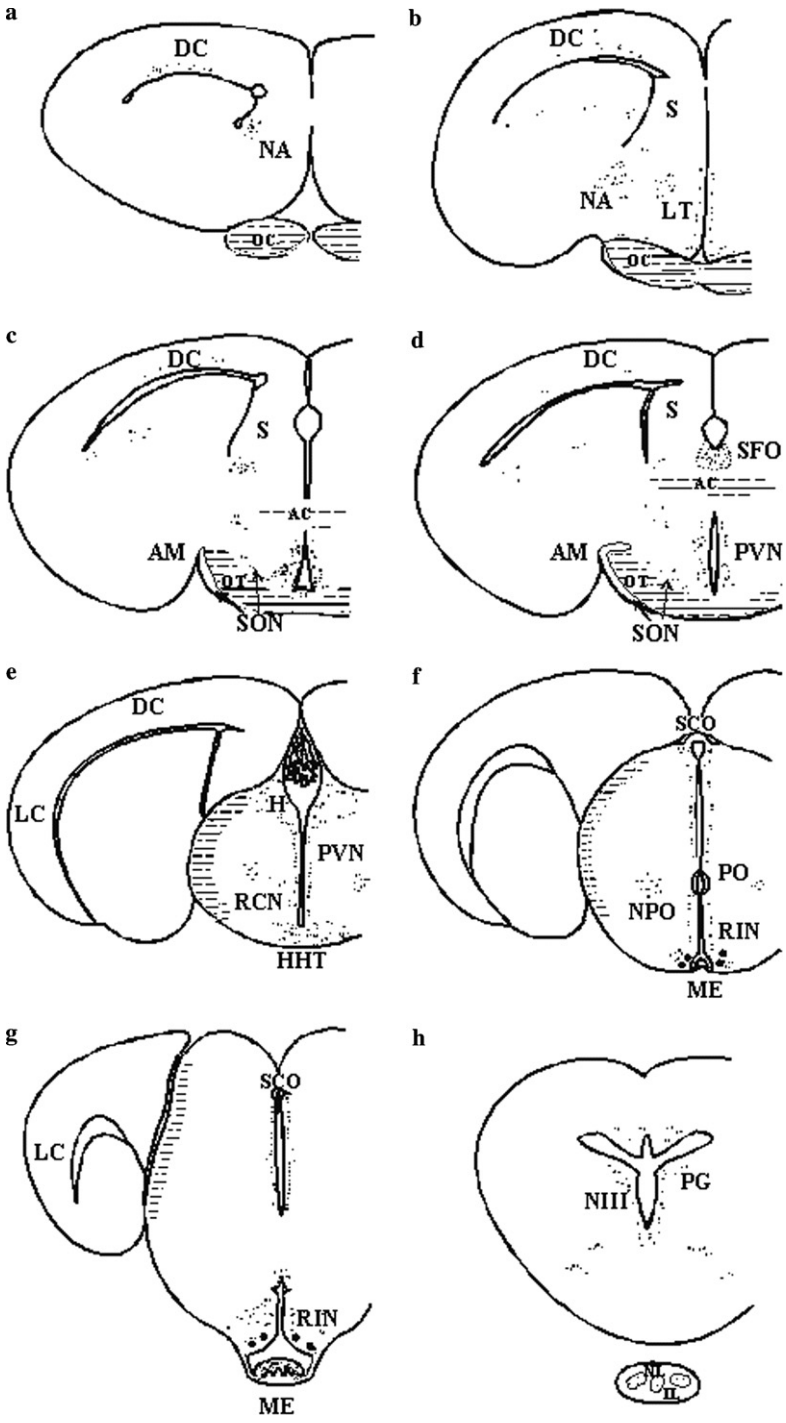

Fig. 1. Schematic drawings of selected transverse sections from rostral (a) to caudal (h) levels throughout the brain of *B. jararaca* showing the distribution of GAL-ir perikarya (circles) and fibers (dots). See abbreviations.

revealed any stained structures in the sections. On the contrary, the use of immunoadsorbed anti-GAL serum with substance P or physalaemin did not abolished the immunoreaction. These findings strongly indicate that GAL and/or GALP must be the substance(s) identified by this antiserum in the present study. To our knowledge, this is the first study on the distribution of GAL-ir in a snake brain. The comparison of GAL-ir between the turtle *M. caspica* (Jiménez et al., 1994) and the snake *B. jararaca* (present study) revealed relevant differences that might not be attributed to different technical quality, since the same anti-GAL and anti-rabbit IgG sera with the same immunohistochemical procedures were employed in both studies.

4.1. Diencephalon

In the snake *B. jararaca* GAL-ir neurons were exclusively found in the infundibular recess nucleus. Thus, these

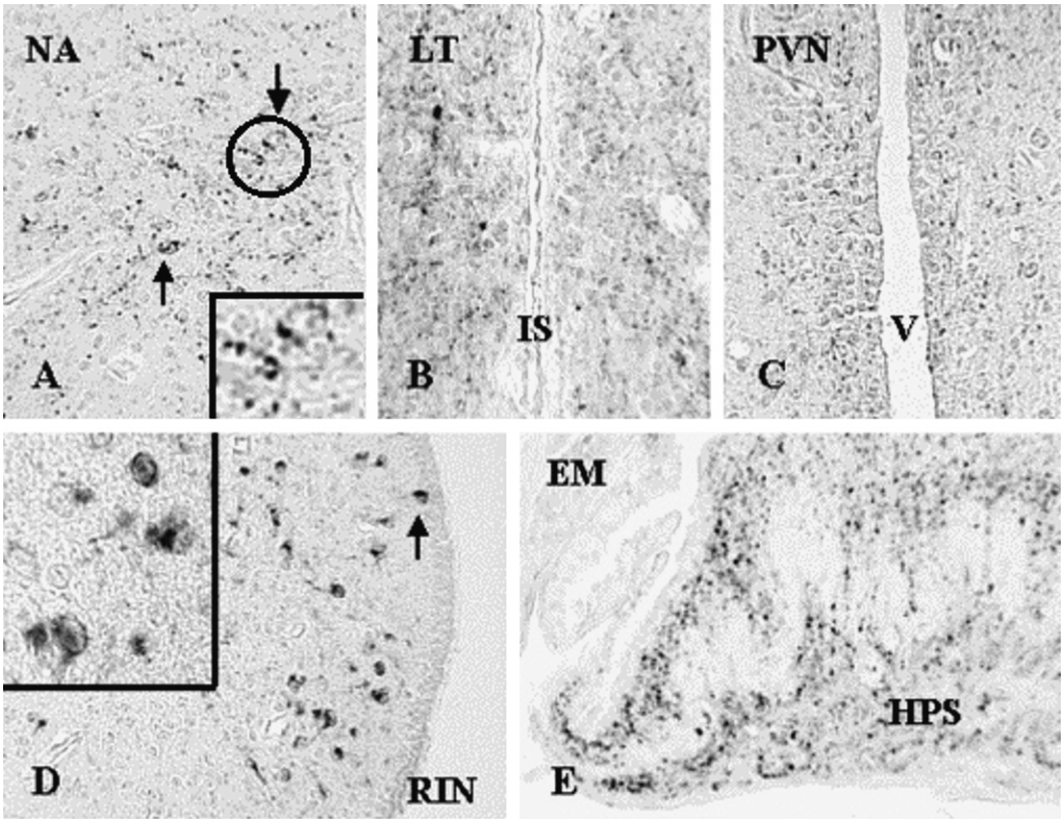

Fig. 2. GAL immunoreactivity in transverse sections of *B. jararaca* brain. (A) GAL-ir fibers in the nucleus accumbens. Some GAL-ir fibers are in contact with immunonegative perikarya (arrows) (300×). Inset: High magnification of the encircled region. (B) GAL-ir fibers in the lamina terminalis (LT) (IS, interhemispheric septum; 300×). (C) Moderate and diffuse galaninergic innervations in the paraventricular nucleus (PVN). (V, ventricle; 350×). (D) GAL-ir perikarya and fibers in the infundibular recess nucleus (RIN), some perikarya close to the ependymal cells lining the infundibular recess (arrow) (300×). Inset: Small and densely stained GAL-ir perikarya in round or pear shapes (700×). (E) A dense network of fibers at the outer median eminence running to the vessels of the hypophyseal portal system (HPS) (500×).

neurons must be the origin of all GAL fibers throughout the brain. In fish, GAL-ir perikarya have been observed in the preoptic and tuberal hypothalamus (Batten et al., 1990a,b; Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991; Rodríguez-Gómez et al., 2000). In amphibians (Lázár et al., 1991; Pieribone et al., 1994; González-Nicolini et al., 1998) and birds (Józsa and Mess, 1993), GAL-ir neurons have been located in the hypothalamus, in addition, GAL-ir perikarya have been found in the telencephalon, mesencephalon and rombencephalon. In mammals, a great number of GAL-ir neurons and fibers have been mapped in diencephalic structures, such as the hypothalamus, in comparison with those found in the telencephalon, mesencephalon and rombencephalon (Rökaeus et al., 1984; Skofitsch and Jacobowitz, 1985; Melander et al., 1986; Palkovits et al., 1987; Beal et al., 1988; Gentleman et al., 1989; Elmquist et al., 1992; Kordower et al., 1992; Perez et al., 2001). The anatomical localization of GAL-ir in the brain of the turtle *M. caspica* appears to resemble that of amphibians rather than that of mammals (Jiménez et al., 1994). So, it seems likely that the number of brain nuclei containing GAL-ir perikarya gradually increases along the vertebrate tree. In this sense, the limited anatomical distribution of GAL-ir perikarya in *B. jararaca* was unexpected. However, it can be hypothesized that peculiar features of feed intake and feeding behavior of snakes could be related to this finding. Conversely, the distribution of CRF, SRIF, AVT-ir and MST-ir in brains of snakes and turtles (Fernández-Llebrez et al., 1988; Smeets et al., 1990; Mancera et al., 1991; López-Avalos et al., 1993; Silveira et al., 2001, 2002; Alponti et al., 2006) has revealed that these peptidergic systems are more specialized in snakes than in turtles. So far, only few species of reptiles have been studied, more studies in other species are needed to support any interpretation.

In rodent and human, GAL neuronal cell bodies are widely distributed in the CNS, particularly in the hypothalamus in the preoptic area, the paraventricular and supraoptic nuclei, the median eminence and rodent arcuate nucleus or infundibular nucleus of the human hypothalamus, the analogue of the rodent arcuate nucleus (Skofitsch and Jacobowitz, 1985; Gentleman et al., 1989; Menyhert et al., 2006). However, GALP neuronal cell bodies are observed only in the arcuate/infundibular nucleus with discrete population also in the median eminence and infundibular stalk that send projections to various hypothalamic nuclei, particularly the paraventricular nucleus, and that make close contacts with luteinizing hormone-releasing hormone

neurons in basal forebrain (Takatsu et al., 2001; Gundlach, 2002). In comparison with rodent and human, *B. jararaca* brain exhibited a pattern of GAL-ir more similar to GALP, with cell bodies concentrated only in the infundibular hypothalamus, also the analogue of the rodent arcuate nucleus in reptiles (Moga et al., 2000). However, GALP remains to be identified in non-mammalian vertebrates.

The location of GAL-ir cell bodies in the infundibular hypothalamus strongly suggest that GAL peptides may participate in the regulation of feeding behavior in *B. jararaca*. Meanwhile, since this location is similar to that of human and rodents it might not be related to the peculiar features of feed intake and feeding behaviour of snakes, which strongly differ from human and rodents. Furthermore, the pattern of GAL-ir observed here in *B. jararaca* might be comparable to that of ad libitum feeding in rodents. Reptiles, including the snake *B. jararaca* feed at infrequent intervals. Many tropical and subtropical reptiles appear to undergo long periods of fasting, even in the absence of seasonal variations. For snakes, the long fasting periods are partially compensated by the impressive ability to ingest very large meals (Holmberg et al., 2003). In captivity, informations about the correct frequence, size and quantities of prey subjects required by snakes have been initially learned by trial and error experience from several technicians. Based on this experience, large snakes are known to may be fed two or three times weekly, while in the case of medium to large snakes such as *B. jararaca*, a meal every 1–2 weeks is adequate, provided the animal is healthy and growing steadily (Breno et al., 1990; Mattison, 1992). The motivational elements involved in predation was identified in *B. jararaca*, including chasing strategies, preferable prey size and frequency of feeding success (Troncone and Silveira, 2001). Those findings provide us the feeding strategy adopted in the present study, which minimizes the occurrence of starvation among captive *B. jararaca*. Some snakes brought from nature to the laboratory starve to death in spite of several efforts to feed them. Since the starvation is the most common sign of poor habituation of *B. jararaca* to captivity, snakes under this condition are not used for experimental studies in our laboratory.

It has been demonstrated that GAL controls the activity of adenohypophyseal cells (Koshiyama et al., 1987; Murakami et al., 1987; Estienne and Barb, 2005). GAL-ir perikarya detected in different hypothalamic nuclei send axons to different adenohypophyseal regions in fish (Batten et al., 1990a,b; Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991; Anglade et al., 1994), and to the portal system of the median eminence in mammals (Skofitsch and Jacobowitz, 1985; Melander et al., 1986; Palkovits et al., 1987; Beal et al., 1988; Merchenthaler, 1991; Kordower et al., 1992; Perez et al., 2001), amphibians (Lázár et al., 1991; Olivereau and Olivereau, 1992; González-Nicolini et al., 1998), birds (Józsa and Mess, 1993), and the turtle *M. caspica* (Jiménez et al., 1994). Similarly, in the snake *B. jararaca*, GAL-ir fibers present in the outer layer of the median eminence could release GAL into the capillaries of the portal system and then control adenohypophyseal activity. In addition, GAL-ir present in the neurohypophysis could release GAL into systemic blood and/or into intercellular spaces to control adenohypophyseal cell activity by a paracrine fashion.

In the snake *B. jararaca*, the supraoptic nucleus and paraventricular nucleus displayed relatively fewer GAL-ir fibers. These two nuclei showed AVT-ir and MST-ir neurons that send their axons to the neurohypophysis (Silveira et al., 2002). In mammals, there are evidences indicating a physiological role of GAL as a neurotransmitter involved in the inhibition of vasopressin release (Kondo et al., 1995). Thus, this localization of GAL-ir fibers is suggestive that GAL exerts an effect on synthesis and/or release of neurohypophyseal hormones in *B. jararaca*.

Many CRF-, SRIF- and neurohypophyseal hormone-containing cerebrospinal fluid (CSF)-contacting perikarya have been reported in the snake *B. jararaca* (Silveira et al., 2001, 2002; Alponti et al., 2006) and in the turtle *M. caspica* (Fernández-Llebrez et al., 1988; López-Avalos et al., 1993). It is thought that these neurons could secrete peptides into the CSF and, in this way, GAL could reach other targets throughout the CNS. It has been demonstrated that the number of CSF-contacting perikarya decreases along the phylogeny (Vigh-Teichmann and Vigh, 1983, 1989). Based on this concept, our study confirms the older evolutionary origin of turtles in relation to snakes, since we observed that *B. jararaca* contain lower number of GAL-ir CSF-contacting neurons than the turtle *M. caspica* (Jiménez et al., 1994). However, compared with mammals, the presence of more abundant CSF-contacting processes of GAL-ir neurons in *M. caspica* (Jiménez et al., 1994) and *B. jararaca* implies that the number of these neurons does not necessarily indicate that GAL plays a lesser central role.

The lamina terminalis of *B. jararaca* exhibited numerous galaninergic innervation. In the lamina terminalis of *M. caspica*, GAL-ir fibers and some GAL-positive perikarya have also been detected (Jiménez et al., 1994). In this location, in addition to this GAL innervation, conspicuous bundles of CRF-, SRIF-, AVT- and MST-ir fibers have been described in *B. jararaca* (Silveira et al., 2001, 2002; Alponti et al., 2006) and other reptilian species (Fernández-Llebrez et al., 1988; Mancera et al., 1991; López-Avalos et al., 1993). In mammals, the organum vasculosum of lamina terminalis appears to represent an important center for neuroendocrine regulation (Buggy and Bealer, 1987). It is not known whether a structure similar to the organum vasculosum of lamina terminalis exists in reptiles (Leonhardt, 1980), but the confluence observed in *B. jararaca* of those peptidergic innervations to the lamina terminalis suggests this possibility.

A network of GAL-ir fibers innervated the habenula of *B. jararaca*. Galaninergic innervation of this region appears to be quite variable among different species. GAL-ir fibers have been observed in the habenula of rats (Skofitsch and Jacobowitz, 1985; Melander et al., 1986), primates (Kordower et al., 1992) and a turtle (Jiménez et al., 1994),

whereas they have been shown to be present (Olivereau and Olivereau, 1991) or absent in teleosts (Holmqvist and Ekström, 1991) and anurans (Lázár et al., 1991).

4.2. Telencephalon and brainstem

In mammals (Melander et al., 1986; Melander and Staines, 1986; Kordower et al., 1992; Perez et al., 2001) and birds (Józsa and Mess, 1993), GAL-ir perikarya and fibers have been found in the septum, which is suggested to be involved in the control of water balance (Miselis, 1981; Tanaka et al., 1988). In anurans and turtles, only GAL-ir fibers have been observed in this region (Lázár et al., 1991; Olivereau and Olivereau, 1992; Jiménez et al., 1994). The absence of GAL-ir in this region of the snake *B. jararaca* is an intriguing feature from a phylogenetic point of view, indicating that this peptide possibly does not influence the septal function in this snake. On the other hand, GAL-ir fibers were present in the nucleus accumbens and in the dorsal cortex of *B. jararaca*. Moreover, in common with *M. caspica* (Jiménez et al., 1994), *B. jararaca* showed GAL-ir fibers in the brainstem. This sharp difference in the distribution of brainstem GAL-ir among vertebrates (Jiménez et al., 1994) makes interpretation difficult.

In mammals, extra-hypothalamic GAL has been involved in the inactivity of cholinergic neurons (Melander et al., 1986; Chan-Palay, 1988), presynaptic inhibition of serotoninergic system (Sundström and Melander, 1988), and modulation of serotonin receptors (Fuxe et al., 1988). Relatively widespread brain GAL-ir distribution observed in the turtle *M. caspica* (Jiménez et al., 1994) and here in the snake *B. jararaca* suggests that this peptide, as in other vertebrates, could also play a central role.

In conclusion, regarding to this unique study on the distribution of GAL in the brain of a snake, any consideration concerning polarity or evolution of the GAL peptidergic system would be incomplete. Our finding that less GAL-ir CSF-contacting neurons are present in *B. jararaca* than in turtle agrees with the older evolutionary origin of turtles in relation to snakes. However, despite of the the widespread distribution of CRF- (Silveira et al., 2001), AVT-, MST- (Silveira et al., 2002) and SRIF-ir (Alponti et al., 2006), the restricted anatomical localization of GAL-ir perikarya in this snake in relation to turtles do not validate the hypothesis that the specialization of peptidergic systems could be a general process related to a more recent evolutionary origin. In addition, the absence of GAL-ir in the septum of this snake is an intriguing feature from a phylogenetic point of view. As found in other vertebrates, the localization of GAL-ir suggests that GAL and/or GALP may participate in the regulation of feeding behavior and/or reproductive functions throughout the activity on the control of adenohypophyseal cells and on the synthesis and/or release of neurohypophyseal hormones in *B. jararaca*. As in turtle, relatively widespread distribution of brain GAL-ir fibers suggests the central role of this peptide in *B. jararaca* snake.

Acknowledgments

The authors are indebted to Dr. P. Fernández-Llebrez for his kind gift of the anti-rabbit IgG antiserum and for his valuable comments and suggestions on this study. Thanks are due to the staff of the Laboratory of Herpetology of the Instituto Butantan for the collection and identification of the snakes and to Dr. M. C. Breno for providing the snake's skulls. Thanks are also due to Mrs. F. Canhoto for her skilled technical assistance. This investigation was supported by a Research Grant 03/13239-0 from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil) and a CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil) Productivity Grant to P.F.S. R.F.A. is the recipient of a CNPq fellowship.

References

Aguila, M.C., Marubayashi, U., McCann, S.M., 1992. The effect of galanin on growth hormone-releasing factor and somatostatin release from median eminence fragments in vitro. Neuroendocrinology 56, 889–894.

Alponti, R.F., Breno, M.C., Mancera, J.M., Martín-del-Río, M.P., Silveira, P.F., 2006. Distribution of somatostatin immunoreactivity in the brain of the snake *Bothrops jararaca*. Gen. Comp. Endocrinol. 145, 270–279.

Ando, R., Bungo, T., Kanwakami, S., Shimojo, M., Masuda, Y., Furuse, M., 2000. Intracerebroventricular injection of mammalian motilin, melanin-concentrating hormone or galanin does not stimulate food intake in neonatal chicks. Br. Poult. Sci. 41, 508–511.

Anglade, I.Y., Wang, J., Jensen, G., Tramu, G., Kah, O., Conlon, J.M., 1994. Characterization of trout galanin and its distribution in trout brain and pituitary. J. Comp. Neurol. 350, 63–74.

Bartfai, T., 1995. Galanin. A neuropeptide with important central nervous system actions. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: The Fourth Generation of Progress. Raven Press, New York, pp. 563–571.

Batten, T.F.C., Cambre, L., Moons, L., Vandesande, F., 1990a. Comparative distribution of neuropeptide-immunoreactive systems in the brain of the green molly, *Poecilia latipinna*. J. Comp. Neurol. 302, 893–919.

Batten, T.F.C., Moons, L., Cambre, M., Vandesande, F., 1990b. Anatomical distribution of galanin-like immunoreactivity in the brain and pituitary of teleost fishes. Neurosci. Lett. 111, 12–17.

Beal, M.F.S., Gabriel, M., Swartz, K.J., MacGarvey, U., 1988. Distribution of galanin-like immunoreactivity in the baboon brain. Peptides 9, 847–851.

Breno, M.C., Yamanouye, N., Prezoto, B.C., Lazari, M.F.M., Toffoletto, O., Picarelli, Z.P., 1990. Maintenance of the snake *Bothrops jararaca* (Wied, 1824) in captivity. The Snake 22, 126–130.

Buggy, J., Bealer, SL., 1987. Physiological regulation by the AV3V region. In: Gross, P.M., Florida, I. (Eds.), Circumventricular Organs and Body Fluids. CRC Press, Boca Raton, FL, pp. 171–190.

Chan-Palay, V., 1988. Neurons with galanin innervate cholinergic cells in the human basal forebrain and galanin and acetylcholine coexist. Brain Res. Bull. 21, 465–472.

Chartrel, N., Wang, Y., Fournier, A., Vaudry, H., Colon, J.M., 1995. Frog vasoactive intestinal polypeptide and galanin: primary structures and effects on pituitary adenylate cyclase. Endocrinology 136, 3079–3086.

DePedro, N., Cespedes, M.V., Delgado, M.J., Alonso-Bedate, M., 1995. The galanin-induced feeding stimulation is mediated via α2-adrenergic receptors in goldfish. Reg. Pept. 57, 77–84.

Díaz-Miranda, L., Pardo-Reoyo, C.F., Martínez, R., Garcı´a-Arraras, J.E., 1996. Galanin-like immunoreactivity in the sea cucumber *Holothuria glaberrima*. Cell Tissue Res. 286, 385–391.

Donkelaar, H.J., Nieuwenhuys, R., 1979. The brainstem. In: Gans, C., Northcutt, R.G., Ulinski, P. (Eds.), Biology of Reptilia, Vol. 10: Neurology. Academic Press, London, pp. 165–200.

Ekblad, E., Rökaeus, A., Hakanson, A., Sundler, F., 1985. Galanin nerve fibers in the rat gut: distribution, origin and projections. Neurosciences 16, 355–363.

Elmquist, J.K., Fox, C.A., Ross, L.R., Jacobson, C.D., 1992. Galanin-like immunoreactivity in the adult and developing Brazilian opossum brain. Develop. Brain Res. 67, 161–179.

Estienne, M.J., Barb, C.R., 2005. The control of adenohypophysial hormone secretion by amino acids and peptides in swine. Domest. Anim. Endocrinol. 29, 34–42.

Fernández-Llebrez, P., Pérez, J., Nadales, A.E., Cifuentes, M., Grondona, J.M., Mancera, J.M., Rodríguez, E.M., 1988. Immunocytochemical study of the hypothalamic magnocellular neurosecretory nuclei of the snake *Natrix maura* and the turtle *Mauremys caspica*. Cell Tissue Res. 253, 435–445.

Fuxe, K., Ogren, S.O., Jansson, A., Cintra, A., Härfstrand, A., Agnati, L.F., 1988. Intraventricular injections of galanin reduces 5-HT metabolism in the ventral limbic cortex, the hippocampal formation and the frontoparietal cortex of the male rat. Acta Physiol. Scand. 133, 579–581.

Gentleman, S.M., Falkai, P., Bogerts, B., Herrero, M.T., Polak, J.M., Roberts, G.W., 1989. Distribution of galanin-like immunoreactivity in the human brain. Brain Res. 505, 311–315.

González-Nicolini, M.V., Orezzoli, A.A., Villar, M.J., 1998. An immunohistochemical study of temperature-related changes in galanin and nitric oxide synthase immunoreactivity in the hypothalamus of the toad. Gen. Comp. Endocrinol. 110, 175–181.

Gundlach, A.L., 2002. Galanin/GALP and galanin receptors: role in central control of feeding, body weight/obesity and reproduction? Eur. J. Pharmacol. 440, 255–268.

Gundlach, A.L., Burazin, T.C., Larm, J.A., 2001. Distribution, regulation and role of hypothalamic galanin systems: renewed interest in a pleiotropic peptide family. Clin. Exp. Pharmacol. Physiol. 28, 100–105.

Hilke, S., Hokfelt, T., Theodorsson, E., 2006. A short estrogen-responsive N-terminal galanin homologue found in rat brain and gut with antiserum raised against rat galanin(1–16). Neurochem. Res. 31, 177–188.

Hokfelt, T., Broberger, C., Diez, M., Xu, Z.Q., Kopp, J., Zhang, X., Holmberg, K., Landry, M., Koistinaho, J., 1999. Galanin and NPY, two peptides with multiple putative roles in the nervous system. Horm. Metab. Res. 31, 330–334.

Holmberg, A., Kaim, J., Persson, A., Jensen, J., Wang, T., Holmgren, S., 2003. Effects of digestive status on the reptilian gut. Comp. Biochem. Physiol. A 133, 499–518.

Holmqvist, B.I., Ekström, P., 1991. Galanin-like immunoreactivity in the brain of teleosts: distribution and relation to substance P, vasotocin, and isotocin in the Atlantic salmon (*Salmo salar*). J. Comp. Neurol. 306, 361–381.

Jiménez, A.J., Mancera, J.M., Pombal, M.A., Pérez-Fı´gares, J.M., Fernández-Llebrez, P., 1996. Distribution of galanin-like immunoreactivite elements in the brain of the adult lamprey *Lampreta fluviatilis*. J. Comp. Neurol. 368, 185–197.

Jiménez, A.J., Mancera, J.M., Pérez-Fı´gares, J.M., Fernández-Llebrez, P., 1994. Distribution of galanin-like immunoreactivity in the brain of the turtle *Mauremys caspica*. J. Comp. Neurol. 349, 73–84.

Józsa, R., Mess, B., 1993. Galanin-like immunoreactivity in the chicken brain. Cell Tissue Res. 273, 391–399.

Jureus, A., Cunningham, M.J., Li, D., Johnson, L.L., Krasnow, S.M., Teklemichael, D.N., Clifton, D.K., Steiner, R.A., 2001. Distribution and regulation of galanin-like peptide (GALP) in the hypothalamus of the mouse. Endocrinology 142, 5140–5144.

Kagstrom, J., Olsson, C., Axelsson, M., Franklin, C.E., 1998. Peptidergic control of gastrointestinal blood flow in the estuarine crocodile, *Crocodylus porosus*. Am. J. Physiol. 274, R1740–R1750.

Kohchi, C., Tsutsui, K., 2000. Galanin: cloning of complementary DNAs and characterization of transcrips in different tissues. J. Exp. Zool. 287, 183–190.

Kondo, K., Murase, T., Otake, K., Ito, M., Kurimoto, F., Oiso, Y., 1995. Galanin as a physiological neurotransmitter in hemodynamic control of arginine vasopressin release in rats. Neuroendocrinology 57, 224–229.

Kordower, J.H., LE, H.K., Mufson, E.J., 1992. Galanin immunoreactivity in the primate central nervous system. J. Comp. Neurol. 319, 479–500.

Koshiyama, H., Kato, Y., Tatsuhide, Y., Murakami, Y., Ishikawa, Y., Yanaihara, N., Imura, H., 1987. Central galanin stimulates prolactin secretion in rats: possible involvement of hypothalamic vasoactive intestinal polypeptide. Neurosci. Lett. 75, 49–54.

Lamanna, C., Assisi, L., Costagliola, A., Vittoria, A., Botte, V., Cecio, A., 1999. Galanin in the lizard oviduct: its distribution and relationships with estrogen, VIP and oviposition. Life Sci. 65, 91–101.

Lázár, G., Liposits, Z., Tóth, P., Trasti, S.L., Maderdrut, J.L., Merchenthaler, L., 1991. Distribution of galanin-like immunoreactivity in the brain of *Rana esculenta* and *Xenopus laevis*. J. Comp. Neurol. 310, 45–67.

Leonhardt, H., 1980. Ependym and circumventriculäre organe. In: Oksche, A., Vollrath, L. (Eds.), Handbuch der mikroskopischen anatotomie des mensche, Band IV Springer, Berlin, pp. 177–665.

López-Avalos, M.D., Mancera, J.M., Pérez-Fı´gares, J.M., Fernández-Llebrez, P., 1993. Immunocytochemical localization of corticotropin-releasing factor in the brain of the turtle, *Mauremys caspica*. Anat. Embryol. 188, 163–171.

Lundquist, C.T., Rökaeus, A., Nässel, R., 1991. Galanin immunoreactivity in the blowfly nervous system: localization and chromatographic analysis. J. Comp. Neurol. 312, 77–96.

Mancera, J.M., López-Avalos, M.D., Pérez-Fı´gares, J.M., Fernández-Llebrez, P., 1991. The distribution of corticotropin-releasing factor-immunoreactive neurons and nerve fibers in the brain of the snake, *Natrix maura*. Coexistence with arginine vasotocin and mesotocin. Cell Tissue Res. 264, 539–548.

Matsumoto, H., Noguchi, J., Takatsu, Y., Horikoshi, Y., Kumano, S., Ohtaki, T., Kitada, C., Itoh, T., Onda, H., Nishimura, O., Fujino, M., 2001. Stimulation effect of galanin-like peptide (GALP) on luteinizing hormone-releasing hormone-mediated luteinizing hormone (LH) secretion in male rats. Endocrinology 142, 3693–3696.

Mattison, C., 1992. Foods and feeding. In: The Care of Reptiles and Amphibians in Captivity, 3rd ed. Blandford, London. Chapter 5.

McDonald, T.J., Dupre, J., Tatemoto, K., Greenberg, G.R., Radziuk, J., Mutt, V., 1985. Galanin inhibits insulin secretion and induces hyperglycemia in dogs. Diabetes 34, 192–196.

McKeon, T.W., Carraway, R.E., Konopka, L.M., Parsons, R.L., 1990. Distribution of galanin-like peptide in various tissues of *Necturus maculosus*. A biochemical and immunocytochemical analysis. Cell Tissue Res. 262, 461–466.

Melander, T., Hökfelt, T., Rökaeus, A., 1986. Distribution of galanin-like immunoreactivity in the rat central nervous system. J. Comp. Neurol. 248, 475–517.

Melander, T., Staines, W.A., 1986. A galanin-like peptide coexists in putative cholinergic neurons of the septum-basal forebrain complex and in acetylcholinesterase-containing fibers and varicosities within the hippocampus in the owl monkey (*Aotus trivirgatus*). Neurosci. Lett. 68, 17–22.

Menyhert, J., Wittmann, G., Hrabovszky, E., Keller, E., Liposits, Z., Fekete, C., 2006. Interconnection between orexigenic neuropeptide Y- and anorexigenic alpha-melanocyte stimulating hormone-synthesizing neuronal systems of the human hypothalamus. Brain Res. 1076, 101–105.

Merchenthaler, I., 1991. The hypophysiotropic galanin system of the rat brain. Neuroscience 44, 643–654.

Merchenthaler, I., Lopez, F.J., Negro-Vilar, A., 1993. Anatomy and physiology of central galanin-containing pathways. Prog. Neurobiol. 40, 711–769.

Miselis, R.R., 1981. The efferent projections of the subfornical organ of the rat: a circumventricular organ within a neural network subserving water balance. Brain Res. 230, 1–23.

Moga, M.M., Geib, B.M., Zhou, D., Prins, G.S., 2000. Androgen receptor-immunoreactivity in the forebrain of the Eastern Fence lizard (*Sceloporus undulatus*). Brain Res. 879, 174–182.

Murakami, I., Kato, Y., Koshiyama, H., Inoue, T., Yanaihara, N., Imura, H., 1987. Galanin stimulates growth hormone (GH) secretion via GH-

releasing factor (GRF) in conscious rats. Eur. J. Pharmacol. 136, 415–418.

Niimi, M., Takahara, J., Kawanishi, K., 1992. Corticotropin releasing factor and galanin-containing neurons projecting to the median eminence of the rat. Neurosci. Res. 14, 295–299.

Ohtaki, T., Kumano, S., Ishibashi, Y., Ogi, K., Matsui, H., Harada, M., Kitada, C., Kurokawa, T., Onda, H., Fujino, M., 1999. Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus. J. Biol. Chem. 274, 37041–37045.

Olivereau, M., Olivereau, J.M., 1991. Immunocytochemical localization of a galanin-like peptidergic system in the brain and pituitary of some teleost fish. Histochemistry 96, 343–354.

Olivereau, M., Olivereau, J.M., 1992. Immunocytochemical localization of a galanin-like peptidergic system in the brain of two urodele and two anuran species (Amphibia). Histochemistry 98, 51–66.

Ottlecz, A., Samson, W.K., McCann, S.M., 1986. Galanin: evidence for a hypothalamic site of action to release growth hormone. Peptides 7, 51–53.

Palkovits, M., Rökaeus, A., Antoni, F.A., Kiss, A., 1987. Galanin in the hypothalamo-hypophysial system. Neuroendocrinology 46, 417–423.

Perez, S.E., Wynick, D., Steiner, R.A., Mufson, E.J., 2001. Distribution of galaninergic immunoreactivity in the brain of the mouse. J. Comp. Neurol. 434, 158–185.

Pieribone, V.A., Brodin, L., Hökfelt, T., 1994. Immunohistochemical analysis of the relation between 5-hydroxytryptamine- and neuropeptide-immunoreactive elements in the spinal cord of an amphibian (*Xenopus laevis*). J. Comp. Neurol. 341, 492–506.

Prasada-Rao, P.D., Subhedar, N., Raju, D., 1981. Cytoarchitectonic pattern of the hypothalamus in the cobra, *Naja naja*. Cell Tissue Res. 217, 505–529.

Rodríguez-Gómez, F.J., Rendón-Unceta, M.C., Sarasquete, C., Muñoz-Cueto, J.A., 2000. Localization of galanin-like immunoreactive structures in the brain of the Senegalese sole, *Solea senegalensis*. Histochem. J. 32, 123–131.

Rökaeus, A., 1987. Galanin: a newly isolated biologically active neuropeptide. TINS 10, 158–164.

Rökaeus, A., Melander, T., Hökfelt, T., Lundberg, J.M., Tatemoto, K., Carlquist, M., Mutt, V., 1984. A galanin-like peptide in the central nervous system and intestine of the rat. Neurosci. Lett. 47, 161–166.

Silveira, P.F., Breno, M.C., Martin-del-Rio, M.P., Mancera, J.M., 2001. Corticotropin-releasing hormone-like immunoreactivity in the brain of the snake *Bothrops jararaca*. Histochem. J. 33, 685–694.

Silveira, P.F., Breno, M.C., Martín-del-Río, M.P., Mancera, J.M., 2002. The distribution of vasotocin and mesotocin immunoreactivity in the brain of the snake *Bothrops jararaca*. J. Chem. Neuroanat. 24, 15–26.

Silveira, P.F., Mimura, O.M., 1999. Concentrating ability of the *Bothrops jararaca* gallbladder. Comp. Biochem. Physiol. 123A, 25–33.

Silveira, P.F., Schiripa, L.N., Picarelli, Z.P., 1992. Hydrolysis of L-cystine-di-β-naphthylamide and neurohypophyseal peptides by the plasma of the snake *Bothrops jararaca*. Comp. Biochem. Physiol. 102B, 119–122.

Skofitsch, G., Jacobowitz, D.M., 1985. Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. Peptides 6, 509–546.

Smeets, J.A.J., Sevensma, J.J., Jonker, A.J., 1990. Comparative analysis of vasotocin-like immunoreactivity in the brain of the turtle *Pseudemys scripta elegans* and the snake *Python regius*. Brain Behav. Evol. 35, 65–84.

Sternberger, L.A., 1986. Immunocytochemistry. Wiley, New York.

Sundström, E., Melander, T., 1988. Effects of galanin on 5HT neurons in the rat CNS. Eur. J. Pharmacol. 146, 327–329.

Takatsu, Y., Matsumoto, H., Ohtaki, T., Kumano, S., Kitada, C., Onda, H., Nishimura, O., Fujino, M., 2001. Distribution of galanin-like peptide in the rat brain. Endocrinology 142, 1626–1634.

Tanaka, J., Saito, H., Seto, K., 1988. Involvement of the septum in the regulation of paraventricular vasopressin neurons by the subfornical organ in the rat. Neurosci. Lett. 92, 187–191.

Tatemoto, K., Rökaeus, H., Jörnvall, H., McDonald, T.J., Mutt, V., 1983. Galanin: a novel biologically active peptide from porcine intestine. FEBS Lett. 164, 124–128.

Troncone, L.R.P., Silveira, P.F., 2001. Predatory behavior of the snake *Bothrops jararaca* and its adaptation to captivity. Zoo Bio. 20, 399–406.

Vallarino, M., Feuilloley, M., Vandesande, M., Vaudry, H., 1991. Immunohistochemical mapping of galanin-like immunoreactivity in the brain of the dogfish *Scyliorhinus canicula*. Peptides 12, 351–357.

Vigh-Teichmann, Y., Vigh, B., 1983. The system of cerebrospinal fluid contacting neurons. Arch. Hist. Jpn. 46, 427–468.

Vigh-Teichmann, Y., Vigh, B., 1989. The cerebrospinal fluid-contacting neurons: A peculiar cell type of the central nervous system. Immunocytochemical aspect. Arch. Histol. Cytol. 52, 195–207.

Vrontakis, M.E., Torsello, A., Friesen, H.G., 1991. Galanin. J. Endocrinol. Invest. 14, 785–794.

Wang, Y., Conlon, J.M., 1994. Purification and primary structure of galanin from the alligator stomach. Peptides 15, 603–606.

Wang, Y., Lance, V.A., Nielsen, P.F., Conlon, J.M., 1999. Neuroendocrine peptides (insulin, pancreatic polypeptide, neuropeptide Y, galanin, somatostatin, substance P, and neuropeptide gamma) from the desert tortoise, *Gopherus agassizii*. Peptides 20, 713–722.

Wolfbauer, C., Skofitsch, G., 1989. Immunohistochemical localization of galanin and calcitonin gene-related peptide in the brain of the frog *Rana temporaria*. Gen. Comp. Endocrinol. 74, 295–307.