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Distribution of somatostatin immunoreactivity in the brain of the snake *Bothrops jararaca*

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Abstract

The distribution of perikarya and fibers containing somatostatin was studied in the brain of the snake *Bothrops jararaca* by means of immunohistochemistry using an antiserum against synthetic somatostatin. Immunoreactive perikarya and fibers were localized in telencephalic, diencephalic and mesencephalic areas. In the telencephalon, numerous immunoreactive perikarya were found in the medial, dorsomedial, dorsal and lateral cortex, mainly in the deep plexiform layer, less so in the cellular layer, but not in the superficial plexiform layer. Immunoreactive perikarya were also observed in the dorsal ventricular ridge, the nucleus of the diagonal band of Broca, amygdaloid complex, septum and lamina terminalis. In the diencephalon, labelled cells were observed in the paraventricular, periventricular hypothalamic and in the recessus infundibular nuclei. In the mesencephalon, immunoreactive perikarya were seen in the mesencephalic reticular formation, reticular nucleus of the isthmus and torus semicircularis. Labelled fibers ran along the diencephalic floor and the inner zone of the median eminence, and ended in the neural lobe of the hypophysis. Other fibers were observed in the outer zone of the median eminence close to the portal vessels and in the septum, lamina terminalis, retrochiasmatic nucleus, deep layers of the tectum, periventricular gray and granular layer of the cerebellum. Our data suggest that somatostatin may function as a mediator of adenohypophysial secretion as well as neurotransmitter and/or neuromodulator which can regulate the neurohypophysial peptides in the snake *B. jararaca*.

Keywords: Neuropeptides; Islet peptides; Hypophysiotrophic hormones; Reptiles

1. Introduction

Somatotropin release-inhibiting factor or somatostatin (SRIF) was discovered in the gut and D-cells of the endocrine pancreas (Brazeau et al., 1973; Polak et al., 1975). SRIF peptides have been recognized as widely distributed in mammals, acting as hormone, autocrine or paracrine factor and neuropeptide (Epelbaum, 1986). In general, SRIF has been involved in the growth, development and metabolism of vertebrates (Slagter and Sheridan, 2004).

In the mammalian brain, two principal bioactive forms of 14 (SRIF-15-28 or SRIF-14) and 28 (SRIF-1-28 or SRIF-28) amino acids have been found (Epelbaum, 1986). Several immunohistochemical studies have demonstrated the wide distribution of SRIF in the central nervous system (CNS) of mammals (Bennett-Clarke et al., 1980; Bennett-Clarke and Joseph, 1986; Campbell et al., 1987; Dierickx and Vandesande, 1979; Finley et al., 1981; Krisch, 1978). The presence of SRIF in the brain has been related to processes such as mediation of adenohypophysial secretion, modulation of neuroendocrine control (Horvath et al., 1989; Murakami et al., 1987), autonomic function, feeding behaviour, neuronal development and neuropathological processes (Delfs and Dichter, 1985; Epelbaum, 1986; Patel and Srikant, 1986).

Immunoreactivity (ir) for SRIF has been detected in the CNS of non-mammalian vertebrates, e.g., fishes (Batten et al., 1985; Stroh and Zupanc, 1993), amphibians

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(Dierickx et al., 1981; Vandesande and Dierickx, 1980), reptiles (Bear and Ebner, 1983; Davila et al., 1988; Doerr-Schott and Dubois, 1977; Fasolo and Gaudino, 1982; Goossens et al., 1980; Perez-Clausell and Fredens, 1988; Reiner, 1992; Weindl et al., 1984) and birds (Anderson and Reiner, 1990; Medina et al., 1998; Takatsuki et al., 1981). SRIF has also been found in neurosecretory cells in invertebrates (Bautz et al., 1980; Schot et al., 1981).

SRIF is considered a phylogenetically ancient family of cyclical peptides. SRIF-14 is conserved with identical structure in representative species of all studied classes of vertebrates (Lin et al., 2000). Using an antiserum raised against synthetic SRIF-14, the distribution of somatostatinergic perikarya and fibers was investigated in the CNS of lizards (Davila et al., 1988; Doerr-Schott and Dubois, 1977; Fasolo and Gaudino, 1982; Goossens et al., 1980; Perez-Clausell and Fredens, 1988; Reiner, 1992) and turtles (Bear and Ebner, 1983; Reiner, 1992; Weindl et al., 1984). The distribution of SRIF in the brain of snakes has not yet been investigated.

Because snakes are adapted to consuming large meals at infrequent intervals, any peculiar pattern of the CNS distribution of SRIF could be revealed in these animals. Furthermore, snakes are adapted to a wide range of habitats, and it is conceivable that differences in the pattern of the central distribution of SRIF could be part of these environmental adaptations. The terrestrial Brazilian pit viper *Bothrops jararaca* is among the most well-studied snakes regarding the predatory behaviour (Troncone and Silveira, 2001), the concentrating ability of the gallbladder (Silveira and Mimura, 1999) and the endocrine compounds related to the control of blood pressure and water and salt balance (Abdalla et al., 1989; Borgheresi et al., 1996; Breno et al., 2001; Gervitz et al., 1992; Hayashi et al., 2003; Silveira et al., 1992a,b; Silveira et al., 1998; Silveira et al., 2001; Silveira et al., 2002).

The primary goal of the present study is to compare the SRIF locations reported in other reptilian species with the distribution of the SRIF-ir perikarya and fibers in the brain of the snake *B. jararaca*. Our study is also undertaken to obtain insight concerning the roles of this peptide in snakes by exploring the presence of SRIF-ir in locations which could indicate that SRIF exerts hormonal effects via the vasculatura or the cerebrospinal fluid, or transmitter and/or modulatory effects via contacts with other neurons. Since SRIF has been reported to influence water intake (Hajdu et al., 2003), blood pressure and heart rate, and to increase plasma vasopressin (Brown, 1988), an additional objective of the present study is to infer whether SRIF could act as a neuromodulator to control the production of neurohypophysial peptides in this snake.

2. Material and methods

2.1. Animal

Adult male ($n = 3$) and female ($n = 3$) snakes *B. jararaca* (Squamata, Serpentes, Viperidae) (about 180 g of body weight and 103 cm in length) were collected in spring from the wild in southern and southeastern Brazil. They were identified by the Laboratory of Herpetology, Instituto Butantan, and then individually housed in a cage (inside length × width × height 35 × 26 × 22 cm) in a restricted-access room where they were acclimated for a minimum of 15 days to a controlled temperature of 25 °C, relative humidity of 65.3 ± 0.9% and 12 h light:12 h dark photoperiod (lights on at 6:00 h AM). 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.

The snakes were anesthetized with sodium pentobarbital (3 mg nembutal/100 g body weight, subcutaneously) between 4:00 and 6:30 h during the light phase. They received an intracardiac injection in bolus of 100 IU sodium heparin in 0.1 ml Ringer's solution for *B. jararaca* (Silveira and Mimura, 1999). Cardiac perfusion was then performed with this Ringer's solution over a period of 15 min and continued with Bouin's fixative 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. Peroxidase-antiperoxidase (PAP) immunohistochemistry

Series of transverse (8 μm thick) sections were hydrated and immunostained according to the peroxidase-antiperoxidase method (Sternberger, 1986) using an antiserum raised against synthetic SRIF-14 in rabbit (kindly provided by Dr. E.M. Rodríguez, Valdivia, Chile). Sections were initially incubated for 15 min at 22 °C in H_2O_2 (0,3% in Tris buffer) 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 second antiserum (anti-rabbit IgG raised in goats, kindly provided by Dr. P. Fernández Llebez, 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. Sections were rinsed three times in Tris buffer after H_2O_2, antisera and PAP incubation. All antisera and the PAP complexes were diluted in Tris buffer, pH 7.8, containing 0.7% nongelling seaweed gelatin Type IV lambda carrageenan (Sigma), 0.5% Triton X-100 (Sigma) and 0.02% sodium azide (Merck). As an electron donor, 0.025% 3.3′-diamonibenzidine tetrahydrochloride (DAB) (Sigma) in Tris buffer, pH 7.8 with 0.007% H_2O_2 (Merck) was used for incubating sections in the dark for 15 min at 22 °C.

The specificity of the antiserum was tested by immunoabsorption with synthetic SRIF-14 (Sigma S-9129) and SRIF-28 (Sigma S-6135). Aliquots of the antiserum were mixed separately with these peptides at concentrations of 10 and 20 mg/ml. These preparations were kept for 18 h at 22 °C. The resulting solutions were employed, in the same staining session, for immunohistochemistry on sections adjacent to those immunostained with the non-absorbed anti-SRIF-14. In order to test the specificity of the immunoreaction and to check the occurrence of endogenous peroxidase, adjacent sections were processed as described above, but incubation in the primary antisera was omitted. Neither the use of immunoabsorbed antiserum nor immunohistochemistry omitting the primary antiserum revealed any stained structures in the sections. Moreover, absorption of the antiserum with the heterologous peptide did not abolish the immunoreaction.

Transverse sections, stained with haematoxylin-eosin and cresyl violet, were used for anatomical examinations. The identification of all the different nuclei was based on those descriptions for other reptiles. The nomenclature of the brain regions and nuclei was adopted from Donkelaar and Nieuwenhuys (1979), Prasada-Rao et al. (1981), Fernandez-Llebrez et al. (1988), Smeets et al. (1990); Silveira et al. (2001, 2002). The positions occupied by SRIF-ir perikarya were determined by examining every tenth immunostained section using a Nikon E600 microscope equipped with a CoolSNAP-PRO digital camera coupled to a microcomputer system. The images were captured and the sizes of cell bodies were measured using the image processor Image-Pro-Plus 4.0 (Media Cybernetics, USA). Morphometric data were presented as means ± standard deviation. Diameter was measured across the long axis of cell bodies. The total number of cells measured for each nucleus was 15–20. In the schematic drawings, the circles represent SRIF-ir perikarya. The number of circles is a relative estimation of the number of SRIF-ir perikarya observed in the immunostained section. The schematic drawings are identical to those shown in earlier reports (Silveira et al., 2001, 2002). All photographs were taken with a CoolPix 990 digital camera (Nikon, Japan).

3. Results

SRIF-ir perikarya and fibers were found in the telencephalon, diencephalon, and mesencephalon. Immunoreactivity to SRIF was not examined in the olfactory bulbs and it was absent in the rostral telencephalon. Neurosecretory nuclei exhibited bilateral symmetry. Since all animals were sacrificed at the same time of the day and the year, circadian and/or seasonal differences could not be evaluated. The distribution of SRIF-ir perikarya and fibers is shown in schematic drawings of representative transverse sections (Figs. 1A–K).

3.1. Neuronal perikarya

In the telencephalon, SRIF-ir perikarya were frequently found in the medial, dorsomedial and dorsal cortex (Figs. 2A–D), and in the the caudal pole of the hemisphere (Fig. 2D). Most perikarya occupied the deep plexiform layer whereas some of them were found in the cellular layer. No immunoreactive perikarya were detected in the superficial plexiform layer. SRIF-ir perikarya showed a strong immunoreactivity. The cells show variability in the size and forms (fusiform or bipolar) (Figs. 2A and B). The dorsal ventricular ridge (DVR) showed isolated, small, strongly immunoreactive, and round or fusiform perikarya located predominantly in the dorsal part (7.84 ± 0.58 μm in diameter) (Fig. 2C). In the caudal part of the DVR, SRIF-ir perikarya were present in the dorsolateral region (Fig. 1C). In addition, some small and round SRIF-ir perikarya (7.64 ± 0.47 μm) were observed in the nucleus of the diagonal band of Broca (NDB)[1] (Fig. 3A) and in the lamina terminalis (LT) (Fig. 3B). The amygdaloid complex also showed small and isolated fusiform- or pear-shaped perikarya (7.73 ± 0.51 μm) having strong immunoreactivities.

In the diencephalon, SRIF-ir perikarya were restricted to the hypothalamus. SRIF-ir perikarya were absent in the supraoptic nucleus (SON), whereas some SRIF-ir cells (8.08 ± 0.55 μm) were scattered around the paraventricular nucleus (PVN) (Fig. 3C). Some of these cells displayed an apical process that appeared to contact the third ventricle (Fig. 3C inset). The periventricular hypothalamic nucleus (PH), ventral to the PVN, showed small, round and strongly immunoreactive perikarya (8.14 ± 0.65 μm) arranged in one or two layers parallel to the ventricle, especially at the ventral regions of the nucleus (Fig. 3D). Numerous strongly immunoreactive perikarya (8.84 ± 0.61 μm) were detected in the recessus infundibular nucleus (RIN). They were small, and round or pear-shaped (Fig. 4A). Many of these neurons displayed an apical process toward the ventricle. In the median eminence (ME), small and round SRIF-ir perikarya (7.79 ± 0.52 μm) were observed close to the ependymal layers (Fig. 4B).

In the mesencephalon, fusiform SRIF-ir perikarya (8.68 ± 0.68 μm) displaying variable immunoreactivities were clustered in the mesencephalic reticular formation

[1] *Abbreviations used:* AC, anterior commissure; AM, amygdaloid complex; DC, dorsal cortex; DMC, dorsomedial cortex; DVR, dorsal ventricular ridge; E, ependyma; EZ, external zone; H, habenula; HHT, hypothalamo-hypophysial tract; IL, intermediate lobe of the hypophysis; IS, interhemispheric connective septum; IZ, internal zone; LC, lateral cortex; LT, lamina terminalis; MC, medial cortex; ME, median eminence; NDB, nucleus of the diagonal band of Broca; NL, neural lobe of the hypophysis; NS, nucleus sphericus; OC, optic chiasm; OT, optic tract; PG, periventricular gray; PH, periventricular hypothalamic nucleus; PO, paraventricular organ; PS, hypophysial portal system; PVN, paraventricular nucleus; RCN, retrochiasmatic nucleus; RF, mesencephalic reticular formation; RIN, recessus infundibular nucleus; RIS, reticular nucleus of the isthmus; S, septum; SCO, subcommissural organ; SFO, subfornical organ; SON, supraoptic nucleus; TS, torus semicircularis; V, ventricle; VMH, ventromedial nucleus of the hypothalamus.

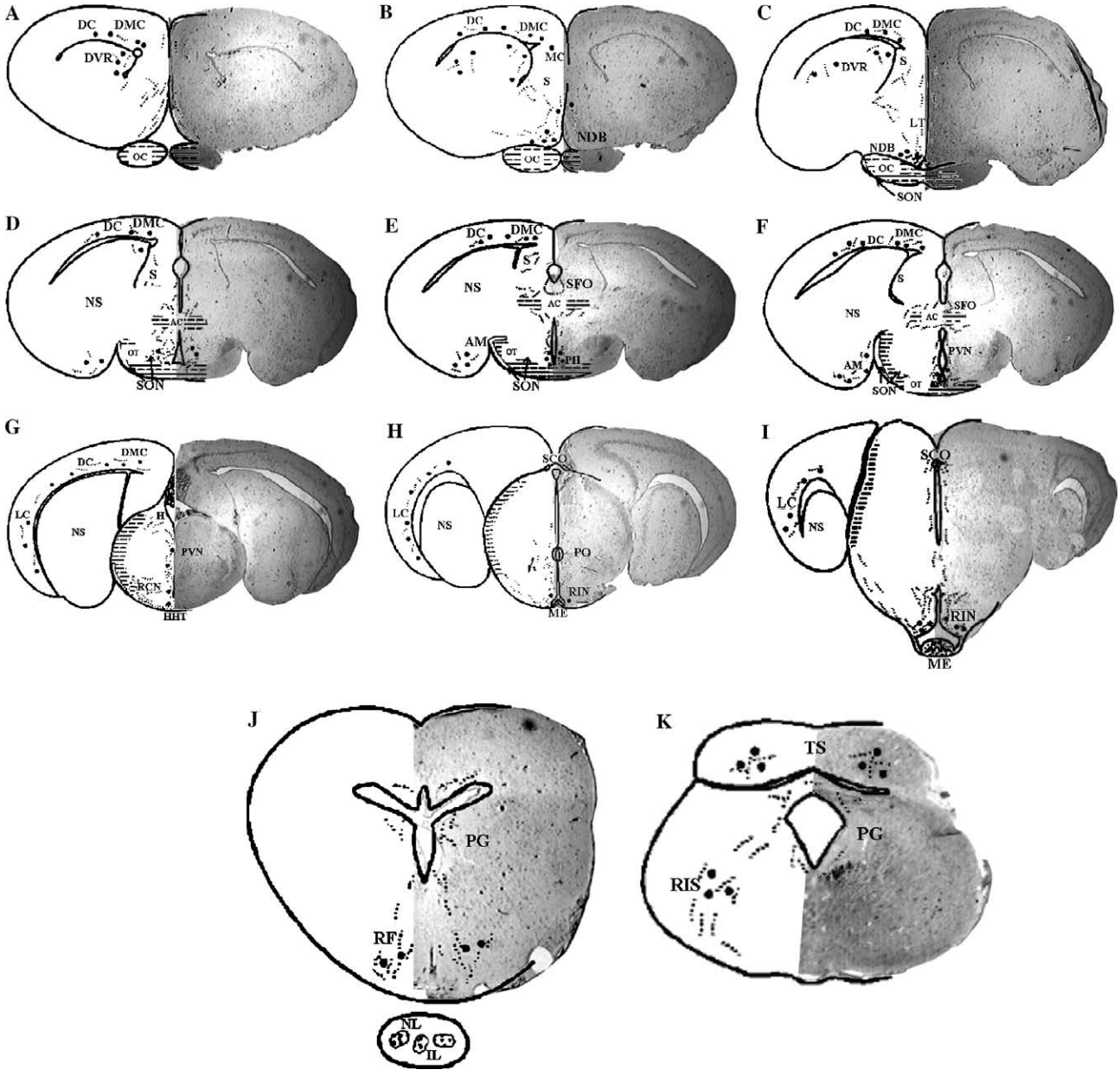

Fig. 1. Series of schematic drawings (left half of each figure) of selected transverse sections (photograph on the right half of each figure) from the rostral (A) to the caudal (K) parts through the brain of *Bothrops jararaca* showing the distribution of somatostatin immunoreactive perikarya (large dots) and fibers (small dots). See Abbreviations.

(RF) (Fig. 4C). In addition, the reticular nucleus of the isthmus (RIS) displayed isolated perikarya, with strong immunoreactivity, which differed in sizes and shapes (Fig. 4D). In the torus semicircularis (TS), numerous fusiform or pear-shaped perikarya (9.74 ± 0.64 μm) showed weak immunoreactivity (Fig. 4E).

3.2. Nerve fibers

In general, all regions displaying SRIF-ir perikarya also showed immunoreactive fibers. However, labelled fibers existed in some other regions lacking SRIF-ir perikarya (Fig. 1). In the telencephalon, SRIF-ir fibers were mainly found in the caudal part of lateral cortex. In general, the cortical fibers displayed varicosities and extended in all directions, mostly perpendicular or parallel to the cortical surface (Fig. 2B). In addition, the septum and the region of the LT showed immunoreactive fibers (Figs. 1B and C and 3B).

In the diencephalon, many SRIF-ir fibers were seen close to the ependyma. Despite the absence of SRIF-ir fibers in the SON, a moderate SRIF-ir innervation was observed in the caudal retrochiasmatic nucleus (RCN) (Fig. 1G). The PH contained a moderate innervation of SRIF-ir fibers (Fig. 1E), whereas the PVN showed fibers specially in its caudal part (Fig. 1F and G). Immunoreactive fibers were observed at all parts of the RIN (Fig. 4A). The diencephalic floor showed numerous

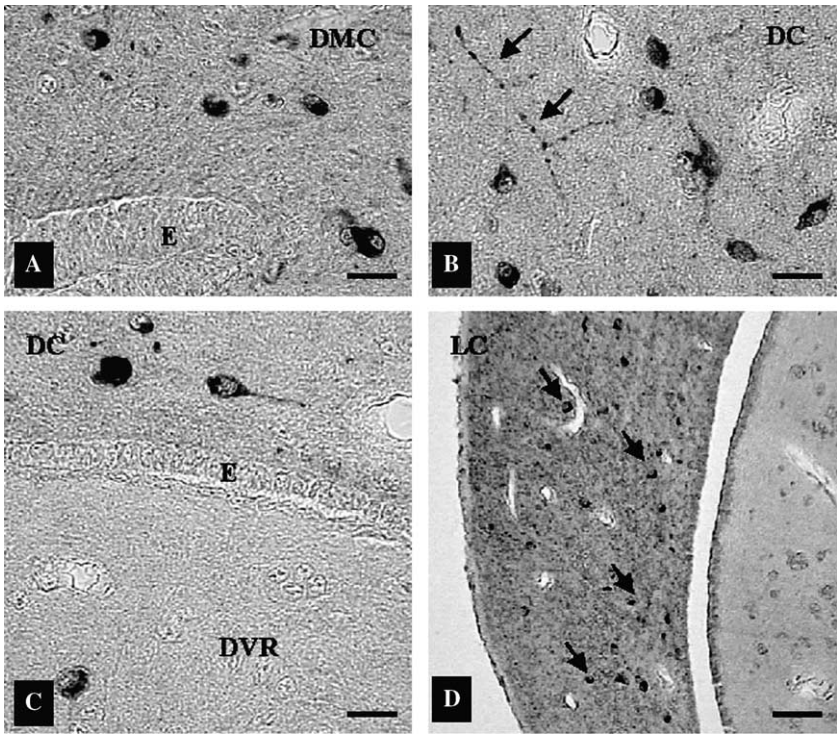

Fig. 2. Somatostatin immunoreactivity are observed in the transverse sections through the cerebral cortex of *Bothrops jararaca*. (A) The dorsomedial cortex (DMC) showing round or fusiform-shaped perikarya with strong immunoreactivity. E, ependyma. (B) The dorsal cortex (DC) presenting numerous round or fusiform-shaped perikarya and varicosity fibers (arrows). (C) A detail view of the DC and the dorsal ventricular ridge (DVR) with immunoreactive perikarya. (D) A general view of the caudal part of the lateral cortex (LC) with numerous immunoreactive perikarya (arrows)—it is controversial that LC could reach this caudal pole of the hemisphere in snakes (see Section 4). Bars = 15 μm for A–C and 22 μm for D.

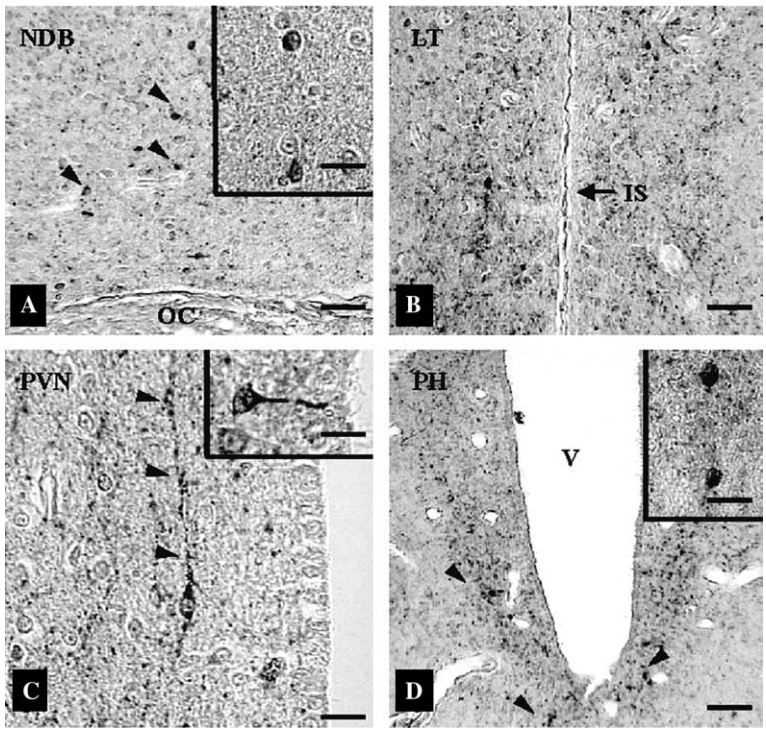

Fig. 3. (A) A transverse section showing the nucleus of the diagonal band of Broca (NDB) with immunoreactive perikarya (arrowheads). Bar = 22 μm. Inset, a detail view of immunoreactive perikarya. Bar = 15 μm. OC, optic chiasm. (B) Immunoreactive fibers in the lamina terminalis (LT). IS, interhemispheric connective septum. Bar = 22μm. (C) Some immunoreactive perikarya are present in the paraventricular nucleus (PVN). Bar = 15 μm. Inset, a detail view of a neuron of the PVN with an apical process contacting the ventricle. Bar = 12 μm. (D) A transverse section through the periventricular hypothalamic nucleus (PH) showing small and round immunoreactive perikarya (arrowheads) and fibers. Bar = 22 μm. Inset, a detail view of immunoreactive perikarya. Bar = 15 μm. V, ventricle.

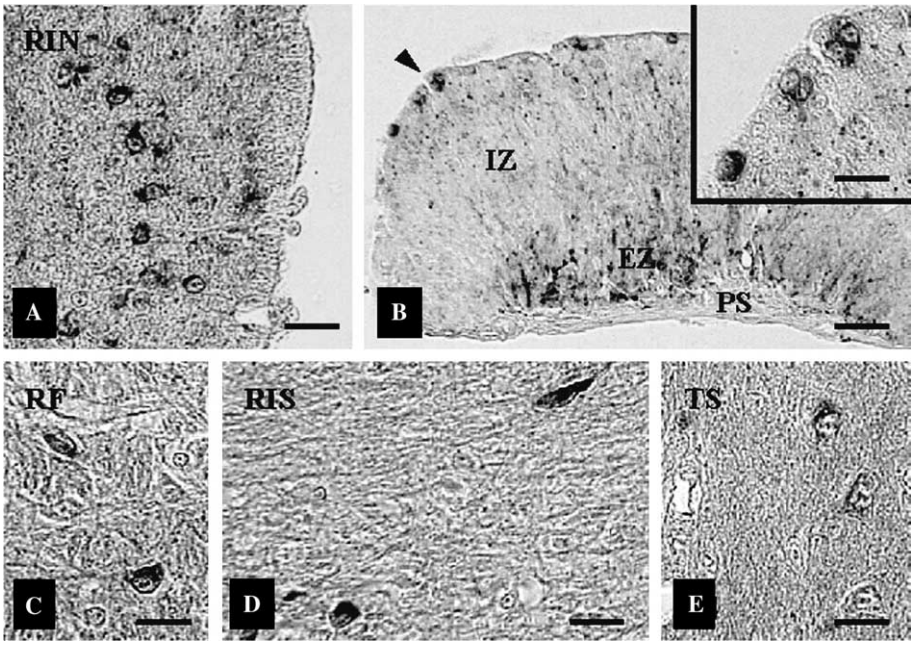

Fig. 4. (A) Numerous small, round or pear-shaped immunoreactive perikarya are present in the recessus infundibular nucleus (RIN). Bar = 22 μm. (B) A transverse section through the median eminence (ME). A dense innervation in the external zone (EZ) close to the vessels of the hypophysial portal system (PS) and more scarcely in the internal zone (IZ). Small and round perikarya are observed among the ependymal cells (arrowheads). Bar = 22 μm. Inset, a detail view of immunoreactive perikarya at ependyma. Bar = 12 μm. Somatostatin immunoreactive perikarya in different sizes, shapes and immunoreactivities are observed at (C) the nucleus of the reticular formation (RF), (D) the reticular nucleus of the isthmus (RIS), and (E) the torus semicircularis (TS). Bar = 15 μm for C–E.

SRIF-ir fibers up to the outer zone of the ME (Fig. 4B). In addition, fibers were also found in the inner zone of the ME and the neural lobe of the hypophysis.

In the mesencephalic and rostral rhombencephalic areas, numerous SRIF-ir fibers innervated the deep layers of the tectum, periventricular gray, and the ventral and lateral portions of the tegmentum, mainly the reticular formation and RIS (Figs. 1J and K). Immunoreactive fibers were also observed in the granular layer of the cerebellum.

4. Discussion

In the snake *Python molurus* (Conlon et al., 1997) and the turtle *Gopherus agassizii* (Wang et al., 1999) the amino acid sequences of SRIF are identical to that of the conserved SRIF-14 first isolated from sheep brain. The anti-SRIF-14 serum used here had also produced immunoreactivity when incubated with the brain tissue of the snake *Natrix maura* (Fernandez-Llebrez et al., 1987). Our results show that this anti-SRIF-14 serum cross-reacted with the perikarya and fibers in the CNS of *B. jararaca* which allowed us to propose the first mapping of SRIF-ir in the brain of a snake. However, the primary structure of SRIF in the snake *B. jararaca* has not yet been characterized. Considering that anti-SRIF-14 could not be an antiserum against native SRIF of *B. jararaca* some reduction of the sensitivity of the labelling may be expected. Since a limited number of specimens was only intermittently available for brain removal, the use of paraffin-embedded section was the only way to conserve this scarce material. However, this procedure may also be a factor diminishing the preservation of the antigen and sensitivity of the immunohistochemical labelling. It would be possible that the absence of immunoreactivity observed in some areas of the brain of *B. jararaca* may be explained by this pitfall. This issue is difficult to resolve, since the conditions of immunohistochemical procedure are usually not ideal in this and other studies on wild species.

In reptiles, the distribution of SRIF-ir perikarya and fibers has been described in the brain of different lizards (*Lacerta muralis*, Doerr-Schott and Dubois, 1977; *Ctenosauria pectinata*, Goossens et al., 1980; *Lacerta sicula*, Fasolo and Gaudino, 1982; *Psammodromus algirus*, and *Podarcis hispanica*: Davila et al., 1988; Perez-Clausell and Fredens, 1988) and turtles (*Pseudemys scripta*, Bear and Ebner, 1983; *Testudo hermanni*, Weindl et al., 1984). Due to the phylogenetic position of reptiles, comparisons of the peptidergic systems (e.g., somatostatinergic system) between three different reptilian radiations (turtles, lizards and snakes) are of great interest. Although many results of the present study on the snake *B. jararaca* agree with those described previously for other reptiles, some particular aspects deserve consideration.

4.1. Telencephalon

As for lizards and turtles (Bear and Ebner, 1983; Davila et al., 1988; Doerr-Schott and Dubois, 1977; Fasolo and Gaudino, 1982; Goossens et al., 1980; Perez-Clausell and Fredens, 1988; Reiner, 1992; Weindl et al., 1984), SRIF-ir

perikarya and fibers were widely distributed over cortical regions of *B. jararaca*. However, morphological differences exist between several reptilian species that have been studied. While only small and multipolar-shaped SRIF-ir cells were seen in the cortex of the lizard *C. pectinata* (Goossens et al., 1980), the present results evidenced distinct shapes of bipolar or pyramidal cells. Similar data were reported in the turtles *P. scripta* and *T. hermanni* (Bear and Ebner, 1983; Weindl et al., 1984), and in the lizards *P. algirus* and *P. hispanica* (Davila et al., 1988; Perez-Clausell and Fredens, 1988). Such a highly diversified morphology agrees to those results described previously in those mentioned reptiles and also in amphibians, birds and mammals (Bennett-Clarke et al., 1980; Takatsuki et al., 1981; Vandesande and Dierickx, 1980).

In *B. jararaca*, SRIF-ir perikarya were present, mainly in the deep plexiform layer of medial and dorsomedial cortex, and in the caudal pole of the hemisphere. This last area can be considered as the lateral cortex, but several authors have reported that the lateral cortex does not reach the caudal pole of the hemisphere in snakes. It would be replaced caudally by the dorsal cortex, as revealed by the olfactory axons (Lohman and Smeets, 1993; Lanuza and Halpern, 1998; Smeets et al., 1986). Thus, in spite of its lateral location, the population of SRIF-ir cells in the caudal cortical area could be located in the dorsal cortex. These results were similar to those described in the lizards *P. algirus* and *P. hispanica* (Davila et al., 1988; Perez-Clausell and Fredens, 1988). However, in another lizard species, *C. pectinata*, SRIF-ir perikarya were observed only in the dorsal cortex (Goossens et al., 1980). In the turtle *P. scripta*, SRIF-ir perikarya were also observed in the cortical regions, but mainly in the cellular layer (Bear and Ebner, 1983). Although the physiological role of SRIF in several brain areas is relatively known, in the cortex it is still unknown. It is thought that the SRIF system could modulate the release of neurotransmitters (Delfs and Dichter, 1985) and a few studies have shown an interaction of SRIF with others neuronal systems in the cortex of several species (Epelbaum, 1986; Nabekura et al., 1989). In the lizards *P. algirus* and *P. hispanica*, SRIF has been shown to colocalize with neuropeptides (e.g., neuropeptide Y), neurotransmitters (e.g., GABA) and calcium-binding proteins (e.g., parvalbumin) in the cerebral cortex (Davila et al., 1993; Martinez-Guijarro et al., 1993). In addition, SRIF and neuropeptide Y were also found in the same neurons in the telencephalon of the turtle *P. scripta* (Reiner, 1992). Further studies on the possible co-existence of SRIF with other neurochemical factors in the cortex of *B. jararaca* will be useful to understand the physiological role of SRIF in this location. It is noteworthy that SRIF-ir neurons were not observed in the striatum of this snake. In mammals (Figueredo-Cardenas et al., 1996; Marin et al., 2000) and amphibians (Marin et al., 1998) the presence of such cells has been described as a subtype of GABAergic inhibitory interneuron that co-express SRIF, neuropeptide Y and nitric oxide synthase. Whether other reptiles, fishes and birds in general do not have somatostatinergic neurons in this area remains to be seen.

The DVR of *B. jararaca* contained SRIF-ir perikarya and fibers. A similar feature has been described in the turtles *P. scripta* and *T. hermanni* (Bear and Ebner, 1983; Weindl et al., 1984) but not in lizards (Doerr-Schott and Dubois, 1977; Fasolo and Gaudino, 1982; Goossens et al., 1980; Perez-Clausell and Fredens, 1988). It is not known whether the DVR of reptiles has any homologous area in mammals, but its caudal part has been related to the amygdaloid complex of mammals (Northcutt, 1978). In according with mammals and some other reptiles, our results in *B. jararaca* show SRIF-ir perikarya and fibers in the amygdaloid complex. In addition, corticotrophin-releasing hormone (CRF) neurons and fibers were also present in the same region of *B. jararaca* (Silveira et al., 2001). According to Fellman et al. (1982), by means of these and some other mediators, the amygdaloid complex could control the hypophysiotropic axis. The literature is absent of data showing the identity of central amygdala in snakes. In the examined reptiles this area seems to be homologue to the striatoamygdalar area (Bruce and Neary, 1995). In mammals, CRF and SRIF are co-located in the bed nucleus of the stria terminalis and also in the central amygdala (Shimada et al., 1989). In the examined reptiles, the bed nucleus of the stria terminalis is located near the striatoamygdalar area (Lohman and Smeets, 1993). In both these aforementioned areas of the amygdaloid complex of *B. jararaca* there was no SRIF-ir, which was located only in the ventral posterior region, in the ventral aspect of the nucleus sphericus. In the amygdaloid complex of this snake, CRF immunolabelling was also located in the ventral aspect of the nucleus sphericus (Silveira et al., 2001). These results do not permit to hypothesize that these CRF-ir and SRIF-ir cells in the brain of the snake *B. jararaca* correspond to the central nucleus of mammals.

As for the lizards *C. pectinata* and *P. hispanica*, and the turtle *T. hermanni* (Goossens et al., 1980; Perez-Clausell and Fredens, 1988; Weindl et al., 1984) the septum of *B. jararaca* showed conspicuous innervation of SRIF-ir fibers. The functional significance of this peptidergic innervation of the septum is not known. On the other hand, in snakes, including *B. jararaca*, and turtles, CRF-, vasotocin-, and mesotocin-immunoreactive fibers were found in the LT (Fernandez-Llebrez et al., 1988; Mancera et al., 1991; Silveira et al., 2001, 2002), where dense SRIF innervations were also observed in *B. jararaca*. It is suggestive that SRIF-ir fibers from the hypothalamus, especially from the preoptic region, pass through LT via the septal region and/or that fibers end and release this substance in the LT proper.

4.2. Diencephalon

The distribution of SRIF-ir perikarya has been studied in the diencephalon of several lizards and turtles (Bear and Ebner, 1983; Doerr-Schott and Dubois, 1977; Fasolo

and Gaudino, 1982; Goossens et al., 1980; Reiner, 1992; Weindl et al., 1984). SRIF-ir perikarya were particularly abundant in the preoptic and infundibular areas. In *B. jararaca* SRIF-ir perikarya were also located in the PH and especially in the RIN. An interesting fact was that the presence of SRIF-ir perikarya occurred very close to the ependymal layer of the ME. This location has never been described before in any other species. It is likely that these neurons are cerebrospinal fluid (CSF)-contacting and concentrate SRIF. These SRIF-ir CSF-contacting neurons sending a dendrite to the third ventricle could get some information about the compositions of the CSF which may influence the SRIF production/release of these cells. Further investigations are required to focuse SRIF-ir of the spinal cord using some representative segments of the various areas.

In *B. jararaca*, the PVN and RCN were highly innervated by SRIF-ir fibers. Since the vasotocinergic and mesotocinergic perikarya of these nuclei contribute to the neurohypophysial fibers tract (Silveira et al., 2002), let us suggest that SRIF acts as a neuromodulator to control the production of vasotocin and/or mesotocin.

In mammals (Bennett-Clarke et al., 1980; Finley et al., 1981), birds (Anderson and Reiner, 1990; Takatsuki et al., 1981), amphibians (Vandesande and Dierickx, 1980) and reptiles (Goossens et al., 1980; Reiner, 1992; Weindl et al., 1984) several studies have demonstrated that SRIF-ir perikarya of the PH project axons to the ME. In *B. jararaca*, the RIN contained more SRIF-ir perikarya than the PH. In addition, neurons from the RIN containing the CRF and mesotocin send their axons to the hypothalamo-hypophysial tract (Silveira et al., 2001, 2002). Hence, it is possible that, similar to other peptidergic systems, SRIF-ir perikarya located in the RIN could also contribute to the hypothalamo-hypophysial tract. However, further tracing studies will be necessary to prove this hypothesis.

In mammals and birds, SRIF-ir fibers were located in the outer zone of the ME, close to the pericapillary spaces of the portal vessels (Bennett-Clarke et al., 1980; Finley et al., 1981; Takatsuki et al., 1981; Vandesande and Dierickx, 1980). Our results show a similar feature and agree with those observed in some lizards (Doerr-Schott and Dubois, 1977; Fasolo and Gaudino, 1982; Goossens et al., 1980) and turtles (Bear and Ebner, 1983; Weindl et al., 1984). It allows us to assume that SRIF could be released into the portal circulation and might control the activity of adenohypophysial cells as observed in mammals (Patel and Srikant, 1986). Some other SRIF-ir fibers, in the inner zone of the ME, might reach the neural lobe and release SRIF that could reach the adenohypophysis via a posterior lobe-adenohypophysial portal system (Bondy et al., 1989). In addition, SRIF present in CSF could be transported across the ME to the portal vessels through tanycytes (Murakami et al., 1987; Patel and Srikant, 1986). The presence in *B. jararaca* of numerous SRIF-ir CSF-contacting neurons in the hypothalamic periventricular areas suggests the release of SRIF in the CNS. These results are coincident with the observations performed in non-mammalian (Batten et al., 1985; Fasolo and Gaudino, 1982; Vandesande and Dierickx, 1980; Vigh et al., 2004). By any of these routes, SRIF could act as a hypophysiotropic factor via the hypothalamo-hypophysial portal system.

4.3. Mesencephalon and rostral rhombencephalon

In mammalian and non-mammalian species (Finley et al., 1981; Reiner, 1992; Vandesande and Dierickx, 1980), SRIF-ir perikarya have been located in different mesencephalic areas. In reptiles, little information exists on the somatostatinergic system in the mesencephalic areas. Poorly labelled SRIF-ir fibers were observed in the cerebellum of the snake *B. jararaca*. In the lizard *C. pectinata*, fibers but not perikarya have been described in the lateral base of the cerebellum, the base of the tegmentum and the caudal parts of the tuber cinereum running toward the optic tectum (Goossens et al., 1980). In the turtle *T. hermanni*, SRIF-ir perikarya were located in the interpeduncular nucleus, the ventral tegmental area and the reticular nucleus of the isthmus, while fibers were widely distributed (Weindl et al., 1984). As for turtles, our results in *B. jararaca* showed SRIF-ir perikarya in the RIS. In addition, SRIF-ir perikarya were also observed in the mesencephalic reticular formation and in the torus semicircularis. The presence of SRIF perikarya and fibers in the mesencephalic areas of mammalian and non-mammalian species, including the snake *B. jararaca*, suggests that SRIF could function as a neurotransmitter or a neuromodulator in these areas.

In conclusion, the pattern of SRIF-ir in the CNS of *B. jararaca* in general is similar to that found in other reptiles. Regarding to this unique study on the distribution of SRIF in the brain of a snake, any consideration concerning polarity or evolution of the SRIF signaling system would be inconsistent. SRIF-ir projections to extrahypothalamic areas suggest this peptide as neurotransmitter and/or neuromodulator in this snake. The presence of SRIF-ir in the amygdaloid complex, the inner and outer zone (close to the pericapillary spaces of the portal vessels) of the ME, and in CSF-contacting neurons in hypothalamic periventricular areas suggest the hypophysiotropic role of SRIF, mainly on adenohypophysial cells. SRIF-ir in the PVN and RCN also indicates the relationship of somatostatinergic with vasotocinergic and/or mesotocinergic neurons in *B. jararaca*.

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