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The distribution of vasotocin and mesotocin immunoreactivity in the brain of the snake, *Bothrops jararaca*

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Abstract

Polyclonal antibodies against vasotocin (AVT) and mesotocin (MST) were used to explore the distribution of these peptides in the brain of the snake *Bothrops jararaca*. Magnocellular AVT- and MST-immunoreactive (ir) perikarya were observed in the supraoptic nucleus (SON), being AVT-ir neurons more numerous. A portion of the SON, in the lateroventral margin of the diencephalon ventrally to optic tract, showed only AVT-ir perikarya and fibers. However, the caudal most portion displayed only mesotocinergic perikarya. Parvocellular and magnocellular AVT- and MST-ir perikarya were present in the paraventricular nucleus (PVN) being AVT-ir fibers more abundant than MST-ir. Vasotocinergic perikarya were also found in a dorsolateral aggregation (DLA) far from the PVN. Mesotocinergic perikarya were also present in the recessus infundibular nucleus and ependyma near to paraventricular organ. Nerve fibers emerging from supraoptic and paraventricular nuclei run along the diencephalic floor, internal zone of the median eminence (ME) to end in the neural lobe. Also a dense network of AVT- and MST-ir fibers was present in the external zone of the ME, close to the vessels of the hypophysial portal system. As a rule, all regions having vasotocinergic and mesotocinergic perikarya also showed immunoreactive fibers. Vasotocinergic and mesotocinergic fibers but not perikarya were found in the lamina terminalis (LT). Moreover AVT-ir fibers were present in the nucleus accumbens and MST-ir fibers in the septum. In mesencephalon and rhombencephalon MST-ir fibers were more numerous than AVT-ir fibers. Vasotocinergic and mesotocinergic fibers in extrahypothalamic areas suggest that these peptides could function as neurotransmitters and/or neuromodulators in the snake *B. jararaca*.

Keywords: Hypothalamus; Extrahypothalamic distribution; Neuropeptides; Reptiles

1. Introduction

The neurosecretory hypothalamo–hypophyseal system of mammalian and submammalian vertebrates has been analysed using histochemical, ultrastructural and immunocytochemical techniques (Dierickx, 1980; Sofroniew, 1983; Castel et al., 1984). Neurohypophysial hormones of both vasopressinergic and oxytocinergic families are synthesized, packed, transported and released into the systemic blood of the neurohypophyseal lobe together with large peptide molecules, the neurophysins (Nps) (Rodríguez, 1984). Arginine-vasotocin (AVT) and mesotocin (MST) are, respectively, the hormones of the vasopressinergic and oxytocinergic family in reptiles (Acher, 1990; Acher and Chauvet, 1995). In addition to the hypothalamo–hypophyseal system, extrahypothalamic projections have also been described in mammalian and in submammalian vertebrates (Buijs, 1978; Smeets et al., 1990; Meurling et al., 1996; González and Smeets, 1997; Panzica et al., 1999).

Immunohistochemical studies have revealed the distribution of AVT-immunoreactive (AVT-ir) and MST-immunoreactive (MST-ir) perikarya and fibers in the central nervous system (CNS) of some reptiles including lizards, snakes and turtles (Goossens et al., 1979; Bons and Pérézi, 1981; Bons, 1983; Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988; Smeets et al., 1990; Propper et al., 1992). However, knowledge of the precise physiological role of AVT and MST in these vertebrates classes remains incomplete

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(Bentley, 1976; Chan, 1977; George, 1977; Dantzler and Braun, 1980; Rice, 1982; Pang et al., 1983; Figler et al., 1989; Fergusson and Bradshaw, 1991; Conklin et al., 1996; Takei, 2000). For instance, in the terrestrial pit viper *Bothrops jararaca*, measures of plasma levels of AVT did not correlated to osmolality (Silveira et al., 1992, 1998).

Studies on the distribution of neuropeptides in submammalian vertebrates are of special interest for comparative and phylogenetic considerations (Oksche, 1976; Moore and Lowry, 1998). Since there are not detailed studies on the distribution of hypophyseal neuropeptides in any viperid snake, we studied the distribution of AVT and MST in *B. jararaca*. The results will be discussed in relation to the pattern of vasotocinergic and mesotocinergic system present in other species and the role of these hormones in nonmammalian species.

2. Material and methods

Adult male ($n = 3$) and female ($n = 3$) snakes *B. jararaca* (Serpentes, Viperidae, Crotalinae) (about 180 g in weight and 103 cm in length) were used in this study. All animals were collected from the wild in south and southeastern Brazil and acclimated for controlled environmental conditions (12-h light:12-h dark photoperiod, relative humidity of $65.3 \pm 0.9\%$ and temperature between 25 and 26 °C) (Breno et al., 1990). Sexual identification was made by gently pressing the tail base below the cloaca, with the consequent exposure of one or both hemipenises characterizing a male (Fitch, 1987). Nonpregnant females were selected by macroscopic examination of the oviduct. They were supported with adequate food (one Swiss mouse to each snake every 15 days) and had free access to water.

The animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (3 mg per 100 g body wt) and subsequently injected transcardially with 0.1 ml of sodium heparin solution (1000 IU/ml of Ringer's solution for *B. jararaca*) (Silveira et al. 1992). This Ringer's solution had, according to plasma ion concentrations, the following composition (mM): NaCl, 180; K_2HPO_4, 5; KH_2PO_4, 0.6; $MgSO_4$, 1.4; $CaCl_2$, 2.5; glucose, 5; and pH 7.2–7.3. Specimens were then perfused with this solution followed by Bouin's fixative for 40–50 min at a flow rate of 2.4–4.8 ml/min. The dissected brains were placed for 48 h in the same fixative and then were dehydrated and embedded in paraffin.

Sagittal and transverse serial (10 μm thick) sections were hydrated and immunostained according to the peroxidase–antiperoxidase (PAP) method (Sternberger 1986). The following primary antisera were used: (i) rabbit anti-AVT antiserum (1:1000) (kindly provided by Professor R.M. Buijs, Amsterdan, Holland), and (ii) rabbit anti-MST antiserum (1:1000) (kindly provided by Professor S. Blähser, Giessen, Germany). Sections were incubated for 15 min at 22 °C in H_2O_2 (0,3% in Tris buffer) in order to avoid endogenous peroxidase activity and then incubated for 18 h at 22 °C in the primary antisera. The second antiserum (anti-rabbit IgG raised in goats from E.M. Rodríguez, Valdivia, Chile) was used at a dilution of 1:40 for 45 min at 22 °C, followed by rabbit-PAP complex (Dakopatts, Copenhagen, Denmark) at a dilution of 1:100 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 complex were diluted in Tris buffer, pH 7.8, containing 0.7% nongelling seaweed gelatin, lambda carrageenan (Sigma), 0.5% Triton X-100 (Sigma) and 0.02% sodium azide (Merck, Germany). As an electron donor, 0.025% 3.3′-diaminibenzidine tetrahydrochloride (DAB) (Sigma) in Tris buffer, pH 7.8 and 0.007% H_2O_2 (Merck) was used under dark conditions during incubation for 15 min at 22 °C.

The specificity of the anti-AVT and anti-MST antisera was tested by immunoabsorption of both antisera with synthetic AVT (Sigma V-0130) and with MST (Bachem H-2505). Aliquots of the antisera were mixed separately with the synthetic AVT or MST at concentration 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 immunocytochemistry on sections adjacent to those immunostained with the non-absorbed anti-AVT or anti-MST. 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 antisera nor immunocytochemistry omitting the primary antisera revealed any stained structure in the sections. Moreover, absorption of the antiserum with the heterologous peptide did not abolish the immunoreaction.

Consecutive sagittal and transverse sections stained with haematoxylin-eosin and cresyl violet were used for anatomical reference. The nomenclature used was according to that used by other authors (Prasada Rao et al., 1981; Fernández-Llebrez et al., 1988; Smeets et al., 1990). The areas occupied by AVT- and MST-ir perikarya were determined by examination of every tenth immunostained section using a Zeiss Jenapol microscope with a DSP Hitachi camera. In the schematic drawings taken from camera lucida, circles represent AVT-ir perikarya and triangles MST-ir perikarya. The number of circles and triangles is a relative estimation of the number of AVT- and MST-ir perikarya observed in the immunostained section. The size of the cells bodies was measured using the image processor HID 4-Advanced program. Morphometric data are presented as mean ± standard deviation

(S.D.). Diameter was measured in the long axis of the cell body. The total number of cells measured for each nucleus and antisera was 15–20.

3. Results

Although we did not perform densitometry, our qualitative morphological and immunocytochemical results indicate that no appreciable differences exist between males and females. Moreover neurosecretory nuclei exhibited bilateral symmetry. Since all animals were sacrificed at the same time of the year and the day, seasonal and/or circadian differences could not be evaluated. Figs. 1 and 2 are schematic drawings of sagittal (Fig. 1) and transverse sections (Fig. 2A–J) through the brain showing the distribution of AVT- and MST-ir perikarya and fibers. Comparison of the distribution of AVT- and MST-ir perikarya and fibers in *B. jararaca* and in other species of snakes is shown in Table 1.

3.1. Neurosecretory perikarya

Vasotocinergic and mesotocinergic perikarya were mostly found in the supraoptic nucleus (SON) and in the paraventricular nucleus (PVN). SON displayed only magnocellular neurons, whereas PVN contained magnocellular and parvocellular neurons. The SON extended rostrocaudally from a region limited by the caudal portion of the optic chiasma and the anterior commissure (Fig. 2B–F). A portion of the SON was located ventrally to the optic tract and close to the ventral brain surface (Fig. 3). Only AVT-ir neurons were observed in this portion of the SON. In addition, some AVT-ir perikarya and fibers were observed inside the optic tract. In the main body of the SON, dorsally to the optic tract, MST-ir neurons occupied positions more ventrally as compared with AVT-ir neurons (Fig. 5). AVT-ir neurons measured 13.29 ± 0.68 μm in diameter and for MST-ir 12.78 ± 1.15 μm. Both were strongly immunoreactive and round, bipolar or pear-shaped (Fig. 4 and Fig. 6).

The PVN first appears at the level of the anterior commissure and extends caudally until the level of the paraventricular organ (Fig. 2D–F). Most immunoreactive cells were distributed on either side of the third ventricle and located close to the ependyma. Some were oriented perpendicular to the ependyma and some displayed an apical dendrite that seemed to protrude through ependyma into the third ventricle. Differences between the locations of AVT- and MST-ir cell bodies in the PVN were not detected. AVT- and MST-immunoreactivity were observed in parvocellular (6.71 ± 1.25 μm for AVT-ir cells and 6.30 ± 1.42 μm for MST-ir cells) as well as in magnocellular (12.44 ± 0.63 μm for AVT-ir cells and 13.00 ± 1.30 μm for MST-ir cells) neurons. These neurons showed strong immunoreactivity and they had fusiform or pear-shaped cell bodies (Fig. 7 and Fig. 8). Immunoreactive nerve fibers run in different directions.

In addition to the SON and PVN, some accessory groups of AVT- or MST-ir perikarya were observed. The dorsolateral aggregation (DLA) is identified as a group of round or pear-shaped AVT-ir neurons (10.4 ± 1.03 μm in diameter) located dorsal to the lateral forebrain bundle (Fig. 9). The recessus infundibularis nucleus (RIN) contained round or pear-shaped MST-ir cells (12.2 ± 0.74 μm in diameter) (Fig. 10). Also some MST-ir cells were detected near paraventricular organ.

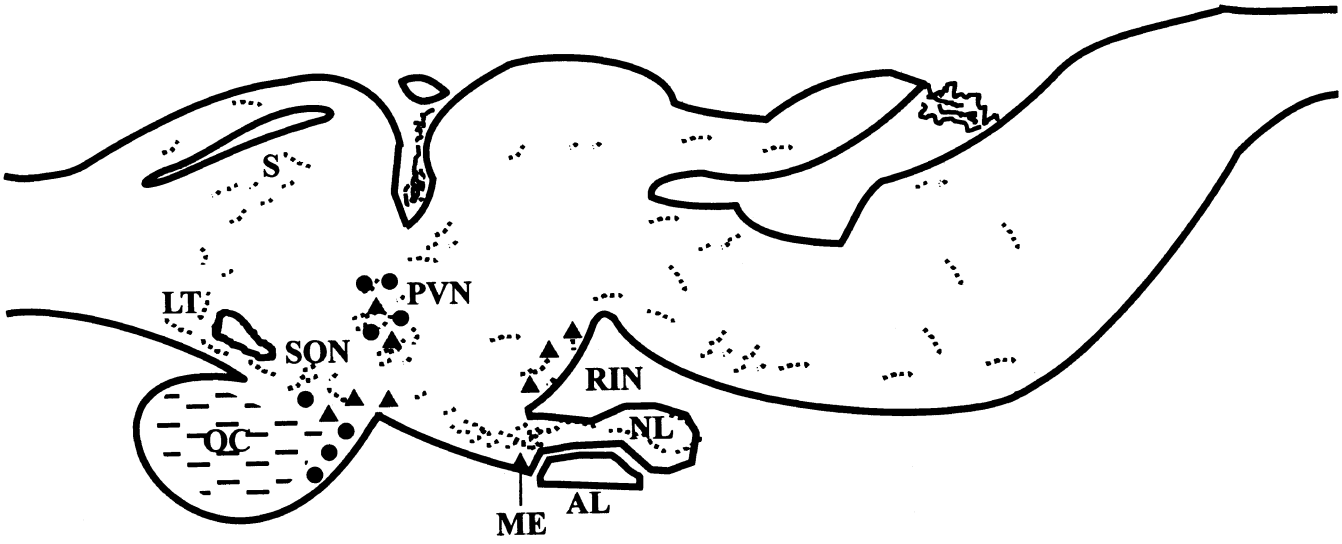

Fig. 1. Camera lucida drawing of a mid-sagittal section showing the distribution of AVT- (circles) and MST-ir (triangles) perikarya, and AVT- and MST-ir fibers (dots). Dashes represent tract of fibers. For abbreviations, see Table 2.

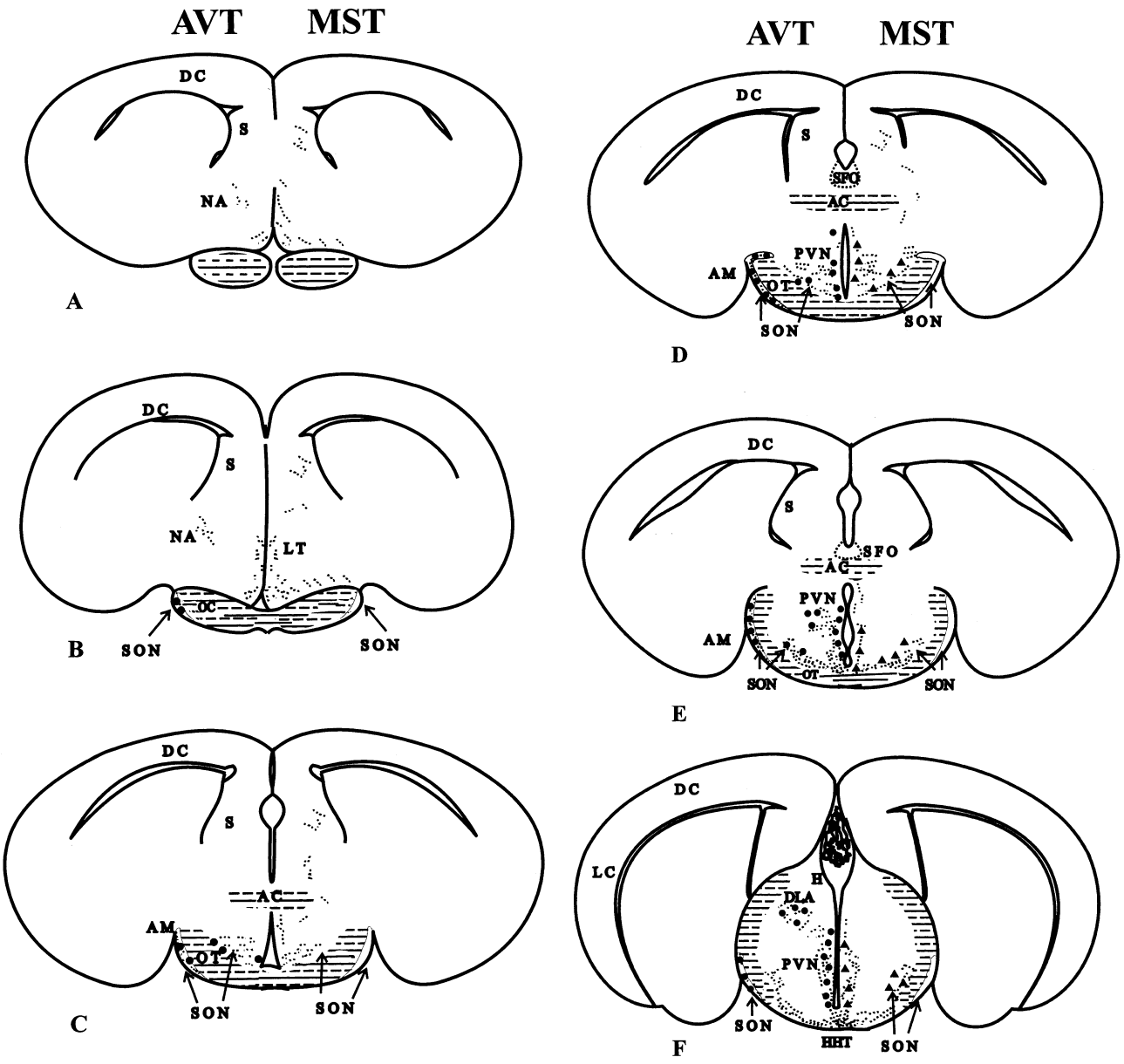

Fig. 2. Schematic drawings of transverse sections from rostral (A) to caudal (I) levels through the brain of *B. jararaca*. AVT-ir perikarya (circles) and fibers (dots) are shown on the left side and MST-ir perikarya (triangles) and fibers (dots) on the right side. Dashes represent tract of fibers. For abbreviations, see Table 2.

3.2. Distribution of immunoreactive fibers

In general, all the regions displaying AVT-ir and/or MST-ir cells also showed immunoreactive fibers (Figs. 1 and 2). In addition, many other regions lacking immunoreactive perikarya displayed a significant number of labelled fibers. MST-ir fibers were found in the septal area and AVT-ir and MST-ir fibers in the lamina terminalis (LT) (Fig. 2A and B).

In the hypothalamus, AVT-ir and, in a lesser amount, MST-ir fibers form bundles of fibers between the PVN and the SON. AVT-ir, but not MST-ir fibers, were found in the ventral neuropil of the SON close to the ventral brain surface (Fig. 2C–F). At variance, in the caudal SON, AVT- and MST-ir fibers were observed in this region. In the PVN, AVT-ir fibers were more abundant than MST-ir fibers. A conspicuous tract of vasotocinergic and mesotocinergic fibers emerged from SON and PVN and run along the diencephalic floor to converge in the median eminence (ME) constituting the hypothalamo–hypophyseal tract. The internal zone of the ME and the neural lobe of the hypophysis showed many AVT- and MST-ir fibers. Also a dense network of fibers was observed in the outer region of the ME close to the vessels of the hypothalamo–hypophysial portal system (Fig. 2F–I). AVT- and MST-ir fibers were also

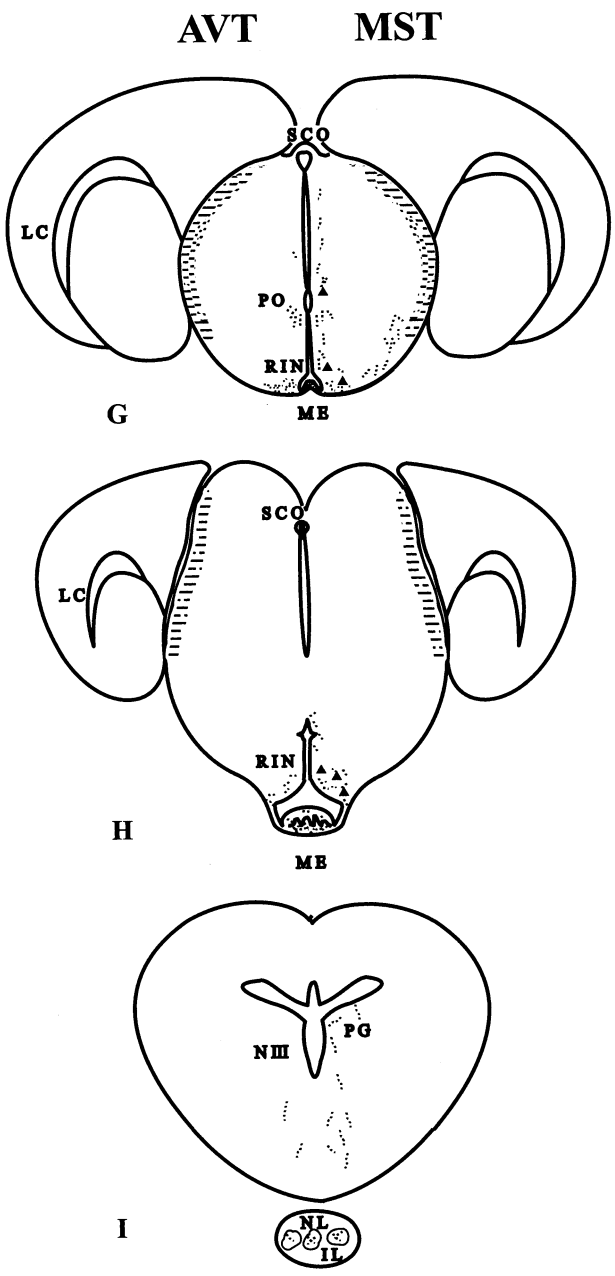

Fig. 2 (*Continued*)

seen close to the paraventricular organ. In the mesencephalon and rhombencephalon MST-ir fibers were more abundant than AVT-ir fibers, and innervate regions such as the interpeduncular nucleus, periaqueductal gray and periventricular region of the fourth ventricle (Fig. 2G–I).

4. Discussion

In general, the distribution of AVT- and MST-ir perikarya and fibers in *B. jararaca* agree with those previously reported in other reptiles using histochemical or immunocytochemical techniques (Philibert and Kamemoto, 1965; Haider and Sathyanesan, 1974; Prasada Rao and Subhedar, 1977; Goossens et al., 1979; Prasada Rao et al., 1981; Bons, 1983; Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988; Smeets et al., 1990; Propper et al., 1992). AVT- and MST-ir perikarya and fibers showed, essentially, the same pattern of distribution. Some authors (Dierickx, 1980; Castel et al., 1984) have used colchicine as a previous treatment to increase the amount of antigen in the neural somata and so to improve the visualization of the peptidergic neurons in reptiles. Since we did not use this strategy in our study, some discrepancies with previously results in other reptiles could be due to this different experimental approach.

Available data on neuropeptidergic systems reveal substantial differences in the distribution of secretory neurons and their projections not only among different classes of vertebrates, but also within a class and even an order (González and Smeets, 1997; Moore and Lowry, 1998). However, as mentioned above, methodological differences such as the use of different antibodies and immunocytochemical techniques could account for the differences reported. Also, differences could depend on the season of sacrifice (Buijs et al., 1986; Voorhuis et al., 1991) or the physiological status of the animal (Mohr et al., 1988; Jirikowski et al., 1991).

In addition, sexual dimorphism of the AVP/AVT-ir system has been reported in mammalian and submammalian species (see Moore and Lowry, 1998). In reptiles, AVT has been reported to predominante in male lizards (*Gekko gecko*: Stoll and Voorn, 1985; Thepen et al., 1987; *Anolis carolinensis*: Propper et al., 1992), turtles (*Pseudemys scripta elegans*) and snakes (*Python regius*) (Smeets et al., 1990). Although we did not perform any quantitative study, our qualitative results suggest that no rough sex-differences seem to exist in *B. jararaca*.

4.1. AVT- and MST-ir perikarya

In *B. jararaca* both magnocellular and parvocellular neurosecretory neurons were labelled by antisera against AVT and MST. In teleost, cartilaginous fishes (Goossens et al., 1977a; Dungen et al., 1982; Meurling et al., 1996) and amphibians (Vandesande and Dierickx, 1976; Conway and Gainer, 1987; González and Smeets, 1997; Lowry et al., 1997) neurosecretory neurons have been mainly found in the preoptic nucleus. At variance, in reptiles (Goossens et al., 1979; Bons, 1983; Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988; Smeets et al., 1990; Propper et al., 1992), birds (Goossens et al., 1977b; Tennyson et al., 1985), and mammals (Vandesande and Dierickx, 1975; Zimmerman, 1981) the SON and PVN have been shown to be the main source of the neurohypophysial peptides. Also

Table 1
Distribution of AVT- and MST-ir perikarya (P) and fibers (F) in some species of snakes. For more details about distribution of AVT and MST-ir perikarya and fibers in other reptilian species see Propper et al. (1992) and text

Brain area	*B. jararaca*[a]		*N. maura*[b]		*P. regius*[c]
	AVT	MST	AVT	MST	
MC	–	–	F(Nps +)	F(Np +)	–
DC	–	–	F(Nps +)	F(Np +)	F
LC	–	–	F(Nps +)	F (Np +)	F
DBB	–	–	–	–	F
AM	–	–	F(Nps +)	F(Nps +)	F
DVR	–	–	F(Nps +)	F(Nps +)	–
STR	–	–	–	–	F
NA	F	–	–	–	F
S	–	F	F(Nps +)	F(Nps +)	F
BNST	–	–	–	–	P, F
SM	–	–	–	–	F
LT	F	F	F(Nps +)	F(Nps +)	F
SON	P, F	P, F	P, F	P, F	P, F
AH	–	–	–	–	F
RCN	F	F	P, F	P, F	–
DLA	P, F	–	P, F	P, F	–
VMH	–	–	–	–	F
LHA	F	F	F	F	F
PVN	P, F	P, F	P, F	P, F	P, F
RIN	F	P, F	–	–	F
PO	F	P, F	P, F	P, F	–
PH	–	–	–	–	P, F
DM	–	–	–	–	F
H	–	–	–	–	F
SCO	–	P, F	–	–	–
ME	F	F	F	F	F
NL	F	F	F	F	F
T	–	–	–	–	F
IP	F	F	F(Nps +)	F(Nps +)	–
PG	F	F	F(Nps +)	F(Nps +)	F
CER	–	–	–	–	F
TRS	–	–	–	–	F
SN	–	–	F(Nps +)	F(Nps +)	F

For abbreviations, see Table 2.
[a] Present study.
[b] Fernández-Llebrez et al. (1988). Extrahypothalamic projections were analysed using antiserum against neurophysin I+II (Nps +).
[c] Smeets et al. (1990). The study was realized only with antiserum anti-AVT.

in *B. jararaca*, SON and PVN are the main neurosecretory nuclei. Moreover, AVT-ir perikarya were found in the DLA and MST-ir cells were present in the RIN.

In the SON of *B. jararaca* AVT and MST neurons show a characteristic distribution that were not observed in the PVN where AVT and MST neurons intermingled. Anatomical segregation of neurosecretory perikarya has also been described in the SON of mammals (Kawata and Sano, 1982; Hou-Yu et al., 1986), birds (Goossens et al., 1977b; Bons, 1980; Blähser, 1981; Panzica et al., 1999) and amphibians (González and Smeets, 1992, 1997). In reptiles, anatomical segregation has been found in the snake *Natrix maura* and turtle *Mauremys caspica* (Fernández-Llebrez et al., 1988) but not in lizard species (Bons, 1983; Stoll and Voorn, 1985; Thepen et al., 1987). A feature reported in the SON of the snake *P. regius* (Smeets et al., 1990) and *B. jararaca* (present results) but not in the snake *N. maura* (Fernández-Llebrez et al., 1988) is the numerous AVT-ir cells occupying the lateral margin of the diencephalon and separated from the main portion of the SON by the optic tract fibers. To present, it is not known the functional significance of the different locations of the neurosecretory perikarya in the SON of some vertebrates.

Regarding the presence of cerebrospinal-fluid (CSF)-contacting neurons in the PVN, it has been reported that their number decreases along the phylogenetic scale (Vigh-Teichmann and Vigh, 1989). The higher number of CSF-contacting neurons in the PVN of turtles as compared with snakes or lizards agree with their more ancient phylogenetic origin (Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988; Smeets et al., 1990; present results).

Table 2
Abbreviations

AC	anterior commissure
AH	anterior hypothalamus
AL	anterior lobe of hypophysis
AM	amygdaloid complex
BNST	bed nucleus of the stria terminalis
CER	locus coeruleus
DC	dorsal cortex
DBB	diagonal band of Broca
DLA	dorsolateral aggregation
DM	dorsomedial nucleus of thalamus
DVR	dorsal ventricular ridge
E	ependyma
H	habenula
HHT	Hypothalamo–hypophysial tract
IL	intermediate lobe of hypophysis
IP	interpeduncular nucleus
LC	lateral cortex
LHA	lateral hypothalamic area
LT	lamina terminalis
MC	medial cortex
ME	median eminence
NIII	nucleus of the oculomotor nerve
NA	nucleus accumbens
NL	neural lobe of hypophysis
OC	optic chiasma
OT	optic tract
PG	periventricular grey
PH	periventricular nucleus of hypothalamus
PO	paraventricular organ
PV	portal vessels
PVN	paraventricular nucleus
RCN	retrochiasmatic nucleus
RIN	recessus infundibular nucleus
S	septum
SCO	subcommissural organ
SFO	subfornical organ
SM	medial septal nucleus
SN	substantia nigra
SON	supraoptic nucleus
STR	striatum
T	tectum
TRS	nucleus of the solitary tract
VMH	ventromedial nucleus of the hypothalamus

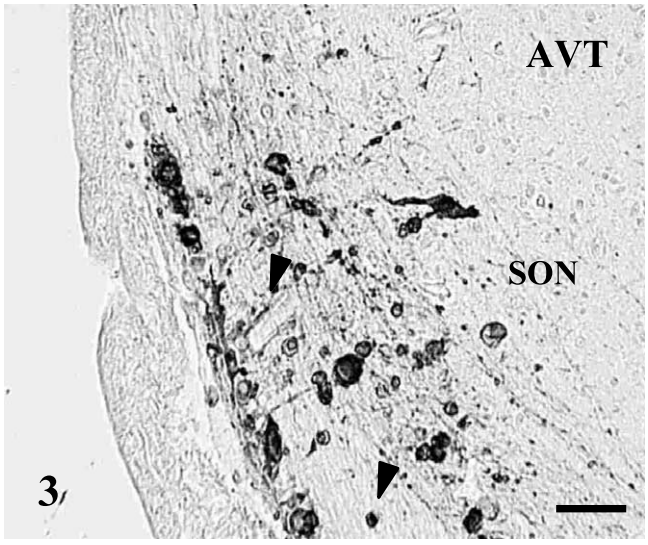

Fig. 3. Transverse section showing the SON immunostained with an antiserum anti-AVT. Immunoreactive perikarya and fibers were observed inside the optic tract (arrowheads). Bar = 33 μm.

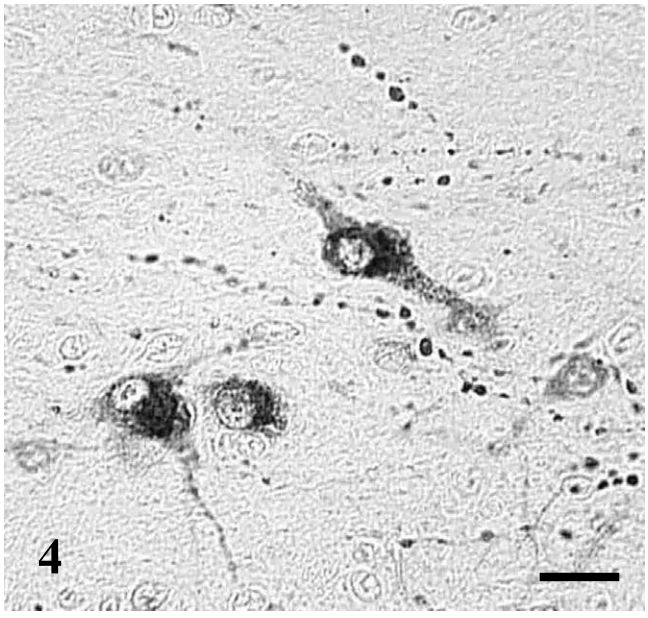

Fig. 4. Transverse section showing the SON immunostained with an antiserum anti-AVT. AVT-ir neurons showed strong immunoreactivity and pear-shaped or bipolar form. Varicose immunoreactive fibers were patent. Bar = 15 μm.

The group of AVT-ir perikarya located dorsal to the lateral forebrain bundle in the snake *B. jararaca* corresponds to the DLA described in *Diadophis punctatus* (Philibert and Kamemoto, 1965). AVT- and MST-ir perikarya have also been described in this location in lizards, turtles and snakes (Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988; Propper et al., 1992). Whether these groups of cells can be considered as a real subnucleus or only a particular portion of SON or PVN is not clear.

A RIN has not been described in some reptilian species (Goossens et al., 1979; Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988). However MST-, but not AVT-ir, perikarya have been reported in the RIN of different lizard species and the snake *P. regius* and *B. jararaca* (Bons, 1983; Smeets et al., 1990; present results). According to these authors axons from these neurons could join the hypothalamo–hypophysial tract or they could reach other locations contributing to the extrahypophyseal neurosecretory pathways.

In reptiles, extrahypothalamic vasotocinergic and mesotocinergic cell groups have been described in different regions of the brain such as the preoptic area and the bed nucleus of the stria terminalis in the telencephalon, and nucleus reticularis inferior parvocel-

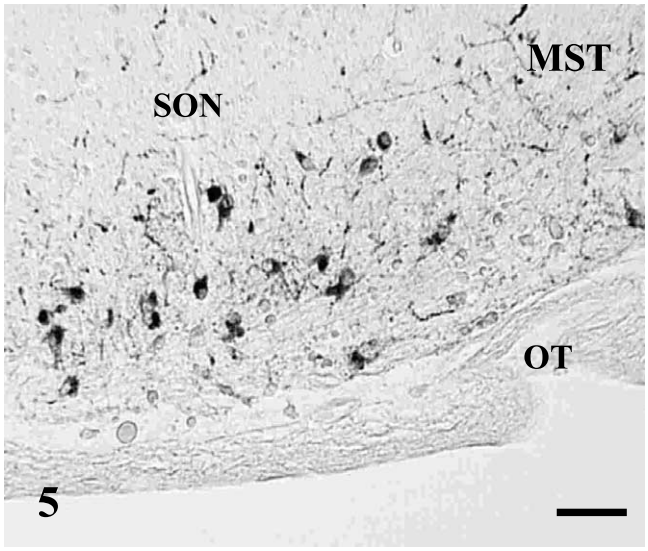

Fig. 5. Transverse section showing the localization of MST-ir perikarya and fibers at the caudal level of SON. Bar = 50 μm.

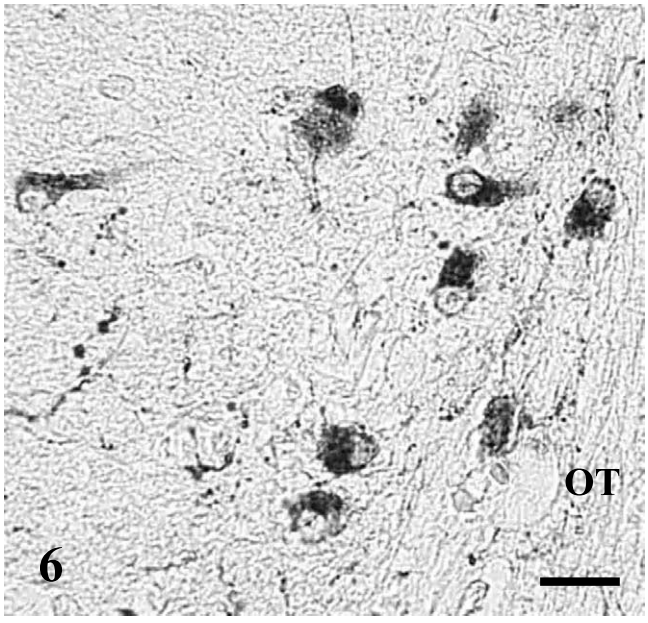

Fig. 6. Strong immunoreactive neurons with round or pear-shaped form and varicose immunoreactive fibers were observed. Detail of SON immunostained with anti-MST serum. Bar = 17 μm.

lularis in the rhombencephalon (Goossens et al., 1979; Bons, 1983; Stoll and Voorn, 1985; Thepen et al., 1987; Smeets et al., 1990; Propper et al., 1992). In the lizards *Lacerta muralis*, *Acanthodactylus pardalis*, *A. boskianus* and *Tarentola mauritanica*, AVT- and MST-ir perikarya have been described in the preoptic area (Bons, 1983). In the lizard *A. carolinensis*, only AVT-ir perikarya was found (Propper et al., 1992). Conversely, in other lizard (*G. gecko*), in turtles (*M. caspica* and *P. scripta elegans*) and in snakes (*N. maura*, *P. regius* and *B. jararaca*) no immunoreactive perikarya have been found in this area

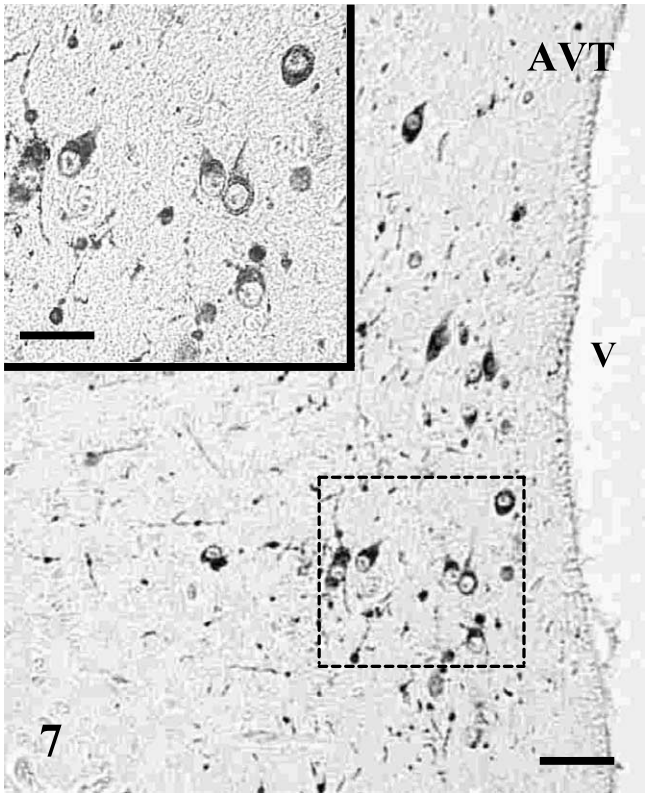

Fig. 7. Transverse sections through the paraventricular nucleus immunostained with antiserum anti-AVT. Fusiform and pear-shaped immunoreactive neurons were located in the subependymal layer. Bar = 50 μm. Inset: Detail of AVT-ir perikarya with varicosed immunreactive fibers. Bar = 17μm.

(Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988; Smeets et al., 1990). Again, discrepancies could be due to methodological reasons or could have an unknown functional significance.

AVT- and MST-ir perikarya have been described in the bed nucleus of the stria terminalis of the lizard *G. gecko*, turtle *P. scripta elegans* and snake *P. regius* (Stoll and Voorn, 1985; Thepen et al., 1987; Smeets et al., 1990). However, no immunoreactive neurons were found in this location of the turtle *M. caspica* and the snake *N. maura* (Fernández-Llebrez et al., 1988) and also the snake *B. jararaca* (present results). It is interesting to note that in all these studies the same anti-AVT antiserum was used (obtained from Dr Buijs). Thus decreasing the possibility of technical reason for discrepancies. Differences observed in the distribution pattern of immunoreactivity, even among species of the same evolutionary radiation, deserves further attention and could have important functional implications.

Another extrahypothalamic AVT-ir cell group has been reported in the nucleus reticularis inferior parvocellularis of the lizard *G. gecko* (Stoll and Voorn, 1985; Thepen et al., 1987) and in the ventromedial nucleus of the thalamus and interpeduncular nucleus of the lizard *A. carolinensis* (Propper et al., 1992), but not in turtle or snake species (Fernández-Llebrez et al., 1988; Smeets et

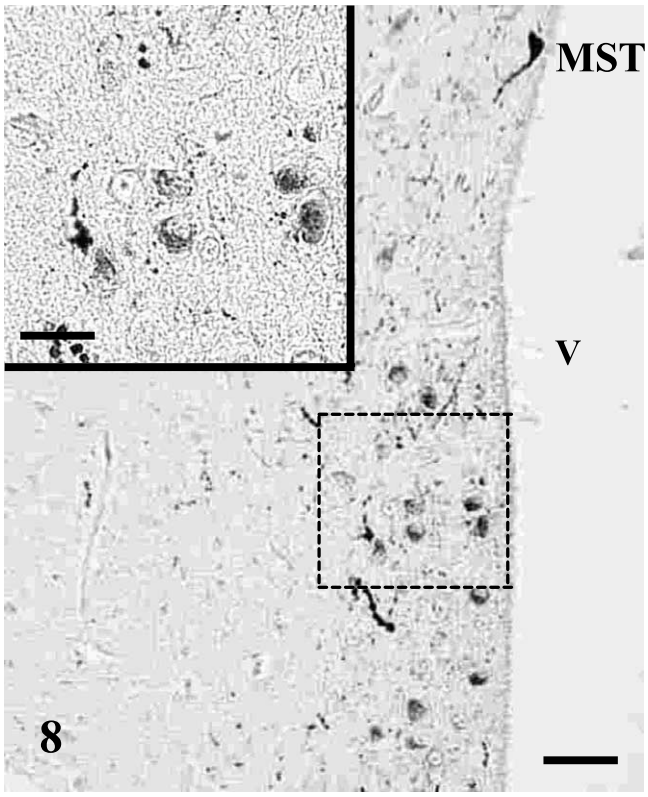

Fig. 8. Transverse sections through the paraventricular nucleus immunostained with antiserum anti-MST. Fusiform and pear-shaped immunoreactive neurons were located in the subependymal layer. Bar = 50 μm. Inset: Detail of MST-ir perikarya with varicosed immunreactive fibers. Bar = 17 μm.

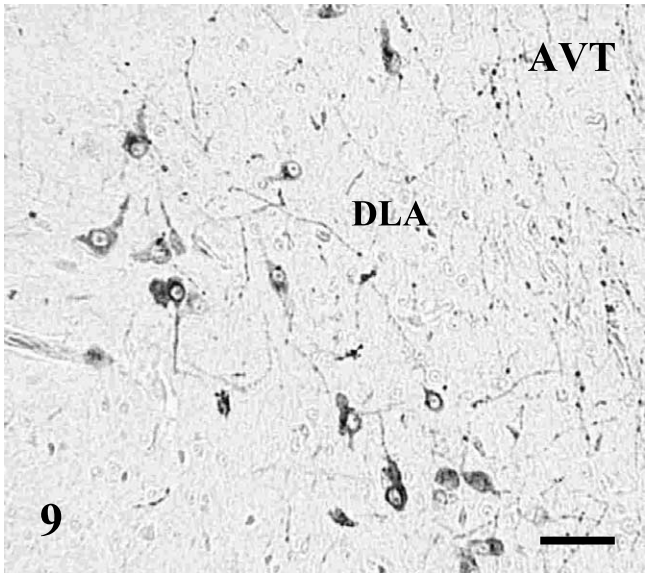

Fig. 9. Detail of AVT-ir perikarya and nerve fibers in the dorsolateral aggregation (DLA). Axons arising from fusiform or pear-shaped perikarya run in all directions. Bar = 50 μm.

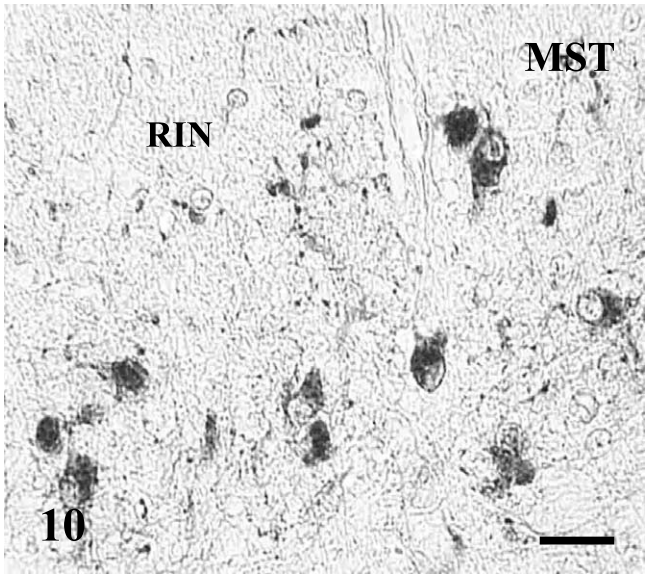

Fig. 10. Transverse section through the recessus infundibular nucleus (RIN) immunostained with antiserum anti-MST. Round or pear-shaped immunoreactive perikarya were observed. Bar = 17 μm.

al., 1990; present results). In addition to that discussed above, differences in peptides expression may represent differences in the physiological state of the individuals examined.

4.2. AVT- and MST-ir fibers

The distribution of AVT- and MST-ir fibers in the hypothalamus agrees with those previously described in reptilian species (Goossens et al., 1979; Bons, 1983; Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988; Smeets et al., 1990; Propper et al., 1992). Most neurons in the SON and PVN send their processes to the ME and neural lobe, forming the classical hypothalamo–hypophysial tract. Axons from the mesotocinergic perikarya of RIN could also contribute to this tract.

Extrahypophysial projections of the neurosecretory system have been described in mammals (Buijs, 1978; Yulis and Rodríguez, 1982) and they have been suggested to be involved in autonomic and humoral cardiovascular control, behaviour and memory processes (Swanson and Sawchenko, 1983; Buijs, 1985). Extrahypothalamic distribution of neurohypophyseal peptides has also been described in birds (Bons, 1980; Blähser, 1981; Panzica et al., 1986, 1999; Kiss et al., 1987), amphibians (Jokura and Urano, 1987; González and Smeets, 1992, 1997), teleost (Dungen et al., 1982; Holmqvist and Ekström, 1995) and cartilaginous fishes (Meurling et al., 1996). In reptiles, extrahypophysial projections have been reported in lizards, snakes and turtles (Bons, 1983; Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988; Smeets et al., 1990; Propper et al., 1992). An extensive extrahypophy-

sial system also occurs in *B. jararaca*, with many AVT- and MST-ir fibers in several extrahypothalamic regions. In general, this distribution agrees with the pattern described previously in other reptiles. The wide distribution of extrahypothalamic neurosecretory fibers in reptiles suggests that, as in mammals, AVT and MST could act centrally as neuromodulators.

Vasotocinergic and/or mesotocinergic innervations of the nucleus accumbens has been described in the lizards *G. gecko* (Stoll and Voorn, 1985; Thepen et al., 1987) and *A. carolinensis* (Propper et al., 1992), the turtle *P. scripta elegans* and the snake *P. regius* (Smeets et al., 1990). *B. jararaca* showed AVT-, but not MST-ir fibers in this location. The physiological significance of this differential pattern of innervation between the different groups is not known.

In mammalian, the septum receives a strong neurophysinergic and vasopresinergic innervation from the PVN (Staiger and Nürnberger, 1989). This innervation has been related to thermoregulatory processes (Nürnberger, 1995) and to sexual behaviour (Hermes et al., 1993). Involvement of the septum in the regulation of paraventricular vasopressin neurons and its relationship with water balance have been reported in the rat (Tanaka et al., 1988). Also Nps- and AVT-ir fibers have been found in the septum of reptiles such as lizard, snakes and turtles (Bons, 1983; Stoll and Voorn, 1985; Fernández-Llebrez et al., 1988; Smeets et al., 1990; Propper et al., 1992). We detected MST-ir, but not AVT-ir, fibers in the septum of the snake *B. jararaca*. Thus MST but not AVT could be involved in septal autonomic functions in *B. jararaca*.

The organum vasculosum of the lamina terminalis (OVLT) of mammals is a circumventricular organ that has been involved in osmoregulation and receives AVP-ir fibers (Weindl and Sofroniew, 1981; Buggy and Bealer, 1987). It is not known whether a OVLT is present in submammalian vertebrates (Leonhardtm, 1980). This area, the LT shows AVT fibers in elasmobranches (Meurling et al., 1996) and birds (Weindl and Sofroniew, 1982). A rich plexus of Nps-ir fibers was described in the LT of the snake *N. maura* and the turtle *M. caspica* (Fernández-Llebrez et al., 1988). In addition, AVT-ir fibers were described in the OVLT of *G. gecko* (Stoll and Voorn, 1985; Thepen et al., 1987), *P. regius* and *P. scripta elegans* (Smeets et al., 1990). Only few AVT- and MST-ir fibers can be observed in the LT of *B. jararaca* (present results).

Unlike mammals (Weindl and Sofroniew, 1981) and birds (Weindl and Sofroniew, 1982), the subcommissural organ of the snake *N. maura* (Fernández-Llebrez et al., 1987, 1988) and *B. jararaca* (present results) displays MST-ir fibers. Some unknown influence on the secretory activity of SCO and the formation of Reissner's fiber should play neurohypophyseal peptides in lower vertebrates. Another diencephalic circunventricular organ, the paraventricular organ seems to be also related to neurohypophyseal neurohormones as judged by the presence of Nps-ir perikarya in the snake *N. maura* (Fernández-Llebrez et al., 1988) and MST-ir perikarya in *B. jararaca* (present study).

In the lizard *G. gecko*, more AVT- than MST-ir fibers were found in the rhombencephalon. In this species, a group of AVT-ir cells has been described in the nucleus reticularis inferior parvicellularis, and it has been suggested that the axonal extension of these perikarya could account for the dominance of vasotocinergic fibers (Stoll and Voorn, 1985; Thepen et al., 1987). Conversely, in the rhombencephalon of *B. jararaca* there are more MST- than AVT-ir fibers. We have not data about the origin of rhombencephalic MST-ir fibers in this snake. It has been suggested that, in reptiles, the PVN is the main source of extrahypothalamic fibers (Stoll and Voorn, 1985; Thepen et al., 1987; Fernández-Llebrez et al., 1988). In *B. jararaca*, the caudal region of the hypothalamus showed more MST-ir (i.e. RCN and RIN) than AVT-ir perikarya. If these neurons had extrahypothalamic projections to the rhombencephalon, this could explain the higher development of mesotocinergic system with respect to the vasotocinergic system at caudal levels of the brain of *B. jararaca*.

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