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Corticotropin-releasing hormone-like immunoreactivity in the brain of the snake *Bothrops jararaca*

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Summary

The distribution of corticotropin-releasing hormone in the brain of the snake *Bothrops jararaca* was studied immunohistochemically. Immunoreactive neurons were detected in telencephalic, diencephalic and mesencephalic areas such as dorsal cortex, subfornical organ, paraventricular nucleus, recessus infundibular nucleus, nucleus of the oculomotor nerve and nucleus of the trigeminal nerve. Immunoreactive fibres ran along the hypothalamo-hypophysial tract to end in the outer layer of the median eminence and the neural lobe of the hypophysis. In general, immunoreactive fibres occurred in the same places of immunoreactive neurons. In addition, immunoreactive fibres were observed in the septum, amygdala, lamina terminalis, supraoptic nucleus, nucleus of the paraventricular organ, ventromedial hypothalamic nucleus and interpeduncular nucleus. These results indicate that, as for other vertebrates, corticotropin-releasing hormone in *B. jararaca* brain, besides being a releasing hormone, may also act as a central neurotransmitter and/or neuromodulator.

Introduction

The superfamily of corticotropin-releasing hormones (CRH) comprises several peptides conserved in phylogeny (Lederis *et al.* 1990, Lovejoy & Balment 1999). *In vivo* and *in vitro* studies have shown that CRH stimulates the secretion of adrenocorticotropin (ACTH), β-endorphin and melanotropin (MSH) in the adenohypophysis (Vale *et al.* 1981, Sakly *et al.* 1982) and inhibits the release of gonadotropin-releasing hormone, growth hormone-releasing hormone, somatostatin, arginine vasopressin and oxytocin (Rivier & Plotsky 1986, Zadina & Kastin 1986).

The distribution of perikarya and fibres containing immunoreactive CRH has been described in the brain of several mammalian species, such as rat (Bloom *et al.* 1982, Merchenthaler *et al.* 1982, Olschowka *et al.* 1982, Kawata *et al.* 1983, Swanson *et al.* 1983, Champagne *et al.* 1998), sheep (Paull *et al.* 1982, Kolodziejczyk *et al.* 1983), dog (Bugnon *et al.* 1984, Stolp *et al.* 1987), cat (Bugnon *et al.* 1984), goat (Kikusui *et al.* 1997) and man (Bugnon *et al.* 1982). In addition to the hypothalamus, the expression of the CRH gene has been detected in the cerebral cortex and in extracerebral sites (Usui *et al.* 1988). The distribution of CRH-immunoreactive elements has also been studied in the brain of non-mammalian vertebrates such as birds (Péczely & Antoni 1984, Yamada & Mikami 1985, Bons *et al.* 1988, Ball *et al.* 1989), amphibians (Tonon *et al.* 1985, Olivereau *et al.* 1987, Miranda & Dezi 1997), and teleost and elasmobranch fishes (Olivereau *et al.* 1984, Yulis *et al.* 1986, Olivereau & Olivereau 1988, Vallarino *et al.* 1989, Mancera & Fernández-Llébrez 1995, Polenov *et al.* 1997, Zupane *et al.* 1999). In mammalian and non-mammalian species, CRH-immunoreactive hypothalamic neurons exert their hypophysiotropic action by projecting axons to the outer layer of the median eminence or the neural lobe. In all vertebrates, CRH-immunoreactive fibres and CRH receptors have been detected in addition to the hypothalamus, in several other areas of the central nervous system (CNS). This suggests that CRH might also act as a neuromodulator or neurotransmitter (Bugnon *et al.* 1984, Zadina & Kastin 1986, Perrin & Vale 1999, Van Pett *et al.* 2000).

In reptiles, the CRH system has been described in the turtles *Pseudemys scripta elegans* (Bugnon *et al.* 1984) and *Mauremys caspica* (López Avalos *et al.* 1993) and in the snake *Natrix maura* (Mancera *et al.* 1991b). Whereas CRH-like immunoreactive neurons have been observed in the nucleus of the paraventricular organ in *P. scripta elegans* (Bugnon *et al.* 1984) and in the paraventricular nucleus of *N. maura* (Mancera *et al.* 1991b) and *M. caspica* (López Avalos *et al.* 1993), CRH-like immunoreactive fibres have also been described occupying hypothalamic as well as extrahypothalamic areas.

By their phylogenetic position, studies on the peptidergic system in snakes are of great interest. Since no detailed anatomical study on the distribution of CRH-like immunoreactive perikarya and nerve fibres exist for the viperid snake, we investigated this distribution in the brain of the terrestrial brazilian pit viper *Bothrops jararaca*. The results are compared with those described in other reptiles and vertebrates.

Material and methods

Adult male ($n = 3$) and female ($n = 3$) *B. jararaca* snakes (Serpentes, Viperidae, Crotalinae) (about 180 g in weight and 103 cm in length) were collected in Spring from the wild in south and southeastern Brazil. They were acclimatized for controlled environmental conditions of photoperiod (12 : 12 h light : dark), relative humidity ($65.3 \pm 0.9\%$) and temperature (25–26 °C) (Breno *et al.* 1990). Their sex was identified by gently pressing the tail base below the cloaca, with the consequent exposure of one or both hemipenises characterizing a male (Fitch 1987). Non-pregnant females were selected by macroscopic examination of the oviduct. The animals were supplied with adequate food and had free access to tap water.

The snakes were anaesthetized with sodium pentobarbital (3 mg/100 g body wt), administered subcutaneously (Silveira *et al.* 1992). They were subsequently injected transcardially by bolus with 0.1 ml sodium heparin solution (1000 IU/ml of Ringer's solution). The composition of the Ringer's solution was similar to the plasma ion concentration of *B. jararaca* as follows: 180 mM NaCl, 5 mM K_2HPO_4, 0.6 mM KH_2PO_4, 1.4 mM $MgSO_4$, 2.5 mM $CaCl_2$, 5 mM glucose, pH 7.2–7.3. The specimens were perfused with this solution, followed by Bouin's fluid 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 dehydrated and embedded in paraffin wax. All the specimens were sacrificed in the Spring during the morning (9:00–12:00 AM).

Sagittal and transverse (10-μm-thick) serial sections were hydrated and immunostained according to the peroxidase–antiperoxidase (PAP) method (Sternberger 1986) using a rat CRH antiserum (1 : 500) obtained in rabbit (kindly provided by Professor P. Fernández-Llebrez, Malaga, Spain). Sections were preincubated for 15 min at 22 °C in H_2O_2 (0.3% in Tris buffer) to inactivate endogenous peroxidase and then incubated in the primary antiserum for 18 h at 22 °C. 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, the rabbit-PAP complex (Dakopatts, Copenhagen, Denmark) was used at a dilution of 1 : 150 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, lambda carrageenan (Sigma), 0.5% Triton X-100 (Sigma) and 0.02% sodium azide. To reveal the immunoreactivity, sections were incubated in the dark for 12 min at 22 °C in 0.04% 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma) in Tris buffer, pH 7.8, containing 0.04% ammonium nickel sulphate hexahydrate (Fluka) and 0.007% H_2O_2 (Merck).

To test the specificity of the immunoreaction, the anti-CRH serum was immunoabsorbed using the same antigen preparation that was employed for raising the antiserum (Mancera *et al.* 1991b). Aliquots of the antiserum, diluted 1 : 500, were mixed separately with free rat CRH at concentrations of 25 and 50 μg/ml. Both preparations were used, in the same staining session, on sections adjacent to those immunostained with the non-absorbed antiserum. Immunoabsorbed anti-CRH did not stain any structure in the sections. Moreover, in order to exclude cross-reactivity of the antiserum with other related peptides (Lovejoy & Balment 1999), preabsorption was also carried out using rat urocortin (Sigma U6631) and rat urotensin II (Sigma U7507) at concentrations of 25 and 50 μg/ml respectively. The immunoreactivity (intensity and distribution) was not affected by using CRH antiserum preabsorbed with either rat urocortin or rat urotensin II. To test possible unspecific immunoreactions, adjacent sections were processed as described above, but incubation in the primary antisera was omitted. No positive structures or cells were found in these sections.

The position of CRH-like immunoreactive perikarya was determined by examination of every tenth immunostained section using a Zeiss Jenapol microscope fitted with a DSP Hitachi camera. In the camara lucida drawings of selected levels, circles represented immunoreactive perikarya and the number of circles correlated with the relative number of cells seen in a given level. The size of cells bodies was measured by using the image processor HID 4-advanced program. Data were presented as mean ± standard deviation. The diameter was measured in the long axis of randomly selected cell bodies, in which the nucleus was evident. The total number of cells measured for each nucleus was 15–20.

Results

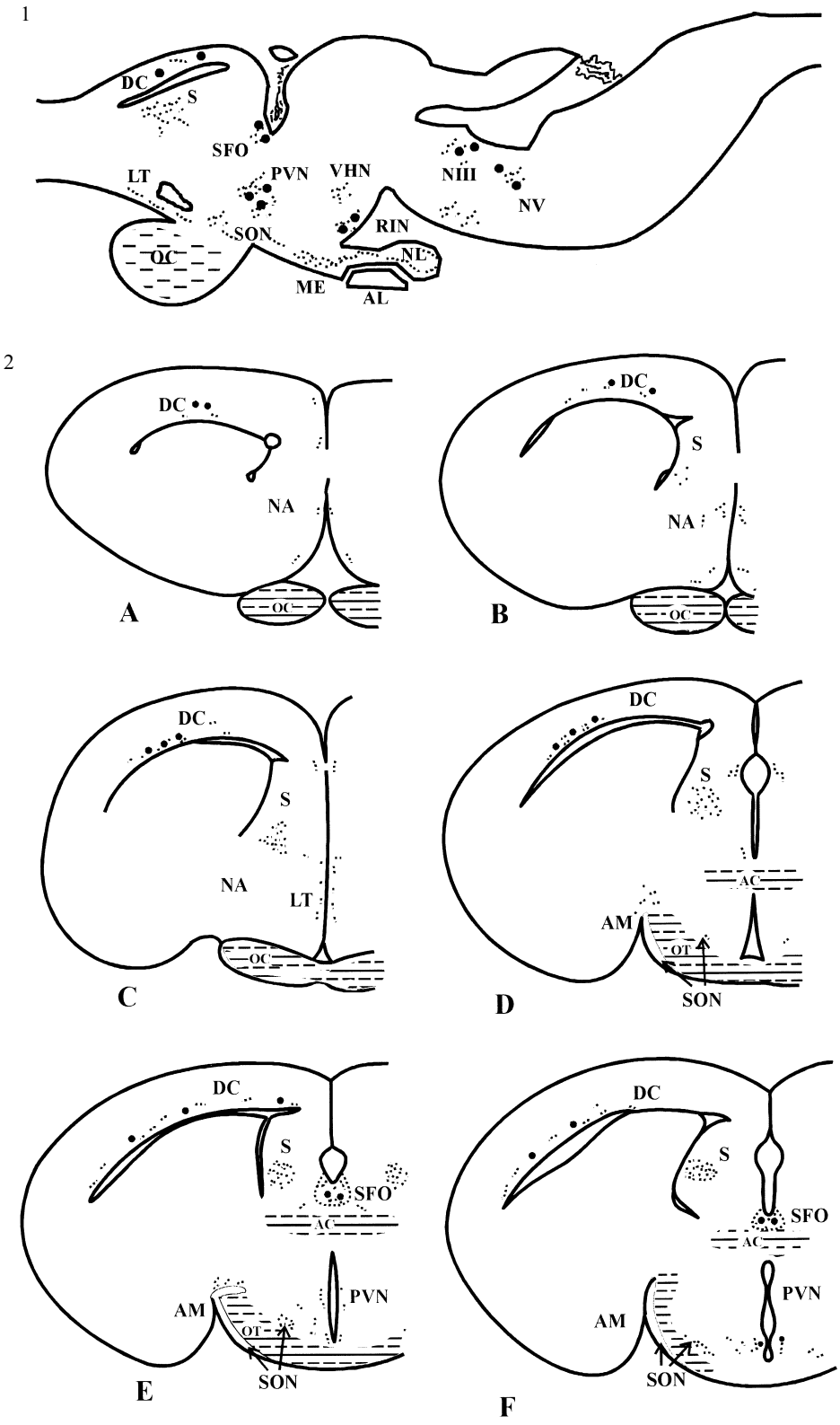
The distribution of CRH-like immunoreactive perikarya and fibres in the brain of *B. jararaca* is represented in the schematic drawings of Figures 1 and 2. Table 1 summarizes the distribution compared with that in other species of reptiles. The nomenclature used in this study for brain regions and nuclei is according to Smeets *et al.* (1990) for the snake *Python regius* and Fernández-Llebrez *et al.* (1988) and Mancera *et al.* (1991b) for the snake *N. maura*. No apparent differences in either the intensity or the distribution of CRH-like immunoreactivity were observed between males and females. Since all animals were sacrificed in the same season and at the same time of day, seasonal and/or circadian differences could not be evaluated.

Neuronal perikarya

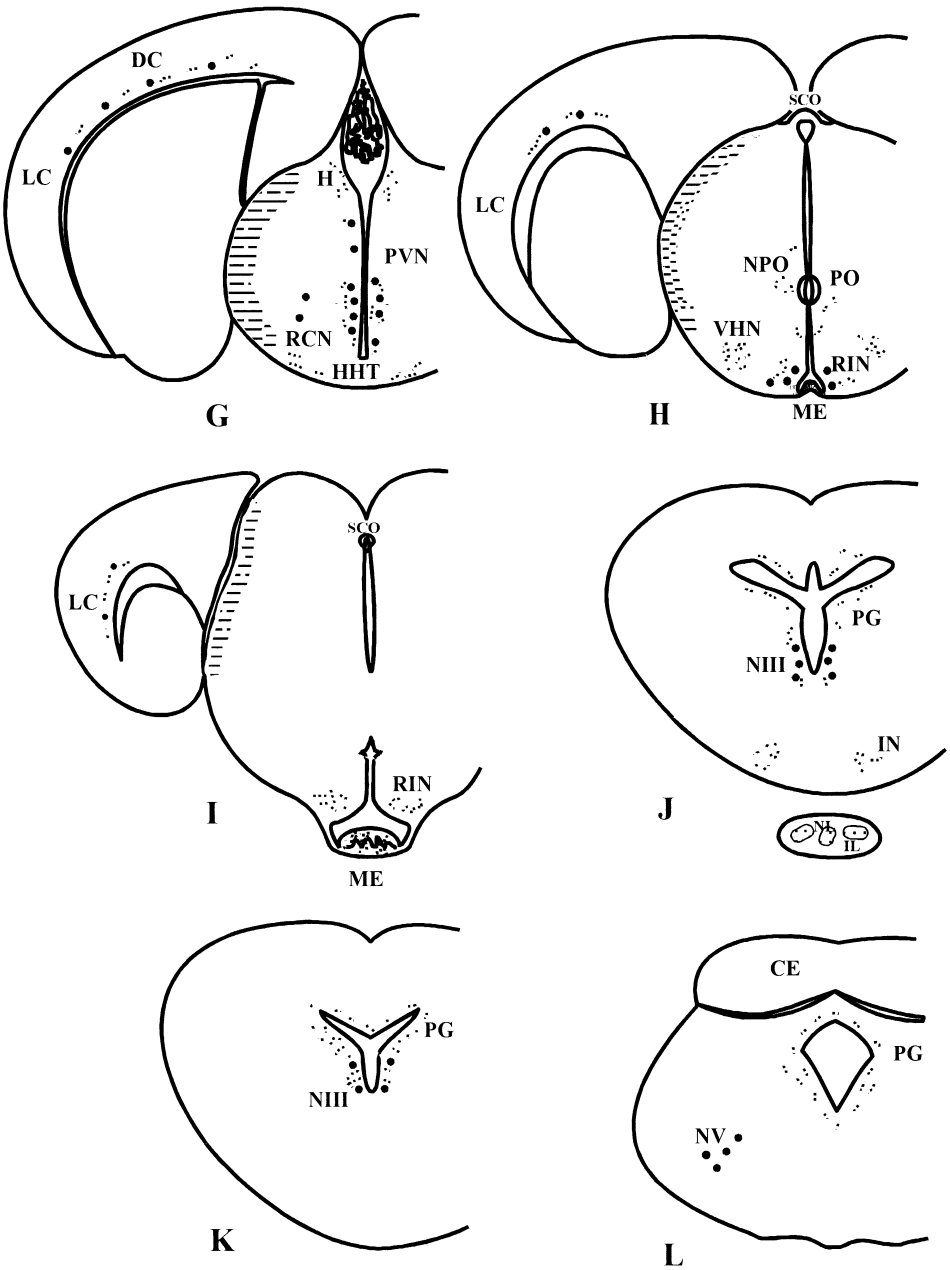
Immunoreactive perikarya were found in telencephalic, diencephalic and mesencephalic regions. In the telencephalon, small, pear-shaped, CRH-like immunoreactive perikarya (7.64 ± 0.47 μm in diameter) were scattered along the inner plexiform layer of the dorsal cortex (Figure 3B). In caudal regions, these cells occupied a more lateral position (Figure 2G–I).

In the diencephalon, small or medium-sized, pear-shaped, CRH-like immunoreactive perikarya were seen in the subfornical organ (10.83 ± 1.28 μm in diameter) (Figure 3A). Also small, round, weakly immunoreactive

neurons ($7.83 \pm 0.48\,\mu m$ in diameter) occupied the recessus infundibular nucleus (Figure 3C). The main population of CRH-like immunoreactive perikarya in the diencephalon were found in the paraventricular nucleus. Most of these cells were small, had a pear shape ($8.27 \pm 0.33\,\mu m$ in diameter) and were arranged in parallel layers close to the ventricle (Figure 3D). However, other cells located deep in the neuropil close to the fibres of the hypothalamo-hypophysial tract.



Figures 1–2. (*Continued*)



Figures 1–2. Schematic drawings of rostrocaudal sagittal (Figure 1) and transverse sections (Figure 2A–L) through the brain of *Bothrops jararaca* showing the localization of CRH-like immunoreactive cell bodies (dark circles) and fibres (dots).

Some labelled neurons of the paraventricular nucleus located near the ventricle projected a process through the ependyma toward the cerebrospinal fluid (Figure 3D).

In the mesencephalon, a few fusiform CRH-like immunoreactive perikarya were found in the motor nucleus of the trigeminal nerve (Figure 4C). Moreover the nucleus of the oculomotor nerve, large, pear-shaped CRH-immunoreactive neurons were observed (24.45 $\pm$ 2.36 μm in diameter) (Figure 4D).

Nerve fibres

CRH-like immunoreactive fibres extended rostrocaudally from the level of the olfactory bulb to the myelencephalon and dorsoventrally from the level of the dorsal cortex to the median eminence (Figures 1 and 2). Except for the motor nucleus of the trigeminal nerve, all locations where CRH-like immunoreactive perikarya were present also showed CRH-like immunoreactive fibres. In addition, many regions lacking immunoreactive perikarya exhibited immunoreactive fibres.

The inner plexiform layer of the cortex showed many CRH-like immunoreactive fibres, mostly at its rostral portion. Also the lateral septum was strongly innervated by CRH-like immunoreactive fibres (Figure 3A). In the lamina terminalis, a prominent bundle of CRH-immunoreactive fibres was observed (Figure 2C). CRH-like immunoreactive fibres were also observed in the subfornical organ, habenula and nucleus of the paraventricular organ (Figures 1 and 2).

Table 1. Distribution of CRF-immunoreactive perikarya (P) and fibres (F) in reptiles.

Brain area	*Bothrops jararaca*[1]	*Natrix maura*[2]	*Mauremys caspica*[3]	*Pseudemys scripta*[4]
Dorsal cortex	P, F	P, F	P, F	
Dorsomedial cortex	—	—	P, F	
Medial cortex	F	—	P, F	
Lateral cortex	P, F	P, F	—	
Nucleus accumbens	—	P, F	P, F	P, F
Nucleus caudatus	—	—	P, F	
Septum	F	F	—	
Lamina terminalis	F	P, F	P, F	
Lateral forebrain bundle	—	F	—	
Amygdaloid complex	F	P, F	P, F	P, F
Subfornical organ	P, F	P, F	P, F	
Supraoptic nucleus	F	F	F	
Retrochiasmatic nucleus	—	F	F	
Paraventricular nucleus	P, F	P, F	P, F	
Dorsolateral aggregation	—	—	P, F	
Habenula	F	F	—	
Nucleus of paraventricular organ	F	P, F	P, F	P, F
Ventromedial nucleus of the hypothalamus	F	F	—	
Recessus infundibular nucleus	P, F	—	P, F	P, F
Median eminence	F	F	F	F
Neural lobe of hypophysis	F	F	F	F
Tectum	—	—	F	F
Pretectal nucleus	—	—	P, F	P, F
Periventricular grey	F	—	P, F	
Torus semicircularis	—	—	F	
Nucleus of oculomotor nerve	P, F	P, F	—	
Interpeduncular nucleus	F	F	P, F	
Motor nucleus of trigeminal nerve	P	P, F	—	
Nucleus of reticular formation	—	P, F	P, F	P, F
Nucleus of the raphe	—	F	P, F	
Reticular substance	—	—	P, F	
Vestibular area	—	F	—	

[1]Present study. [2]Mancera *et al.* (1991a,b). [3]López Avalos *et al.* (1993). [4]Bugnon *et al.* (1984) (brief report).

Although the supraoptic nucleus did not show CRH-like immunoreactive perikarya, it exhibited a dense plexus of CRH-like immunoreactive fibres, except in its caudal most portion, the retrochiasmatic nucleus (Figure 2E–G).

In the paraventricular nucleus and adjacent areas, CRH-like immunoreactive fibres were abundant and coursed in a latero-ventral direction to join the hypothalamo-hypophysial tract. The recessus infundibular nucleus and the ventromedial hypothalamic nucleus also contributed fibres to this tract, that run along the lateral hypothalamus, diencephalic floor and the outer and inner zones of the median eminence (Figure 2G–I). Most CRH-like immunoreactive fibres procceding from the paraventricular nucleus approach the capillary plexus of the portal vessels in the outer zone of the median eminence (Figure 4A,B).

In the mesencephalon, CRH-like immunoreactive fibres run following a latero-dorsal direction and were seen in the periventricular grey, nucleus of the oculomotor nerve and interpeduncular nucleus (Figure 2J–L).

Discussion

The CRH family of vertebrate regulatory peptides comprises CRH, urotensin I, urotensin II, sauvagine and urocortin. In the brain of vertebrates, one or more of these peptides can be expressed (see Lovejoy & Balment 1999). Hence, cross-reactivity among these related peptides would be expected in an immunocytochemical study. In our study, the immunoabsorption test performed with urocortin II and urotensin showed no changes in the pattern of immunoreactivity; thus, at least for these two peptides, cross-reactivity did not seem to occur.

We used an antiserum against rat CRH to detect CRH in the snake *B. jararaca*. Unlike that of rat CRH, the aminoacid composition of *B. jararaca* CRH is not known. However, comparison of aminoacid sequences among mammalian and non-mammalian species has revealed a remarkable phylogenetic conservation of CRH (Lederis *et al*. 1990, Lovejoy & Balment 1999). Thus, we think that in *B. jararaca* the perikarya and fibres shown in this study indeed contain CRH. The anti-CRH serum used in the present investigation has been used before and observed to bind to perikarya and fibres of the snake *N. maura* (Mancera *et al.* 1991b) and the turtle *M. caspica* (López Avalos *et al.* 1993). The distribution of CRH-like immunoreactive perikarya and fibres in *B. jararaca* agree with those previously reported in other reptiles (see Table 1).

CRH-like immunoreactivity in the hypothalamo-hypophysial system

In mammals, parvocellular elements in the paraventricular nucleus have been reported to be the main source of CRH fibres of the hypothalamo-hypophysial tract (Bloom *et al.* 1982, Merchenthaler *et al.* 1982, Hoffman *et al.* 1986). At variance with this distribution, other authors found CRH immunoreactivity in magnocellular neurons of the paraventricular nucleus and the supraoptic nucleus (Paull *et al.* 1982, Kawata *et al.* 1983, Stolp *et al.* 1987). In birds, the hypothalamic CRH system was found to be quite similar

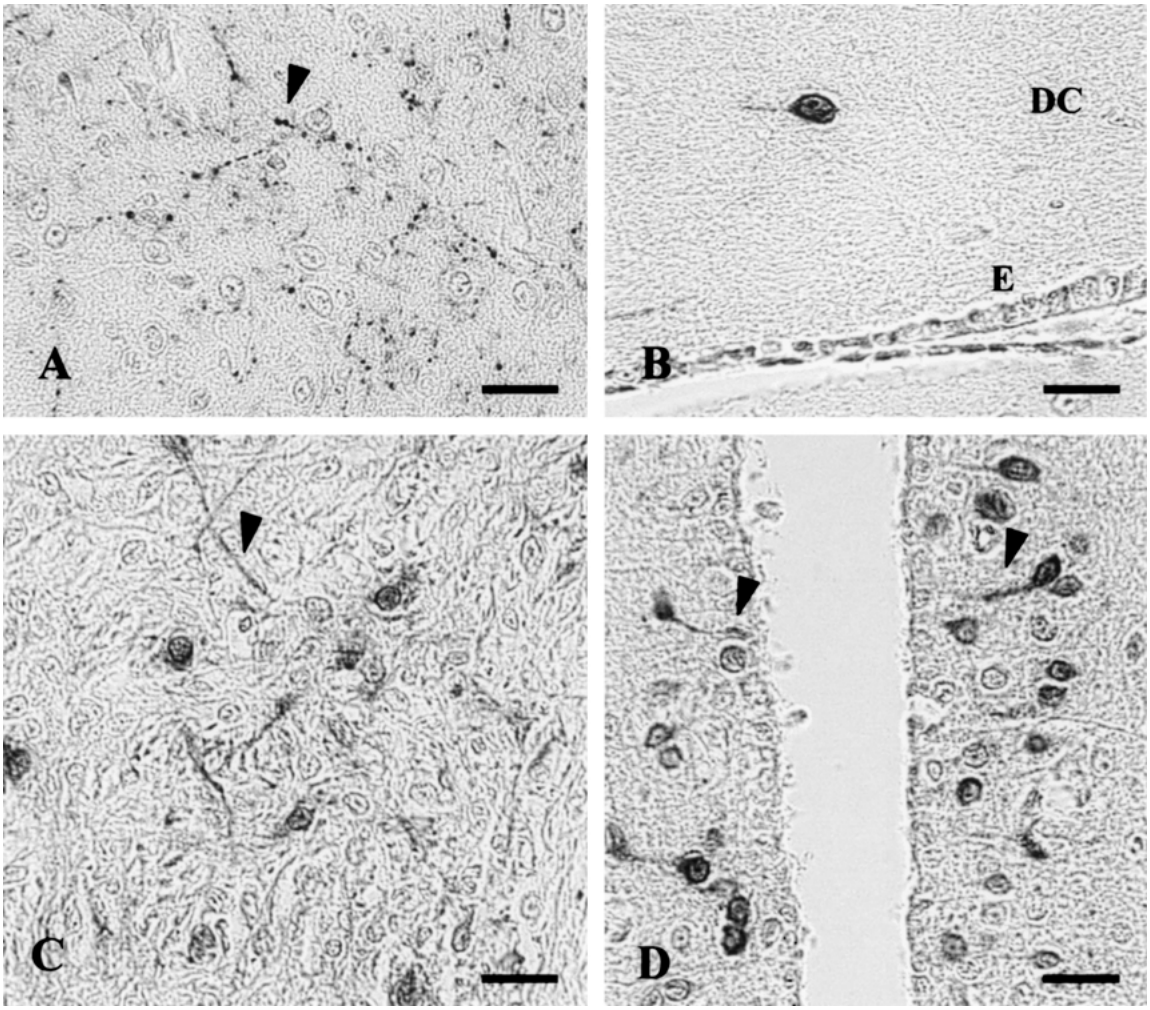


Figure 3. (A) Immunoreactive nerve fibres in the septum (arrowhead). Transverse section. Bar = 18 μm. (B) Detail of the inner portion of the dorsal cortex (DC) with an immunoreactive perikarya. Transverse section. E: ependyma. Bar = 18 μm. (C) Detail of immunoreactive fibres (arrowhead) and perikarya in the subfornical organ. Transverse section. Bar = 18 μm. (D) CRH-like immunoreactive neurons in the paraventricular nucleus. Some periventricular fibres toward the cerebrospinal fluid are seen (arrowheads). Transverse section. Bar = 18 μm.

to mammals, although the CRH-like immunoreactive neurons extended far beyond the boundaries of the paraventricular nucleus proper (Bugnon *et al.* 1984, Péczely & Antoni 1984, Yamada & Mikami 1985, Bons *et al.* 1988, Ball *et al.* 1989). In amphibians and fish, CRH-like neurons in the preoptic nucleus and/or paraventricular nucleus have been found to project to the median eminence and/or the neural lobe of the hypophysis (Bugnon *et al.* 1984, Olivereau *et al.* 1984, Tonon *et al.* 1985, Olivereau & Olivereau 1988, Vallarino *et al.* 1989, Mancera & Fernández-Llébrez 1995).

The anatomical distribution of CRH-like immunoreactive neurons in the paraventricular nucleus of *B. jararaca* was similar to that found in *N. maura* (Mancera *et al.* 1991b) and *M. caspica* (López Avalos *et al.* 1993) and contrasted with that described for the turtle *P. scripta elegans* (Bugnon *et al.* 1984) in which CRH-like immunoreactive neurons were present in the nucleus of the paraventricular organ instead of the paraventricular nucleus. As for *N. maura* and *M. caspica* the supraoptic nucleus of *B. jararaca* lacked CRH-like immunoreactive perikarya but displayed numerous CRH-like immunoreactive fibres. In agreement with Mancera *et al.* (1991b) and López Avalos *et al.* (1993), CRH could control the release or synthesis of arginine vasotocin and/or mesotocin via this innervation.

Regarding the presence of CRH-like immunoreactive neurons in the infundibular nucleus, it has been reported in the pigeon *Columba livia domestica* (Peczely & Antoni 1984), the frog *Rana ribibunda* (Tonon *et al.* 1985) and the turtle *M. caspica* (López Avalos *et al.* 1993). At variance with this distribution, the infundibular nucleus lacked CRH-like immunoreactive perikarya, in the turtle *P. scripta elegans* (Bugnon *et al.* 1984) and the snake *N. maura* (Mancera *et al.* 1991b). In the snake *B. jararaca*, the infundibular nucleus also contained CRH-like immunoreactive neurons. As in other species, the axons from these neurons are able to join the hypothalamo-hypophysial tract or distribute throughout other regions of the CNS.

In fish, CRH fibres have been found very close to the adenohypophysial ACTH- and MSH-cells, suggesting a

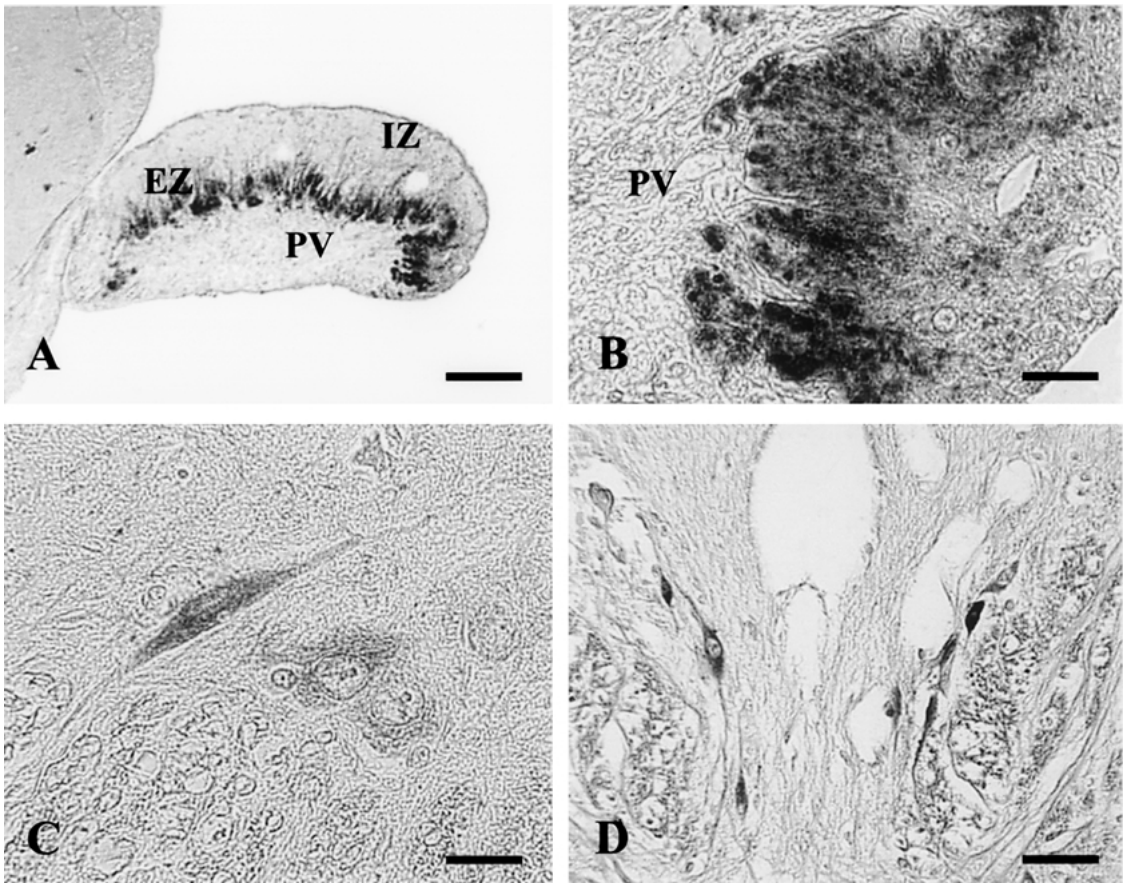


Figure 4. (A) Transverse section through the median eminence. Note the dense network of fibres in the external zone (EZ) running toward the vessels of the hypophysial portal system (PV). IZ: internal zone of the median eminence. Bar = 66 μm. (B) Detail of the external zone of the median eminence. PV: portal vessels. Bar = 22 μm. (C) Transverse section of the nucleus of the oculomotor nerve where large neurons displayed immunoreactivity for CRH. Bar = 18 μm. (D) Transverse section through the trigeminal nerve with CRH-like immunoreactive neurons. Bar = 40 μm.

paracrine control of the activity of these cells by CRH (Olivereau *et al.* 1984, Yulis *et al.* 1986, Olivereau & Olivereau 1988, Vallarino *et al.* 1989, Mancera & Fernández-Llébrez 1995). In mammals, birds and amphibians, an hypothalamo-hypophysial portal system is present and CRH-immunoreactive fibres mostly end in the outer layer of the median eminence close to the capillary portal vessels. Thus, the control of the activity of ACTH-cells seems to be mainly via the portal system. In addition, a small portion of CRH fibres may reach the neural lobe of the hypophysis and, hence, CRH could control the activity of ACTH-cells of the adenohypophysial lobe and/or MSH-cells of the neighbouring intermediate lobe by a paracrine fashion or by a hypothetical posterior-lobe–adenohypophysial portal system (Peczely & Antoni 1984, Tonon *et al.* 1985, 1986, Olivereau *et al.* 1987, Lederis 1987, Verburg-van Kemenade *et al.* 1987, Bons *et al.* 1988). A similar situation has been described in the snake *N. maura* (Mancera *et al.* 1991b) and the turtle *M. caspica* (López Avalos *et al.* 1993), where CRH-like immunoreactive fibres reached the outer layer of the median eminence and the hypophyseal neural lobe. Also in *B. jararaca*, CRH-like immunoreactive fibres were seen in the median eminence and the neurohypophysis, thus suggesting a role of CRH in the paracrine and/or endocrine control of the release of ACTH, MSH and/or β-endorphin from the anterior and intermediate hypophysial lobes.

Comparisons among reptilia reveal that the number of hypothalamic CSF-contacting CRH immunoreactive neurons is greater in turtles than in snakes (Mancera *et al.* 1991b, López Avalos *et al.* 1993, present results). Peptidergic CSF-contacting neurons were considered to be a primitive type of secretory neurons, since their number decreased in phylogeny (Vigh-Teichmann & Vigh 1983, 1989). This consideration is in agreement to the more ancient origin of turtles as compared with snakes. However, CRH-like immunoreactive neurons contacting CSF have also been described in the paraventricular nucleus of the pigeon (Péczeli & Antoni 1984). In this respect, turtles and birds thus seem to retain a somehow primitive character.

CRH-like immunoreactivity outside the hypothalamo-hypophysial system

CRH-like immunoreactive perikarya and fibres have been identified in extrahypothalamic regions of mammals (Merchenthaler *et al.* 1982, Olschowka *et al.* 1982), birds (Ball *et al.* 1989, Bons *et al.* 1988), turtles (Bugnon *et al.* 1984, López Avalos *et al.* 1993) and snakes

(Mancera *et al.* 1991b). In contrast, fish and amphibians lack extrahypothalamic CRH (Tonon *et al.* 1985, Yulis *et al.* 1986, Olivereau *et al.* 1987, Olivereau & Olivereau 1988). In *B. jararaca*, as in other vertebrates, the presence of CRH outside the hypothalamus suggests that it could act as a neurotransmitter and/or neuromodulator.

CRH-like immunoreactive cells and fibres have been reported in telencephalic regions such as the cortex, nucleus accumbens, amygdala and septum in rats (Merchenthaler *et al.* 1982, Fellmann *et al.* 1982), pigeons (Bons *et al.* 1988), turtles (Bugnon *et al.* 1984, López Avalos *et al.* 1993) and snakes (Mancera *et al.* 1991b). In the telencephalon of *B. jararaca*, CRH-like immunoreactive perikarya were detected in the cortex while CRH-like labelled fibres distributed more widely in the cortex, amygdala and, specially, the septum. In mammals, projections from the paraventricular nucleus and the subfornical organ to the septum have been involved in the control of osmotic balance (Miselis 1981, Tanaka *et al.* 1988). Since in *B. jararaca* both the paraventricular nucleus and the subfornical organ displayed CRH-immunoreactive neurons, as in mammals, they could be the origin of the CRH-like immunoreactive fibres observed in the septum and also these fibres could be involved in osmoregulation. This suggestion, however, awaits experimental verification.

In the diencephalon of *B. jararaca*, the lamina terminalis shows a strong CRH innervation. These results agree with those reported in *N. maura* (Mancera *et al.* 1991b) and *M. caspica* (López Avalos *et al.* 1993). Whether CRH-like immunoreactive fibres end in this area or are in transit between the telencephalon and diencephalon is not yet clear. The physiological significance of the CRH innervation of the lamina terminalis in lower vertebrates is not known. In mammals, the organum vasculosum of the lamina terminalis has been considered as an important centre for endocrine regulation. However, the existence of an organum vasculosum in the lamina terminalis of reptiles has not yet been demonstrated (Leonhardt 1980, Buggy & Bealer 1987).

An interesting fact is the presence of CRH-like immunoreactivity perikarya and fibres in the subfornical organ of the snakes *B. jararaca* (present results) and *N. maura* (Mancera *et al.* 1991b) and the turtle *M. caspica* (López Avalos *et al.* 1993) but not in the turtle *P. scripta* (Bugnon *et al.* 1984). The subfornical organ is a central integrator of water balance and also participates in the control of the hypothalamo-hypophysial-adrenal axis (Tanaka *et al.* 1987, Ferguson 1988, Plotsky *et al.* 1988). Therefore, the presence of CRH-like immunoreactive neurons in the subfornical organ of reptiles could be related to these known functions of the organ (Mancera *et al.* 1991b, López Avalos *et al.* 1993).

In the mesencephalon of *B. jararaca*, CRH-like immunoreactive neurons occur in the nucleus of the nerve oculomotor and nucleus trigeminal nerve. CRH-like immunoreactive neurons have also been reported in these nucleus in *N. maura* (Mancera *et al.* 1991b) but not in *M. caspica* (López Avalos *et al.* 1993). In mammals and birds, a strong innervation, but not perikarya, has been observed in these two nuclei (Merchenthaler *et al.* 1982, Bons *et al.* 1988). The physiological significance of these projections is not known. In *B. jararaca* it is likely that CRH-like immunoreactive cells of the nucleus of the nerve oculomotor and nucleus trigeminal nerve may send their axons to regions where CRH could act as a neurotransmitter or neuromodulator.

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