The aim of this study was to perform an immunohistochemical study of the angiotensinergic pathway from the arcuate nucleus (AN) to the posterior lobe of the hypophysis (PLH) of 10-week-old matched normotensive Wistar Kyoto rats (WKY), using our own polyclonal antibody against Angiotensin II (mouse-antiangiotensin II, MAAII). Cells and fibers in the rostroventral and dorsocaudal parts of the AN, the internal zone of the median eminence and PLH showed immunoreactive material for anti-angiotensin II. Angiotensin II fibers originating in the anteroventral part of the AN, crossing median eminence (ME) and infundibular stem and arriving at the PLH were also observed.

Key words: Posterior lobe – Hypophysis – Angiotensin II – Immunohistochemistry

INTRODUCTION

Angiotensin II is an integrative hormone/neurotransmitter that coordinates the activity of several physiological agents involved in body fluid balance (Ferrario, 1983). Parts of this complex action depend on the brain angiotensin system, which is an essential factor in regulating thirst, sodium appetite, and vasopressin release (Phillips, 1987; Muders et al., 1997).

Angiotensin II (AII) receptors have been classified into two subtypes: type-2 (AT2) and type-1 (AT1). AT2 receptors are located in some lobes of the cerebellum, layer VI of the cerebral cortex, the ventral lateral septal nucleus, the superior colliculus (Moulik et al., 2002) and the inferior olive, and this type-2 receptor remains unchanged during hypertension (Saavedra et al., 1986; Tsutsu and Saavedra, 1991). However, interference between AT2 and AT1 receptors has been described, since it has been suggested that the expression of AT1 receptors may depend on AT2 receptor expression (Armando et al., 2002). AT1 receptors (Lenkei et al., 1995; Moulik et al., 2002; Nuyt et al., 2001) are located in many brain areas involved in the salt-water balance and cardiovascular regulation: several hypothalamic nuclei, circumventricular organs, ventrolateral medulla, and nucleus of the solitary tract (Phillips et al., 1993; Moulik et al., 2002). AT1 receptor have also been observed in the periolivary region, dorsolateral nucleus of the lateral lemniscus and dorsal raphe, structures of similar distribution of monoaminergic neurons (Phillips et al., 1993; Moulik et al., 2002).

In the subfornical organ (SFO), a circumventricular organ, AGII acts on AT1 receptors to influence the sympathetic nervous system (Mckinley et al., 2001) and increases the number of AT1 receptors in experimental or genetic models of hypertension (Castren and Saavedra, 1989; Tsutsu and Saavedra, 1991). The SFO is involved in the increase in plasma vasopressin and in the rise in arterial blood pressure observed in response to angiotensin II (Lind et al., 1983; Muders et al., 1997), and is also involved in the control of drinking behavior (Simpson, 1981).

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Angiotensin and vasopressin are found in the magnocellular and parvocellular neurons in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus, possibly in the same cells (Imboden and Felix, 1991). Imboden et al. (1989) have also demonstrated the existence of a dense plexus of fibers and terminals in the neurohypophysis of the Wistar Kyoto rat, using their own purified angiotensin II antiserum (Imboden et al., 1987), confirming the findings (Lind et al., 1983) of an angiotensinergic paraventricular hypophysial pathway. They described a colocalization of angiotensin and vasopressin in neurons as well as in the fibers of the hypothalmo-neurohypophyseal system, suggesting the possibility that the vasopressin and angiotensin systems interact in the hypophysis as well as in the hypothalamus (Imboden and Felix, 1991; Leong et al., 2002).

In a previous work (Castañeyra-Perdomo et al., 1999) we reported a decrease in immunoreactive material (IRM) for vasopressin and an increase in IRM for angiotensin II in the posterior lobe of the hypophysis (PLH) (Perez-Delgado et al., 2000) in a group of spontaneously hypertensive rats (SHR) as compared with a control group of Wistar-Kyoto rats (WKY).

The purpose of the present work is to describe part of the angiotensinergic fibers that might originate in the AN and arrive at the PLH in order to define the possible role of this pathway in the regulation of the salt-water balance.

**MATERIALS AND METHODS**

Antiserum was raised in the mice as follows: after coupling to a carrier (thyroglobulin), angiotensin II (Sigma) was emulsified with complete Freund’s adjuvant and injected subcutaneously into 10 sites of the male mouse back. Each mouse received the equivalent of 10 µg of angiotensin II. Twenty days later, each mouse received the equivalent of 5 µg emulsified with incomplete Freund’s adjuvant in 4 to 8 subcutaneous injections, and fourteen days later each mouse received the equivalent of 5 µg in an intraperitoneal injection without coadjuvant. Seven days later, the mice were sacrificed by intracardial exsanguination. For laboratory purposes, the antiserum obtained was named MAAII. The antiserum specificity of angiotensin II was evaluated by means of an absorption test; incubating the antiserum overnight with the homologous antigen. The antigen was able to abolish the immunostaining (Perez-Delgado et al., 2000).

Ten normotensive male rats (Wistar-Kyoto rats, WKY), (Letica S/A, Barcelona SPAIN) were killed at the 10th week of life. The animals had been kept under lighting conditions of 12:12, and food and water were provided ad libitum. During the two weeks before sacrifice, water intake was recorded daily, and body weight weekly. Rats were anaesthetized with chloral hydrate, and their brains were fixed by vascular perfusion with Bouin’s fluid. Embedding was in paraffin. Ten mm thick frontal and sagittal serial sections of the brains were obtained. One of the series was stained using the Klüver-Barrera method, while the others were processed using the immunoperoxidase method (Sternberger et al., 1970). The polyclonal antibody raised in the mice against angiotensin II (MAAII) was used as the primary antibody containing MAAII at a 1:100 dilution. Incubation was for 24 h at room temperature. Peroxidase-labelled anti-mouse IgG (whole molecule, Sigma) was used as secondary antibody at a dilution of 1:100 for 2 h at room temperature. The peroxidase reaction product was visualized through the diaminobenzidine reaction. All antibodies were diluted in Tris buffer, pH 7.8, containing 0.7% lambda carrageenan (Sigma), 0.5% Triton X-100 and 0.1% sodium azide. Method specificity was controlled by omitting the primary antibody.

**RESULTS**

Our results showed, in a frontal view (Fig. 1), that angiotensin II immunoreactivity was much more intensive in the ME than in the AN. In the ME, the IRM was located in numerous fibers and terminals with varicoce of the whole internal zone (Fig. 1A, B). The subependymal zone and pial and subpial part of the ME was also weakly immunoreactive for AII (Fig. 1B). In two small areas located in a ventral (Fig. 1B, C) and in a dorsal part of the NA (Fig. 1B, D), a small amount of the IRM was also found in the frontal sections.

In a sagittal view (Figs. 2, 3), the angiotensin II-immunoreactive material was quite intensive in the internal zone of the ME, located in fibers and terminals (Figs. 2A, 3A). A weak immunoreactive activity was present in the subependymal layer of the internal zone and pial and subpial part of the external zone of the ME (Figs. 2A, 3A). In the NA, the IRM was observed in two restricted areas (Figs. 2A, 3A): one located in the dorso-caudal part of the NA in the fibers and perivascular space (Fig. 3A, C), and the other one in the rostroventral part in a neuronal group (Fig. 3A, B). We also found IRM (Figs. 2, 3) along the fibers that appeared to originate in these neurons in the rostroventral part of the AN along the median eminence and infundibular stem, and arrive at the posterior lobe of the hypophysis (Figs. 2A, B, 3A).
Fig. 1. (Coronal section of the AN and ME). The figure shows the angiotensin II immunoreactive material localized in fibers of the internal zone of the ME (A,B); cells and fibers of the ventral part of the AN (B,C) and dorsal part of the AN (B,D). AN = arcuate nucleus; IZ = internal zone of the ME; ME = median eminence; V = ventricle; ◀️ = ventral part of the AN; ➤️ = dorsal part of the AN. Scale bars: A: 540 μm; B: 270 μm; C, D: 40 μm.
DISCUSSION

The structures of the brain with a high concentration of angiotensin AT1 receptors consist of three distinct nuclei: the median preoptic nucleus, the subfornical organ, and the organum vasculosum of the lamina terminalis (OVLT) (Mckinley et al., 2001). However, angiotensin II receptors are also present in supraoptic, paraventricular and arcuate hypothalamic nuclei and the median eminence (Johren et al., 1997). The hypothalamic angiotensin II (AII) system plays an important role in hypophysial hormone release. AII- and vasopres-
sin-immunoreactive neurons and fibers are detected in the paraventricular, accessory magnocellular, and supraoptic nuclei; in the retrochiasmatic part of the supraoptic nucleus and in the median eminence (Johren et al., 1997).

Stimulation of the anterior medial parts of the arcuate nucleus causes a dual pressor/depressor response and moderate bradycardia. (Mastriani et al., 1989). The direct pathway from the AN to SFO may have a role in the modulation of neural and/or humoral events related to cardiovascular regulation and body fluid homeostasis by influencing the activity of SFO neurons (Rosas-

![Diagram of the brain structures](image-url)
Fig. 3. (Sagittal section of the NA, ME). Shows the angiotensin II immunoreactive material localized in the rostroventral part (A,B) and dorsocaudal part (A,C). AN = arcuate nucleus; ME = median eminence; ← rostroventral part of the AN; → dorsocaudal part of the AN. Scale bars: A: 180 µm.; B,C: 40 µm.
Arellano et al., 1996a,b). The inputs from AN neurons converge onto SFO neurons, which alter their discharge rate during changes in the plasma concentrations of AI or Na⁺. The AN may be involved in modulating the activity of SFO neurons that act in the detection of blood-borne signals from the depletion of intra- and extracellular fluid volumes. Stimulation of the caudal AN region inhibits SFO zones that respond to angiotensin II. These data suggest that AN may be involved in some stimulating activity via its rostral region and inhibiting activity via its caudal region (Rosas-Arellano et al., 1996a,b).

On the other hand, angiotensin II is localized in the cells of the SON and PVN and in their pathways to the median eminence and neurohypophysis (Imboden et al., 1989; Lind et al., 1983; Perez-Delgado et al., 2000). Therefore, it appears that the PVN contributes fibers that are responsible for the neurohypophyseal terminal field described by Imboden et al. (1989). Nevertheless, the same author (Imboden et al., 1989) described a secondary angiotensinergic pathway that terminated in the neurohypophysis whose origin is undetermined. We observed AII-ir neurons in the rostroventral part of the AN and AII-ir fibers originating in the same rostroventral part of the AN extending along the median eminence, infundibular stem, and arriving at the posterior lobe of the hypophysis; this could be the secondary pathway found by Imboden et al. (1989).

In conclusion, the present study suggests that there are two angiotensinergic pathways ending in the posterior lobe of the hypophysis: the first and largest one originating in the SON, PVN and another secondary one in part appears to originate in the rostroventral neuronal group of the AN. These findings could explain the dual role played in cardiovascular regulation by the AN (Rosas-Arellano et al., 1996a,b).

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