

## **Effect of hypertension on the angiotensin II fibres arriving at the posterior lobe of the hypophysis of the rat. An immunohistochemical study**

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**Summary.** We studied immunohistochemically the posterior lobe of the hypophysis (PL) of 15-week-old spontaneously hypertensive rats (SHR) and of matched normotensive Wistar Kyoto rats (WKY), by using our own polyclonal antibody raised in mice against Angiotensin II (mouse-antiangiotensin II, MAII). The blood pressure, water intake and volume of the PL were also recorded. The SHR rats were hypertensive, drank more water and showed a clear hypertrophy of their hypophysial PL. Also the PL of the SHR animals showed an increase in the immunoreactivity to the anti-angiotensin II antibody in the fibres arriving at the PL, with respect to the PL of WKY rats. This increase is compatible with the hyperactivity of the brain RAS, depletion of vasopressin content in the PL and increase in plasmatic levels of vasopressin described in SHR rats with respect to normotensive animals, as angiotensin II could locally stimulate vasopressin release to plasma from the neurohypophysis.

**Key words:** Posterior lobe, Hypophysis, Hypertension, Angiotensin, Immunohistochemistry

### **Introduction**

Angiotensin is an integrative hormone/neurotransmitter which coordinates the activity of several physiological agents implicated 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).

The hormone vasopressin, well known for its pressor and antidiuretic effects, is primarily synthesized in the magnocellular neurons of the hypothalamus. The axons originating in these neurons pass through the internal zone of the median eminence and reach the posterior lobe of the hypophysial stalk (PL) (Holmes et al., 1986).

The secretion of this hormone is controlled, at least in part, by angiotensinergic inputs that come from the subfornical organ (SFO) and reach the paraventricular (PVN) and supraoptic (SPO) nucleus of the hypothalamus (Sgro et al., 1984).

Immunocytochemical data indicate that both angiotensin and vasopressin are found in the magnocellular and parvocellular neurons in the PVN and SPO of the hypothalamus, possibly in the same cells (Imboden and Felix, 1991). Imboden et al. (1989) have also demonstrated a dense plexus of fibres and terminals in the neurohypophysis of the Wistar Kyoto rat, using their own purified angiotensin II antiserum (Imboden et al., 1987), confirming the findings of Lind et al. (1985) of an angiotensinergic paraventriculohypophysial pathway. They described colocalization of angiotensin and vasopressin in neurons as well as in fibres of the hypothalamo-neurohypophysial system, suggesting the possibility that vasopressin and angiotensin systems interact in the pituitary as well as in the hypothalamus (Imboden and Felix, 1991).

In a recent study, we have described a decrease in immunoreactive material for vasopressin in the posterior lobe of the hypophysis (PL), in a group of spontaneously hypertensive rats (SHR) in comparison with a control group of Wistar-Kyoto rats (WKY) (Castañeyra Perdomo et al., 1999). The purpose of this study was to examine the possibility that angiotensinergic fibres and terminals described in the PL (Imboden et al., 1989) were altered by spontaneous hypertension in relation with the described alterations in the hypophysial-vasopressin content (Castañeyra Perdomo et al., 1999). For this purpose we have used immunocytochemical methods involving our own antiserum against angiotensin II produced in mice.

### **Materials and methods**

#### **Antisera**

Antisera was raised in mouse as follows: angiotensin II (Sigma), after coupling to a carrier (thyroglobulin), was emulsified with complete Freund's adjuvant and

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injected subcutaneously into 10 sites of the male mouse back. Each mouse received the equivalent to 10  $\mu\text{g}$  of angiotensin II. Twenty days later each mouse received the equivalent to 5  $\mu\text{g}$  emulsified with incomplete Freund's adjuvant in 4 to 8 subcutaneous injections. Fourteen days later each mouse received the equivalent to 5  $\mu\text{g}$  in an intraperitoneal injection without adjuvant. Seven days later the mice were killed by intracardial exsanguination. For laboratory purposes, the antiserum obtained was named MAAII.

The specificity of the antisera was evaluated by means of absorption test incubating the antisera overnight with the homologous antigen. The antigen was able to abolish the immunostaining.

#### Tissue sample

Five normotensive male rats (Wistar-Kyoto rats, WKY), and 5 spontaneously hypertensive male rats (SHR) (Letica S/A, Barcelona, Spain) were sacrificed at the 15th week of life. Animals were kept under lighting conditions of 12:12, and food and water were provided ad libitum. Systolic and diastolic blood pressures were assessed in conscious rats by an indirect tail-cuff method. During the last two weeks before sacrifice, water intake was recorded daily, and body weight weekly. At the end of the experiment the rats were anaesthetised with chloral hydrate, and their brain fixed by vascular perfusion with Bouin's fluid. Embedding was in paraffin. Frontal serial sections, 10  $\mu\text{m}$  thick, through the region of the hypophysis were obtained. The sections were mounted in two parallel series. One of the series was stained with the Klüver-Barrera method, while the other one was processed for the immunoperoxidase method of Sternberger et al. (1970).

#### Immunohistochemistry

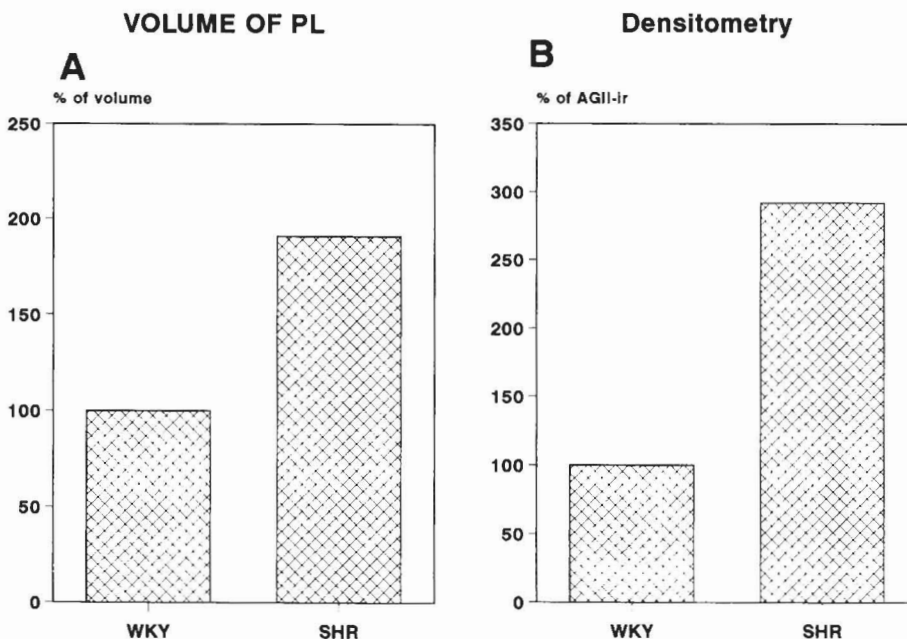
The polyclonal antibody raised in mouse against the angiotensin II (MAAII) was used as primary antibody. All sections from the WKY and SHR rats were incubated simultaneously in the same coplin jar containing MAAII at a 1:100 dilution. Incubation was for 24 h at room temperature. Anti-mouse IgG (whole molecule) peroxidase labelled (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.

#### Densitometry

The intensity of the immunoreaction of the posterior lobe of the hypophysis with MAAII was measured by recording the optical density of the immunostained sections of the PL, using a Magiscan image analysis system, and the Genias program (Joyce Loebel, Newcastle, UK). Six sections of each rat, corresponding to the rostral, intermediate and caudal thirds of the PL were used for the analysis. The mean resulting from the values of the six sections of each rat was regarded as the animal's value. The values of the 5 control rats, and the 5 hypertensive rats were plotted, and a statistical analysis (Student's t-test) was performed.

#### Volume determination

We also measured in the Klüver-Barrera serial, the volume of the posterior lobe of the hypophysis, using the



**Fig. 1.** This figure shows graphically the difference (in %) in the volume of the PL of the hypophysis (A) and the proportion of immunoreactive material for angiotensin II (B) in both SHR and WKY rats. AGII-ir: immunoreactive material for Angiotensin II. SHR: spontaneously hypertensive rats. WKY: Wistar Kyoto rats.

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serial reconstruction system "SSRS" (Eutectic Electronics Inc. North Carolina USA). The mean value of the 5 animals in each group of WKY and SHR rats was used for comparison.

### Results

#### Drinking

The amount of water intake in hypertensive animals was significantly higher than in control rats ( $p < 0.05$ ).

#### Systolic blood pressure

The hypertensive SHR rats had higher systolic blood

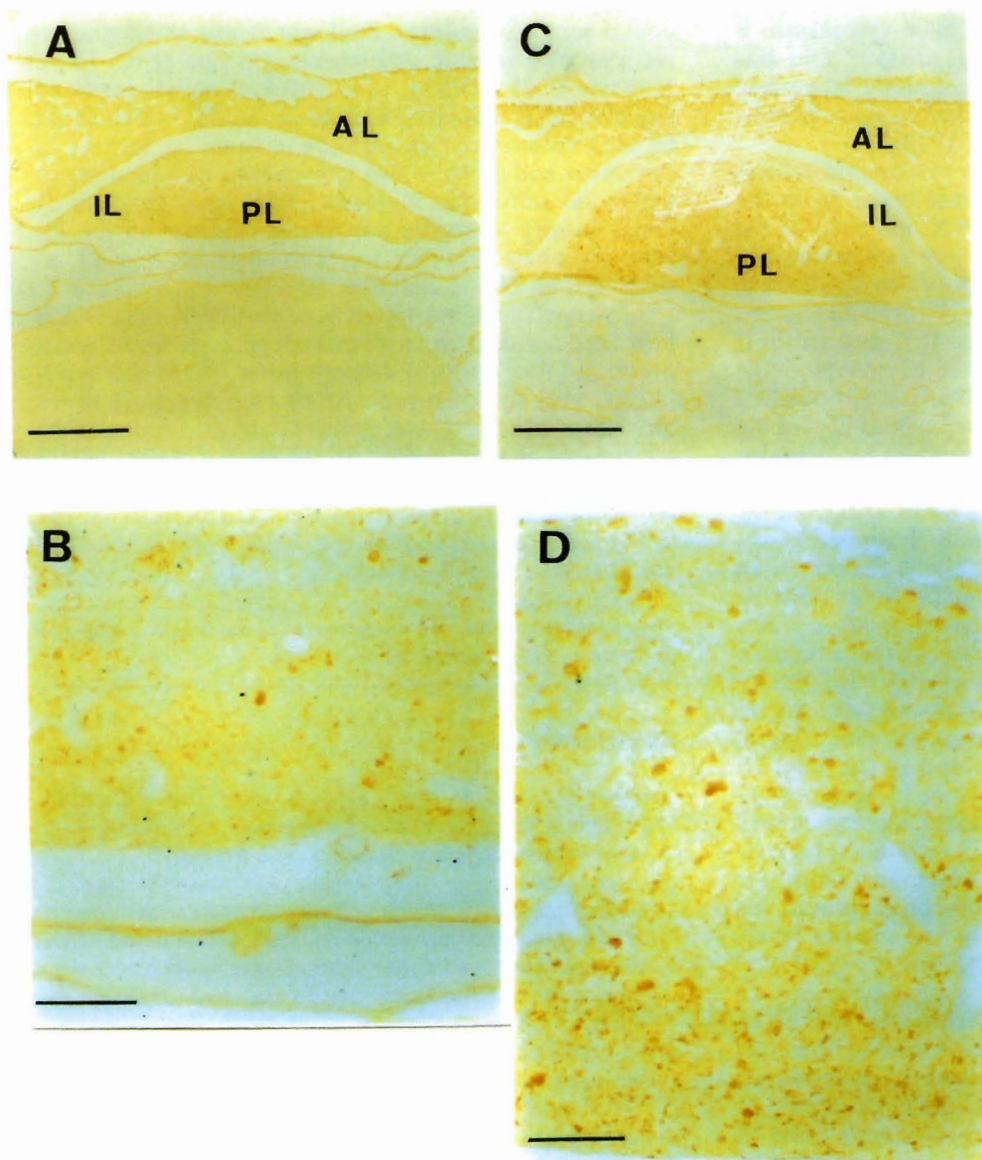
pressure values than WKY control rats ( $p < 0.01$ ).

#### Global volume of the posterior lobe of the hypophysis

The posterior lobe of the hypophysis in the SHR rats showed a 91% bigger size of its volume compared to WKY control animals (Figs. 1A, 2).

#### Immunohistochemistry (densitometry)

The amount of MAII-ir, determined by densitometry, in the fibres of the posterior lobe of the hypophysis of the SHR rats was nearly three times bigger than that found in the PL of WKY rats (Figs. 1B, 2).



**Fig. 2.** This figure shows the angiotensin II-immunoreactive material localized in the posterior lobe of the pituitary. **A and B.** Control group (WKY). **C and D.** Spontaneously hypertensive rats (SHR). AL: anterior lobe. PL: posterior lobe. IL: intermediate lobe. Bars: A, C, 550  $\mu\text{m}$ ; B, D, 100  $\mu\text{m}$ .

## Discussion

The existence of morphological and physiological differences in the brain of SHR rats compared to WKY rats has been described by many authors (Nelson and Boulant, 1981; Zini et al., 1997; Castañeyra Perdomo et al., 1999). We confirm here the pituitary hypertrophy described in previous studies in the hypertensive rats (Horie et al., 1991; Castañeyra Perdomo et al., 1999), but we have also detected a clear increase in angiotensin II immunoreactivity in the posterior lobe of the hypophysis in these SHR rats, compared to WKY control animals. This increase agrees with our previous study in which we found a decrease in vasopressin immunoreactivity in this same area of the hypertensive SHR rats. We suggested then that the decrease in vasopressin-ir could be an expression of an alteration in vasopressin release and/or production in these animals (Castañeyra Perdomo et al., 1999), since vasopressin is known as a hormone producing pressor and has antidiuretic effects, and plasma vasopressin levels had already been described to be increased in these genetic hypertensive animals (Crofton et al., 1978).

Angiotensin II regulates in part vasopressin release through angiotensinergic inputs that come from the subfornical organ and reach the PVN and SPO of the hypothalamus where the vasopressin is synthesized (Sgro et al., 1984). But angiotensin II is also localized in the cell bodies of the SPO and PVN (probably in the same cells that produce vasopressin), and in their pathways to the median eminence and neurohypophysis (Lind et al., 1985; Imboden et al., 1989). It appears that the PVN contributes fibres which are responsible for the neurohypophysial terminal field described by Imboden et al. (1989), suggesting the possibility that angiotensin and vasopressin interact in the pituitary as well as in the hypothalamus (Kilcoyne et al., 1980; Imboden et al., 1989). In this sense, Lind et al. (1985) described an enhanced immunostaining of angiotensin II fibers in the internal lamina of the median eminence after water deprivation in the rat, but a decrease of this immunostaining in the Brattleboro rat (genetically vasopressin-deficient animal), compared to control rats. So, in conditions where plasma vasopressin increases (water deprivation), angiotensin II increased in the internal lamina of the median eminence, while in vasopressin-deficient animals fiber immunostaining for angiotensin II was diminished (Lind et al., 1985). According to these authors, we have found here that immunostaining for angiotensin II increased in the neurohypophysis in the SHR rats, animals in which plasma vasopressin levels are increased (Crofton et al., 1978; Kohara et al., 1993).

Chevillard and Saavedra (1982) suggested an inverse relation between vasopressin and angiotensin systems in the posterior lobe of the pituitary gland, as they found an increase in ACE activity in this area of Brattleboro diabetic rats which was reversed by vasopressin treatment. However, our results together with those of other authors, would indicate that this inverse relation

does not exist in SHR rats. So, in these SHR animals, and compared to control WKY rats, plasmatic vasopressin levels are increased (Crofton et al., 1978; Kohara et al., 1993), vasopressin content in the posterior lobe is decreased (Castañeyra Perdomo et al., 1999), but ACE or aminopeptidase A activities do not change (Zini et al., 1997), while angiotensin II nerve terminals arriving at the neurohypophysis are clearly increased compared to control rats. In this sense, a hyperactive brain renin-angiotensin system (RAS) has been previously described in hypertensive SHR rats (Campbell et al., 1995).

On the other hand, an increase of the metabolic response in the neural lobe after dehydration has been described, that is paralleled by depletion of vasopressin content in this PL, enhanced secretion of vasopressin into plasma and also by an increase in brain angiotensin II formation (Kadekaro et al., 1992). The SHR rats, which presented a depletion in vasopressin content in the neural lobe (Castañeyra Perdomo et al., 1999) and an increase of plasmatic vasopressin (Crofton et al., 1978; Kohara et al., 1993), also showed an increase of angiotensin II immunoreactivity in the PL, which could be the expression of an increase of brain angiotensin II formation and perhaps also expression of an increase of metabolic activity in the neural lobe, as angiotensin II could locally stimulate vasopressin release in the PL (directly or by previous transformation to Ang III, as suggested by Zini et al. (1996, 1997)).

In conclusion, we report here that SHR rats, which presented an increase in plasma vasopressin levels and a decrease in vasopressin content in the PL of the hypophysis, also show an increase of angiotensin II fibres arriving at the PL. These fibres would then probably be responsible, at least in part, for the increased vasopressin secretion to plasma. It could also be another morphological expression of the hyperactivity of the brain RAS described in the SHR with respect to normotensive related animals.

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