

## Effect of hypertension and captopril treatment on the vasopressin in the rat median eminence and posterior lobe of the hypophysis. An immunohistochemical study

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**Summary.** The present study analyses the effects of hypertension and/or its oral treatment with captopril (angiotensin-converting enzyme inhibitor) on the rat median eminence (ME) and the posterior lobe of the hypophysis (PL). After an immunohistochemical reaction using an antibody against arginine-vasopressin, we compared by densitometry the amount of vasopressin immunoreactive material (vasopressin-ir) of these centers in 4 groups of animals: control Wistar Kyoto rats (WKY), spontaneously hypertensive rats (SHR), WKY rats treated with captopril (WKY-T) and SHR rats also treated with the same drug (SHR-T). Captopril was administered at a dosage of 0.1 mg/ml in the drinking water from the 8th to the 15th weeks. We have found that the rats showing the lowest level of vasopressin-ir, in both ME and PL, were those from the SHR group, the concentration increasing after oral captopril treatment (SHR-T), although without reaching the values of WKY rats. Then, ACE inhibition by captopril influences vasopressin content in brain areas where the hormone is concentrated before being released, which supports the hypothesis that suggests a central modulatory effect of ACE inhibitors, contributing to their therapeutic action on hypertension.

**Key words:** Hypertension, Captopril, Median eminence, Posterior lobe, Hypophysis, Vasopressin

### Introduction

The hormone vasopressin is primarily synthesized in the magnocellular neurons of the hypothalamus, and the axons originated in these neurons pass through the internal zone of the median eminence (ME) and reach the posterior lobe of the hypophysial stalk (PL) (Holmes et al., 1991). This hormone is well known for its pressor

and antidiuretic effects and its secretion is controlled, at least in part, by angiotensinergic inputs that come from the subfornical organ (SFO) and reach the paraventricular (PVN) and the supraoptic nucleus (SPO) of the hypothalamus (Sgro et al., 1984; Tanaka, 1989). In this sense, central administration of angiotensin II has been reported to stimulate vasopressin release from the posterior pituitary gland (Phillips, 1987).

The spontaneously hypertensive rat (SHR) is the most widely studied animal model of genetic hypertension and it has been described that plasma vasopressin concentration is elevated in SHR rats (Crofton et al., 1978), and vasopressin contribution in the hypertensive process has been examined in different animal models of hypertension (Share and Crofton, 1982).

Captopril (an angiotensin-converting enzyme inhibitor) is a commonly used drug in the treatment of hypertension. The hypotensive effect of captopril could be due not only to the blockade of formation of angiotensin II in plasma, but also to a local blockade of the brain renin-angiotensin system (Dzau, 1987; Fabris et al., 1990).

The aim of the present study was to analyze possible changes induced by hypertension and its oral treatment with captopril in the amount of vasopressin immunoreactivity in the rat median eminence and pituitary posterior lobe.

### Material and methods

We have studied 20 male rats, that were all sacrificed at the 15th week of life. Ten control rats (Wistar-Kyoto rats, WKY) were divided in two groups: one control group of 5 animals (WKY) and another group that was treated with oral captopril from the 8th to the 15th week of life (WKY-T). Another group of ten spontaneously hypertensive rats (SHR) (Leica S/A Barcelona, Spain), were also subdivided into a subgroup of 5 animals without treatment (SHR) and another 5 animals (SHR-T) that also received captopril during the

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same period as the WKY-T group.

All animals received water and food ad libitum. Captopril was added to the drinking water of the treated groups (WKY-T and SHR-T rats) after the 8th week of life until sacrifice (15th week), at a dosage of 0.1 mg/ml (Thunhorst et al., 1987). We weekly measured the blood pressure by an indirect tail-cuff method, that allowed us to determine systolic and diastolic blood pressure. We also registered the daily fluid ingestion.

Animals were fixed by perfusion with Bouin's fluid, postfixed during 24 hours in the same fixative, dehydrated and embedded in paraffin under standard conditions. Brains were cut in two alternative serial coronal sections. One of the serial sections was stained with the Klüver-Barrera method. In the other series of sections we selected the slides corresponding to the regions in which we were interested, i.e., the ME and the PL of the hypophysis. All sections corresponding to the areas of our interest from the different groups of animals (WKY, SHR, WKY-T and SHR-T) were mounted on the same slide and incubated simultaneously in the same coplin jar containing rabbit anti-arginine-vasopressin antibody (INC Immunobiologicals) for 24 hours at a dilution of 1:2000 in a solution of phosphate-buffered saline 0.01M, triton X-100 0.2% and sodium azide 0.1%, pH 7.4. Anti-rabbit IgG (whole molecule) peroxidase conjugate (Sigma) at a dilution of 1:100 in the same solution as the primary antisera was used as a second antibody for 2 hours. The peroxidase reaction product was visualized through the diaminobenzidine reaction.

The content of immunoreactive material (vasopressin-ir) was determined by the optical densitometry, using the Magiscan Analysis System and the Genias Program (Newcastle, UK). We also measured the volume of the posterior lobe of the hypophysis in the

Klüver-Barrera stained serial using the serial reconstruction system "SRSS" (Eutectic Electronic Inc., North Carolina, USA). The values of 5 rats of each group were statistically analyzed by a two-way ANOVA and a post hoc Bonferroni test.

## Results

### Drinking

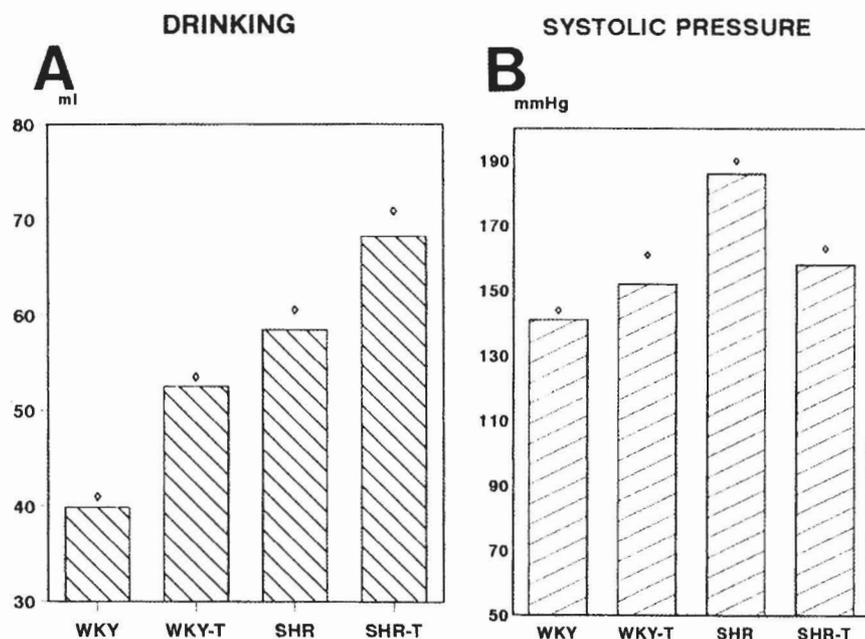
The amount of water intake in hypertensive groups was significantly higher than in control animals ( $p < 0.05$ ). Control animals treated with captopril (WKY-T) drank more water than WKY animals ( $p < 0.05$ ). Although hypertensive animals treated with captopril (SHR-T) tended to drink a little more water than SHR rats, the difference was not significant in these hypertensive animals (Fig. 1A).

### Systolic blood pressure

SHR rats showed higher systolic blood pressure (SBP) values than WKY rats ( $p < 0.01$ ) and SHR-T rats ( $p < 0.02$ ). No significant differences in SBP was detected between WKY, WKY-T and SHR-T rats (Fig. 1B).

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

Figure 2A shows the comparison of the PL volume in the four groups of animals. If we assume that the WKY group shows the normal value of PL volume (100%), we detected that captopril treatment of control rats, i.e., the WKY-T group showed a smaller value of its volume (81%) than WKY animals. SHR rats showed a 91% higher PL volume (191%), while SHR-T showed



**Fig. 1.** The figure shows graphically: (A) the mean value of the daily water ingestion during the last week of life; (B) the values of systolic blood pressure at the 15th week in the four groups of rats. As the previous determinations of these parameters from week 8 to 14 followed a similar pattern, we will only refer here to the results in this last week of life.  $\diamond$ : standard error. WKY: Wistar Kyoto control rats; WKY-T: Wistar Kyoto rats, treated with captopril; SHR: spontaneously hypertensive rats; SHR-T: spontaneously hypertensive rats, treated with captopril.

a 41% higher one (141%) than control rats.

#### Immunohistochemistry (densitometry)

The SHR group showed the lowest amount of vasopressin-ir along the analyzed structures of the hypothalamo hypophysial axis (Figs. 2B,C, 3C,G). The highest concentration of vasopressin-ir was shown in the WKY-T and WKY groups (Figs. 2B,C, 3A,B,E,F). The treatment of the hypertensive rats with captopril (SHR-T) produced an increase in IRM in both structures (Figs. 2B,C, 3D,H).

#### Discussion

In this study we have found that blood pressure in WKY rats does not change with captopril treatment, while in SHR-T rats, blood pressure decreases to normal levels, according to the results described in hypertensive mice by Webb et al. (1986).

Several studies have revealed pituitary hypertrophy in the hypertension and that this hypertrophy is aggravated with emotional stress (Horie et al., 1991). We have also detected an increase in the volume of the posterior lobe of the hypophysis in the hypertensive animals which is followed by a partial reduction after captopril treatment.

It has been suggested that endogenous vasopressin plays a role in the central control of blood pressure (Pittman et al., 1982) and in the present study, the concentration of vasopressin-ir has been shown to be

lower in the SHR rats than in the control WKY rats. This could indicate that in the development of the hypertension of the SHR rats, additionally to the reported implication of the renin-angiotensin system (Harrap et al., 1990), the decrease of vasopressin-ir as expression of an alteration in vasopressin release and/or production could be another factor conditioning the high values of blood pressure, since vasopressin is well known as a hormone-producing pressor and antidiuretic effects. In fact, plasma vasopressin levels have been described to be increased in these genetic hypertensive animals (Crofton et al., 1978). Also, the control system of vasopressin release of the SHR rats has been reported to be more sensible than that of WKY rats (Crofton et al., 1981).

In a previous work, performed in male normotensive mice, we described an increase in immunoreactive material for arginine-vasopressin in the ME, after chronic oral captopril treatment (Castañeyra-Perdomo et al., 1998). In the present study, captopril treatment for seven weeks also produced an increase in the concentration of vasopressin-ir in ME and PL of the SHR rats, while in the normotensive WKY rats there was a small increase in vasopressin content which was not statistically significant. However, Muders et al. (1997) have recently reported that chronic ACE inhibition by quinapril in normotensive rats reduced brain ACE activity, but also reduced vasopressin content (determined by micropunch technique) in several areas involved in central cardiovascular regulation, including the ME. Plasma levels of vasopressin were significantly

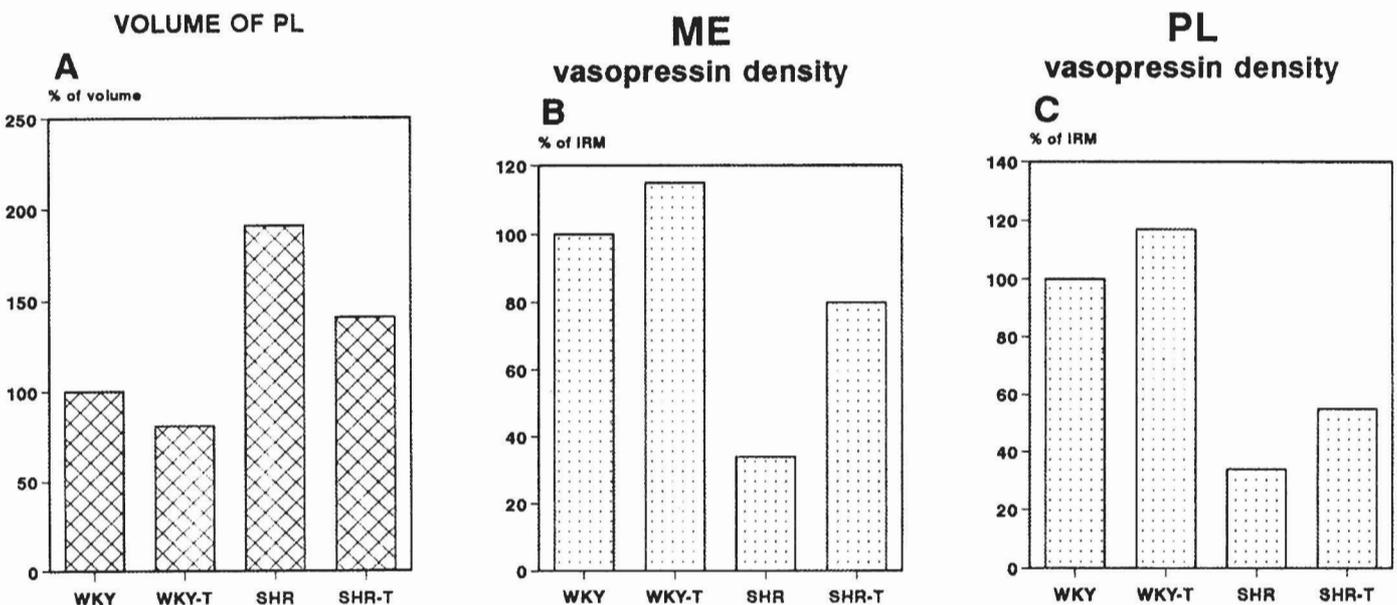


Fig. 2. This figure shows graphically the differences (in %) in the volume of the posterior lobe of the pituitary (A), the proportion of immunoreactive material (IRM) for vasopressin in the median eminence (ME) (B) and the posterior lobe of the pituitary (PL) (C) in the four groups of rats. IRM: immunoreactive material (vasopressin-ir); WKY: Wistar Kyoto rats; WKY-T: Wistar Kyoto rats, treated with captopril; SHR: spontaneously hypertensive rats; SHR-T: spontaneously hypertensive rats, treated with captopril.

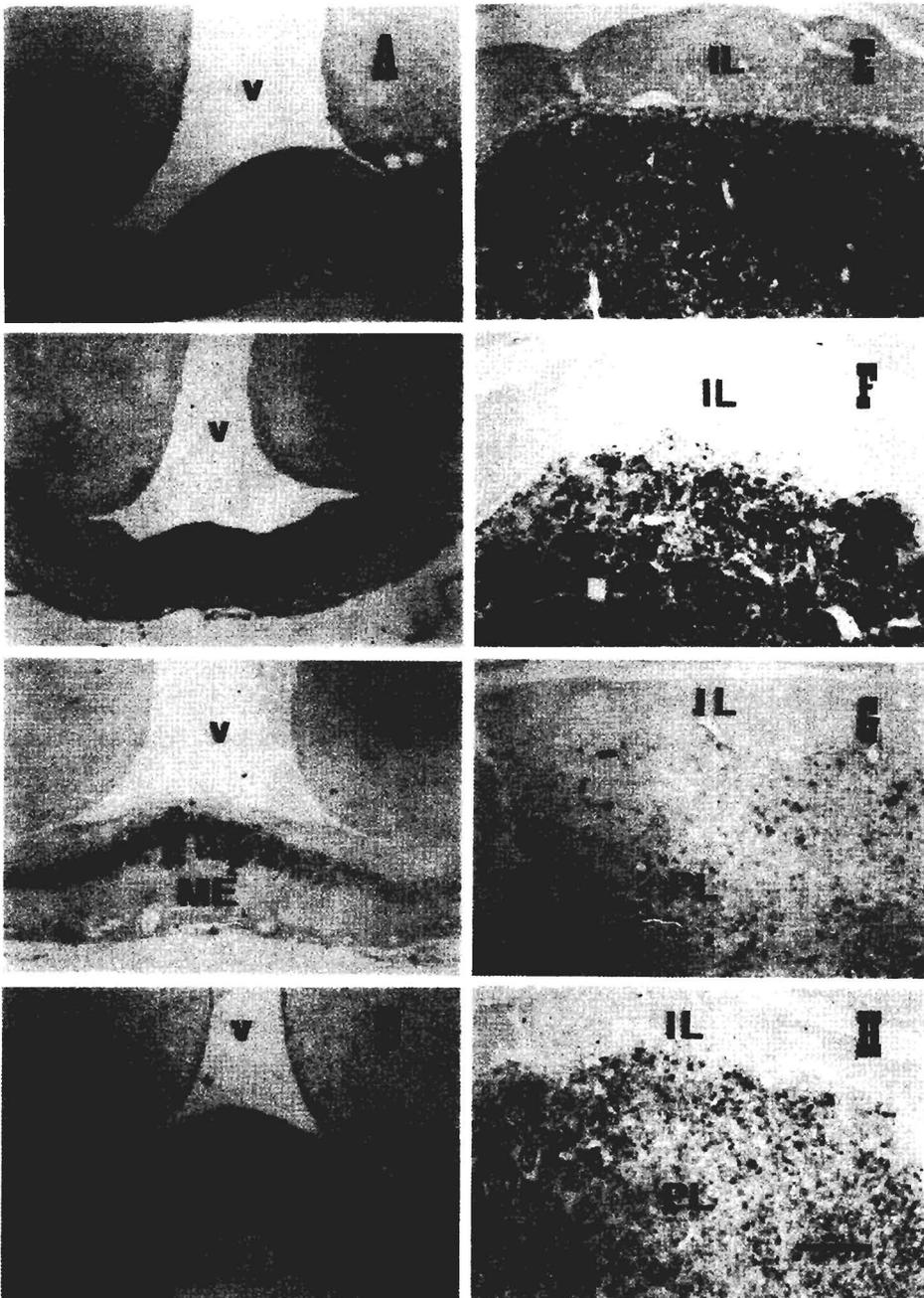
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decreased in these animals (Muders et al., 1997). The different technique used for determining vasopressin content and the different ACE inhibitor employed by Muders et al. (1997), could justify the different results regarding vasopressin content in the ME.

Captopril decreases peripheral and central production of angiotensine II that, as we have mentioned before, regulates in part vasopressin release. We think that our present results agree with those authors that have reported interference of ACE inhibitors with the

release of vasopressin (Williams, 1988; Muders et al., 1997; Castañeyra-Perdomo et al., 1998), and with the reported decrease of vasopressin plasmatic levels by quinapril treatment (Muders et al., 1997). The hormone, that is not released, accumulates along the hypothalamo-hypophysial tract.

The angiotensine II receptors are increased in the SFO of SHR (Nazarali et al., 1989; Saavedra et al., 1986; Tsutsumi and Saavedra, 1991) and this could augment the angiotensinergic input on magnocellular hypo-



**Fig. 3.** This figure shows the vasopressin immunoreactive material localized in the internal zone of the median eminence (A-D) and posterior lobe of the pituitary (E-H). A, E: control group (WKY); B, F: control treated group (WKY-T); C, G: spontaneously hypertensive group (SHR). D, H: spontaneously hypertensive treated group (SHR-T); V: 3rd ventricle; ME: median eminence; IL: intermediate lobe; PL: posterior lobe; IL: intermediate lobe. Bars: 90  $\mu$ m.

thalamic neurons that would then induce a higher vasopressin release from the neurohypophysis. Enalapril (another ACEI) treatment has been reported to decrease the angiotensin II receptors in the SFO of SHR (Nazarali et al., 1989; Saavedra et al., 1986; Tsutsumi and Saavedra, 1991). Captopril could then also interfere with the central angiotensinergic stimulus, and consequently could reverse the effect of hypertension on vasopressin-ir shown in our results.

In conclusion: 1) hypertension decreases the vasopressin-ir of the two studied components of the hypothalamus-hypophysial axis (ME and PL), probably in relation to alterations of the central angiotensinergic inputs that control the production and/or release of this hormone in the hypertensive animals. We suggest that the currently described decrease in vasopressin-ir could be produced by an increase in vasopressin release by the neurohypophysis in the SHR rats, since an increase in plasmatic vasopressin levels has been previously reported in these animals (Crofton et al., 1978). 2) The angiotensinergic control is influenced by the action of captopril, which decreases the peripheral and central production of angiotensin II, and this decrease is one possible reason for the increase in vasopressin-ir detected in both ME and PL.

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