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Effect of the arterial hypertension and captopril treatment on the angiotensin II content in the subfornical organ. A study in SHR rats

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Summary. We studied the effects of spontaneous high blood pressure and the captopril treatment on the subfornical organ (SFO) of rats. The brains of control Wistar-Kyoto rats (WKY), WKY rats treated with captopril (WKY-T), spontaneously hypertensive rats (SHR) and SHR rats treated with captopril (SHR-T) were processed immunohistochemically using anti-angiotensin II as primary antibody. Immunorective material (IRM) for angiotensin II was observed in a group of neurons and some cells of the ependymal layer of the SFO in WKY rats. The angiotensin II immunoreactive (AGII-ir) in the SHR rats was decreased, showing positive reaction only in a few neurons, while captopril treatment induced an increase in immunoreactive material in hypertensive rats, but contrarily, the expression of AGII-ir in the WKY-T group was scarce. The variations of the angiotensin II observed in the SFO could be owing to an interaction between the hypertension and its captopril treatment.

Key words: Hypertension, Captopril, Angiotensin II, Subfornical organ

Introduction

The Subfornical organ (SFO) is a circumventricular organ located below the commissura fornici, entering the rostral wall of the third ventricle (Castañeyra-Perdomo et al., 1992). This organ is a neurogliovascular structure containing neurons, glia and plexus of fenestrated capillaries and characterized by the absence of a blood-brain barrier (Akert and Steiner, 1970). The SFO has connections with the brain regions involved in the central regulation of drinking, salt appetite, blood pressure and cardiovascular function, among them the anteroventral region of the third ventricle (Xu et al., 2001; Vialou et al., 2004) and is also involved in the control of drinking behaviour (Simpson, 1981; Lenkei et al., 1995). In addition, neurons of the caudal ventrolateral medulla, which are involved in the maintenance and reflex regulation of arterial pressure and in the modulation of the intra-extracellular signals of body fluid balance of the circumventricular organ neurons, have direct connection to the SFO (Babic et al., 2004).

In the subfornical organ the AGII acts on AT1 receptors to influence the sympathetic nervous system (Mckinley et al., 2001) and to increase the number of AT1 receptors in experimental or genetic models of hypertension (Saavedra et al., 1986a; Castren and Saavedra, 1989; Tsutsumi and Saavedra, 1991). SFO is implicated in the increase of the plasma vasopressin and in the rise in arterial blood pressure observed in response to angiotensin II (Lind et al., 1983; Muders et al., 1997).

Type-1 angiotensin II receptors (AT1) are placed in brain structures implicated in the salt water balance and cardiovascular regulation, such us; subfornical organ and other circumventricular organs, hypothalamic nucleus, and nucleus of the solitary tract (Nuyt et al., 2001; Moulik et al., 2002; Grob et al., 2004), but the AT1 receptors have also been observed in the periolivary region, dorsolateral nucleus of the lateral lemniscus and dorsal raphe (Phillips et al., 1993; Moulik et al., 2002). Type-2 angiotensin II receptors (AT2) are located in some lobes of the cerebellum, layer VI of the cerebral cortex, ventral lateral septal nucleus, superior colliculus (Moulik et al., 2002). It is proposed that the function of AT1 receptors can be dependent on AT2 receptor expression (Armando et al., 2002).

The circulating peptide hormone angiotensin II has access to specific receptors (AT1) in the subfornical organ and OVLT and act on the subfornical organ to stimulate water drinking in the rat (McKinley et al 2004). Angiotensin II is also found in the paraventricular

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(PVN) and supraoptic (SPO) hypothalamic nuclei, probably into the vasopressinergic cells (Imboden and Felix, 1991). The co localization of angiotensin and vasopressin in the same cells and fibers of the hypothalamo-neurohypophyseal system would signify that vasopressin and angiotensin systems interact in the hypophysis and in the hypothalamus (Imboden et al. 1989; Imboden and Felix, 1991; Leong et al., 2002).

The aim of the present work is to detect changes of the AGII in the SFO of spontaneously hypertensive rats and their captopril treatment, by using specific antibody against the angiotensin II, in order to investigate the association among the increase and depletion of AGII, AT1 receptor and arterial hypertension.

Material and methods

Antisera was raised in the mice as follows: angiotensin II (Sigma) after coupling to a carrier (thyroglobulin), 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. 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. Antisera specificity of the angiotensin II was evaluated by means of absorption test, by incubating the antisera overnight with the homologous antigen. The antigen was able to abolish the immunostaining (Perez-Delgado et al., 2000).

Twenty male rats, fed with a standard diet, were sacrificed in groups of five at 15 postnatal weeks. There were four groups: Group 1, control Wistar-Kyoto rats (WKY); Group 2, WKY treated with captopril (WKY-T); Group 3, spontaneously hypertensive rats (SHR) and Group 4, SHR treated with captopril (SHR-T). The 5 members of each group shared the same cage and drank water and saline (0.3 molar) simultaneously from the beginning of the study. The positions of the drinking bottles were switched every day to avoid the development of position preferences. The captopril treatment was administered in the drinking fluid from 8 postnatal weeks at a dosage of 0.1 mg /ml (according to Thunhorst et al., 1987). Brains were fixed by perfusion through the rat's left ventricle with Bouin's fluid and postfixed in the same fluid, dehydrated and embedded in paraffin under standard conditions. Four (A, B, C and D) serial coronal sections of 10 microns were cut. The A series was stained with the Klüver-Barrera method.

The B, C and D brain series were processed by immunohistochemistry. The sections were preincubated, in PBS-Triton with 2% normal goat serum, for 2 hours. The polyclonal antibody raised in the mice against the angiotensin II (Perez-Delgado et al., 2000) was used as the primary antibody at a 1:100 dilution in PBS-Triton with 2% normal goat serum, the incubation was for 24 h, at room temperature. Followed by "DAKO StreptABCcomplex/HRP Duet, Mouse/Rabbit" procedure. The reaction product was visualized by diaminobenzidine reaction. Method specificity was controlled by omitting the primary antibody.

Results

The SHR group only showed quite a strong AGII reaction in the cytoplasm of several neurons located in the dorsolateral part of the SFO, and a weak immunohistochemical reaction in some groups of cells in the SFO ependymal layer (Fig. 1B,E). In the SHR-T group the positive reaction was found in more cells than in the hypertensive rats, the immunoreactive material (IRM) was located in a large groups of cells in the dorsal part of the SFO. The immunoreactive material in ependymal cells and perivascular spaces of the SHR treated rats was also found (Fig. 1C,F). The WKY group (Fig. 1A,D) showed IRM in the perivascular spaces, in cells located in the dorsolateral parts of the SFO and in the ependymal layer but the AGII-ir expression was lighter than SHR-T (Fig. 1C,F) . In the WKY-T the SFO cells IRM was practically not found. The plexus choroideus of the III ventricle and some ependymal cells of the WKY and SHR groups also showed a small amount of IRM.

Discussion

The angiotensin II receptors are increases in experimental or genetic models of hypertension in the SFO and not in the other brain structures (Castren and Saavedra, 1989; Tsutsumi and Saavedra, 1991). The AGII in the SFO, in the supraoptic and paraventricular hypothalamic nuclei and in the median eminence plays an important role in hypophysis hormone release (Johren et al., 1997). We have previously demonstrated (Castañeyra-Perdomo et al., 1999), that SHR rats show the lowest level of vasopressin immunoreactive material, in the median eminence (ME) and hypophysis posterior lobe (PL), and the concentration of the vasopressin increases after oral captopril treatment, although it does not reach the values of WKY rats, therefore, ACE inhibition by captopril influences the vasopressin content in brain areas where the hormone is concentrated before being released (Castañeyra-Perdomo et al., 1999).

The SHR animals showed an increase in the AGII-ir in the fibres arriving at the hypophysis posterior lobe (PL), with respect to the PL of WKY rats (Perez-Delgado et al., 2000). This increase is compatible with the hyperactivity of the brain RAS, the depletion of vasopressin content in the PL and the increase in plasmatic levels of vasopressin described in SHR rats (Crofton et al., 1978), since angiotensin II could locally stimulate vasopressin release to plasma from the neurohypophysis.

On the other hand, injections of AGII cause a greater increase of the excitatory response of the SFO in SHR

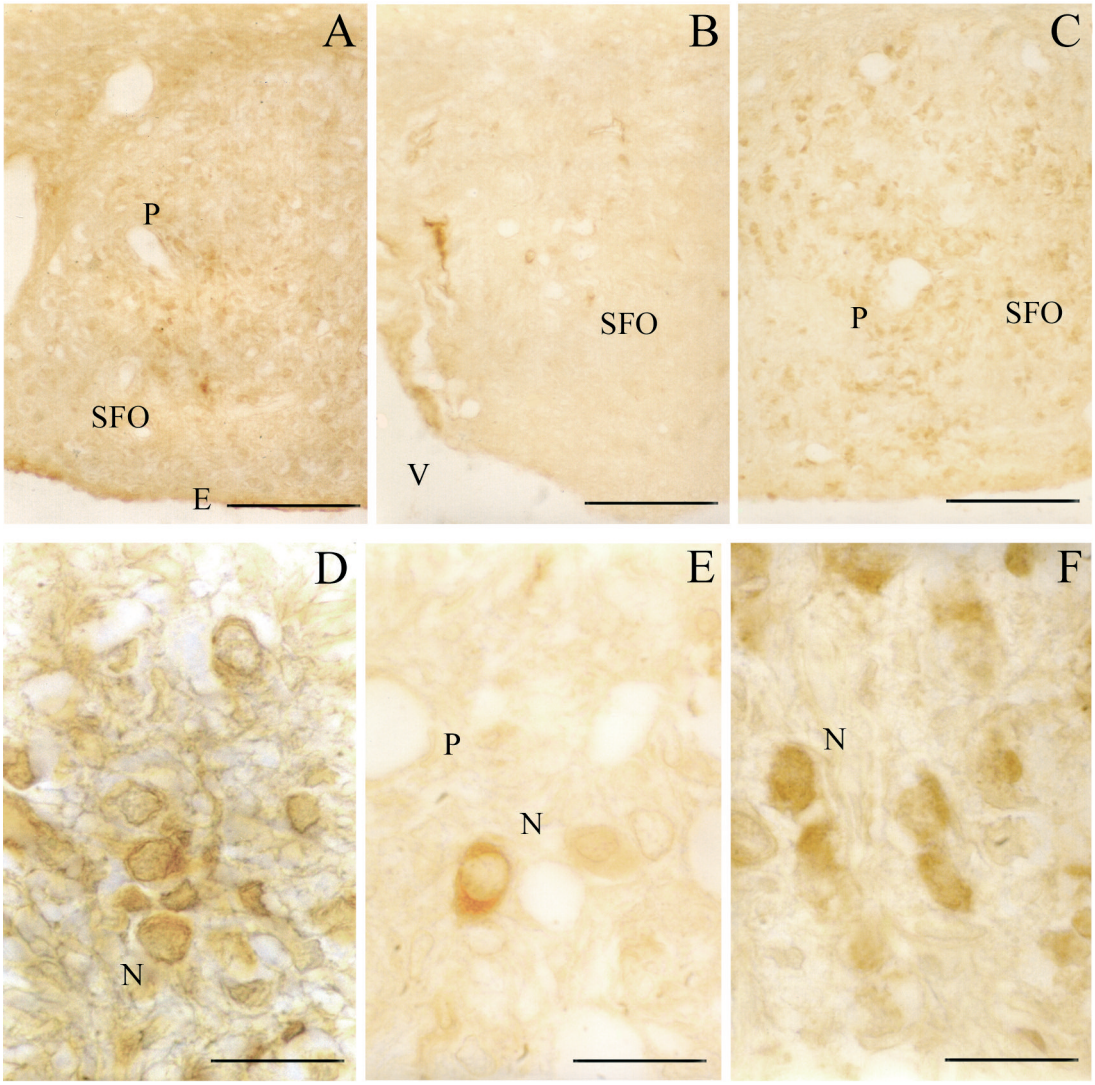

Fig. 1. Coronal view of the subfornical organ). Shows the angiotensin II immunoreactive material in: neurons, ependymal layer and perivascular space of the WKY rats (**A, D**); SHR rats (**B, E**) and SHR treated rats. E: ependymal cells; N: neuron; SFO: subfornical organ; P: perivascular space; V: III ventricle. Bars: A, B, C, 150 µm; D, E, F, 15 µm.

than in WKY rats (Miyakubo et al., 2002). In the present work we found a decrease in AGII-ir in the SFO of the SHR compared to the WYK rats, this decrease is possibly an expression of the high release of AGII in the SHR, that could be caused by the differences in the spontaneous discharge rate of SFO neurons projecting to the PVN and in their response to circulating AGII found by Miyakubo et al. (2002) between WKY and SHR. Thereby, it could be a hyperfunction of SFO neurons in the spontaneous hypertension, that produces an increase of the AGII release, expressed by a decrease of the AGII-ir content in the SFO neurons, that is accompanied by an increase in the number of AGII receptors (Saavedra et al., 1986b; Castren and Saavedra, 1989; Tsutsumi and Saavedra, 1991).

Moreover, this decrease of AGII and increase of AGII receptor are avoided by angiotensin converting enzyme inhibitor (ACEI) treatment, as we found in the present work that, the AGII immunoreactive reaction in the SFO of the SHR rats is increased by captopril treatment and the AGII receptors are decreased by enalapril treatment (Nazarali et al., 1989). Therefore, we could conclude that the AGII and AGII receptors interact in the SFO and could be related to the physiopathological mechanisms of this kind of hypertension.

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