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Short communication

Asymmetrical effect of captopril on the angiotensinase activity in frontal cortex and plasma of the spontaneously hypertensive rats: Expanding the model of neuroendocrine integration

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ABSTRACT

There is a reciprocal connection between the frontal cortex (FC) and cardiovascular function, and this connection is functionally lateralized. The possible pathophysiological impact of neuroendocrine asymmetries is largely underestimated. Our aim was to examine the activity of soluble (SOL) and membrane-bound (MB) aminopeptidases (APs) involved in the renin-angiotensin system in the peripheral plasma and in the left and right FC, in both untreated (control) and captopril-treated spontaneously hypertensive rats (SHRs). Enzymatic activities were measured fluorometrically using arylamide derivatives as substrates. Captopril reduced systolic blood pressure, but no differences in plasma AP activity were observed between the control and treated SHRs. In contrast, whereas the bilateral pattern (left vs. right differences) of SOL activities did not substantially change in the FC after captopril treatment, the asymmetries observed for MB activities in the FC markedly increased compared with the control group. Moreover, correlations between the AP activities in the plasma and those in the left or right FC were observed. In the control rats, the plasma AP activities correlated significantly with those in the right FC, whereas they correlated with those in the left FC in the captopril-treated group. In both groups (control and captopril), these correlations were negative for the SOL activity but positive for the MB activity. The present results reveal a pattern of bilateral behavior between the nervous and cardiovascular systems. The inverted bilateral behavior after captopril treatment suggests a systematized, lateralized neuroendocrine response representing a regular bilateral behavior that has yet to be analyzed.

The frontal cortex (FC) is not only involved in cognitive functions but also connected to cardiovascular function [25]. It has been postulated that this connection is functionally lateralized [12]. However, the possible impact of this connection on the pathophysiology of neuroendocrine asymmetries is largely underestimated. The brain renin-angiotensin system (RAS), through the action of its constitutive neuropeptides, is involved in the regulation of behavior [31]. The metabolism (biotransformation to other active forms or inactivation) of neuropeptides may be accomplished by soluble (SOL) or membrane-bound (MB) enzymes [21], and some of the aminopeptidases (APs) involved in the RAS enzymatic cascade appear to be asymmetrically distributed in the brain [2,22]. Among the APs involved in the RAS, aspartyl AP (AspAP) metabolizes angiotensin I (Ang I), whereas glutamyl AP (GluAP) metabolizes Ang II and cholecystokinin (CCK). Ang III is metabolized to Ang IV by alanyl AP (AlaAP), an enzyme that also acts as an enkephalinase [5]. Angiotensin IV binds specifically to the AT_4 receptor, which is identical to insulin-regulated AP (IRAP) [24]. Cystinyl AP (CysAP), also called oxytocinase/vasopressinase, is considered the human variant of IRAP and is inhibited following Ang IV binding [24]. All of the aforementioned neuropeptides, as well as the neuropeptidases involved in their metabolism, participate in several behaviors, including anxiety and depression [2,28,30,31]. Captopril was reported to cross the blood-brain barrier (BBB) [13], modifies the activities of the above-mentioned APs in the hypothalamus as it does in peripheral tissues [29] and improves cognition and depressed mood [7,20]. Alternatively, other authors reported that captopril did not cross the BBB and suggested that its central actions might be due to an elevated central Ang II formation [10]. Our aim was to analyze the activities of these neuropeptidases, in their SOL and MB forms, in the left and right FC of spontaneously hypertensive rats (SHRs) and to compare these activities with the activities

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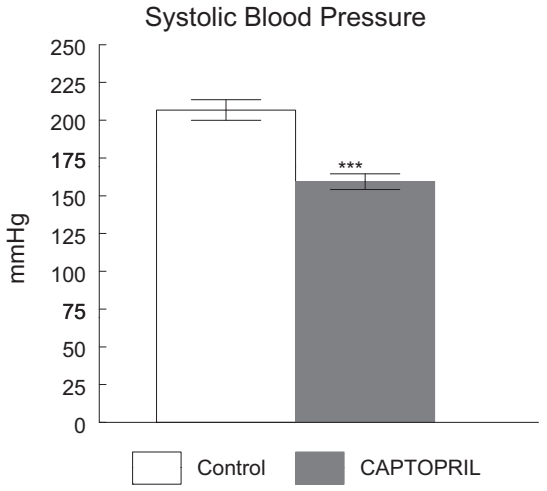

Fig. 1. Systolic blood pressure (mm Hg) in control and captopril-treated animals, as measured at the end of the treatment period (four weeks). The values represent the mean ± SEM of 10 animals in each group. *** $p < 0.001$.

in the plasma of these rats. In addition, we studied the relationship between these activities in the plasma and the same activities in the left or right FC in control and captopril-treated SHRs. Systolic blood pressure (SBP) was also monitored throughout the experiments.

All of the experimental procedures involving animals were performed in accordance with the European Communities Council Directive 86/609/EEC and were approved by the bioethics committee of the University of Jaén. Twenty adult male SHRs were grouped as either control ($n = 10$) or captopril-treated ($n = 10$) animals. Captopril (100 mg/kg p.o.) was administered daily in drinking water (0.5 ml/100 g body weight) for 4 weeks. The SBP was monitored by the plethysmographic method throughout the experimental period [29]. At the end of the treatment period, after recording the SBP, blood samples were obtained, and plasma was isolated by centrifugation for 10 min at 2000 *g* and stored at −20 °C. Each rat was subsequently perfused with saline under equithesin anesthesia, and the left and right FC were obtained as previously described [23]. Briefly, the brain samples were dissected according to the stereotaxic atlas of Paxinos and Watson [19]. For each group, the left and right frontal lobes 11.20 mm anterior to the interaural line were collected separately [23]. Aspartyl- (AspAP), glutamyl- (GluAP), alanyl- (AlaAP) and cystinyl-aminopeptidase (CysAP) activities were measured fluorometrically using arylamides as substrates, as previously described [2,23]. Student's *t*-test was used to compare the data from control and captopril-treated SHRs, and the paired Student's *t*-test was used for left FC vs. right FC comparisons. *P*-values below 0.05 were considered significant. The Pearson correlation coefficient of the left or right frontal cortex and plasma AP activities was computed using SPSS13.0 and STATA 90. *P*-values below 0.05 were considered significant.

The SBP of captopril-treated SHRs was 47 mm Hg (or 30%) lower than that of control rats ($p < 0.001$) (Fig. 1). Compared with the basal conditions, the main left vs. right changes in the AP activities in the brain after captopril treatment were observed for MB APs (Fig. 2). SOL GluAP activity did not change after captopril treatment. Although SOL CysAP activity increased by 36% ($p < 0.05$) in the left FC after captopril treatment, no left-right differences were observed in the control and captopril-treated animals. In contrast, SOL AlaAP activity was 31% higher in the right FC of control animals ($p < 0.05$). After captopril treatment, the right FC SOL AlaAP activity decreased ($p < 0.05$; 40% lower), which resulted in a loss of the asymmetry observed in the controls (Fig. 2). Regarding the MB activities, the bilateral pattern of GluAP did not change substantially after captopril treatment compared with the basal conditions. However, captopril markedly increased the asymmetry in the CysAP and AlaAP activities observed in the control group (Fig. 2). GluAP activity exhibited an asymmetrical distribution in the frontal cortex of the control group with a left predominance ($p < 0.01$). This left-right difference was slightly reduced ($p < 0.05$) after captopril treatment. In contrast, there was a right frontal predominance (50% higher than the left side) for AlaAP activity in the control group ($p < 0.05$). This right predominance increased even further after captopril treatment, with the AlaAP activity double that of the left side ($p < 0.001$). Similar to AlaAP, CysAP activity slightly predominated in the right frontal cortex of the control group ($p < 0.05$; 50% higher on the right side), and this difference increased in magnitude after captopril treatment ($p < 0.001$; 100% higher on the right side). No detectable levels of AspAP were observed in the brain in these experiments.

Modifications in the basal anatomical and functional FC asymmetries have been associated with changes in behavior, including emotional state [1,9,11,15,17,18,22,27]. Vancassel et al. [27] reported that rats with a diet-induced deficiency of n-3 polyunsaturated fatty acids displayed a modified asymmetrical PUFA distribution in their brains, leading to changes in motor behavior and possibly to cognitive disturbances. Unilateral prefrontal cortex lesions can alter emotional and cardiovascular autonomic responses depending on which hemisphere is injured, with a predominant parasympathetic activation by the left prefrontal cortex but a sympathetic inhibition by the right prefrontal cortex [15]. There is a relationship between depression and frontal asymmetry in which greater left frontal activity is associated with fewer depressive symptoms [11]. Tryptophan depletion, which reduces brain serotonin levels and may induce acute depressive symptomatology, leads to changes in frontal EEG asymmetry [1]. Correlations between changes in EEG asymmetry in the lateral frontal cortex and alterations of mood have been reported in humans, suggesting that state-dependent changes of lateralized cortical activity may underlie certain cognitive-emotional interactions [18]. It has been reported that socially anxious patients showed a significant shift after cognitive behavioral therapy, from a greater EEG activity in the right FC to greater resting activity in the left FC [17]. In addition, changes in emotional state after captopril treatment have been reported [7,8,20]. In a model of myocardial infarction in rats, captopril therapy appeared to decrease anxiety in the infarcted group but increase anxiety in the sham-operated rats. This response, however, differed depending on the test used to measure anxiety [20]. Captopril administered p.o. but not i.c.v. significantly modified conditioned avoidance responses [8]. The authors suggested that the decrease in Ang II and/or the increase in the neuropeptides bradykinin, substance P, enkephalin and neurotensin in the brain following ACE inhibition may be involved in this response. It has also been reported that the ACE inhibitors captopril and enalapril improve cognition and depressed mood in hypertensive patients [7]. We previously postulated that anatomical and functional asymmetries underlie neurochemical left-right differences [22]. Therefore, because the activities of the neuropeptidases GluAP, AlaAP and CysAP regulate the functional status of angiotensin metabolites, CCK, oxytocin/vasopressin or enkephalin, changes in the bilateral brain pattern of these molecules after captopril treatment may reflect variation in emotional state in these experimental conditions.

In contrast to brain, although captopril reduced SBP, no differences in the plasma AP activities were observed between the control and captopril SHR groups (Fig. 3). Nevertheless, it was particularly interesting to observe a correlation between the left or right FC and the plasma AP activities (Table 1). In the control SHRs, the correlations between the AP activities in the right FC and plasma were mainly negative (the higher the right FC AP activity, the lower the plasma AP activity and vice versa) and were specific for SOL AP activities. However, some correlations were positive (the

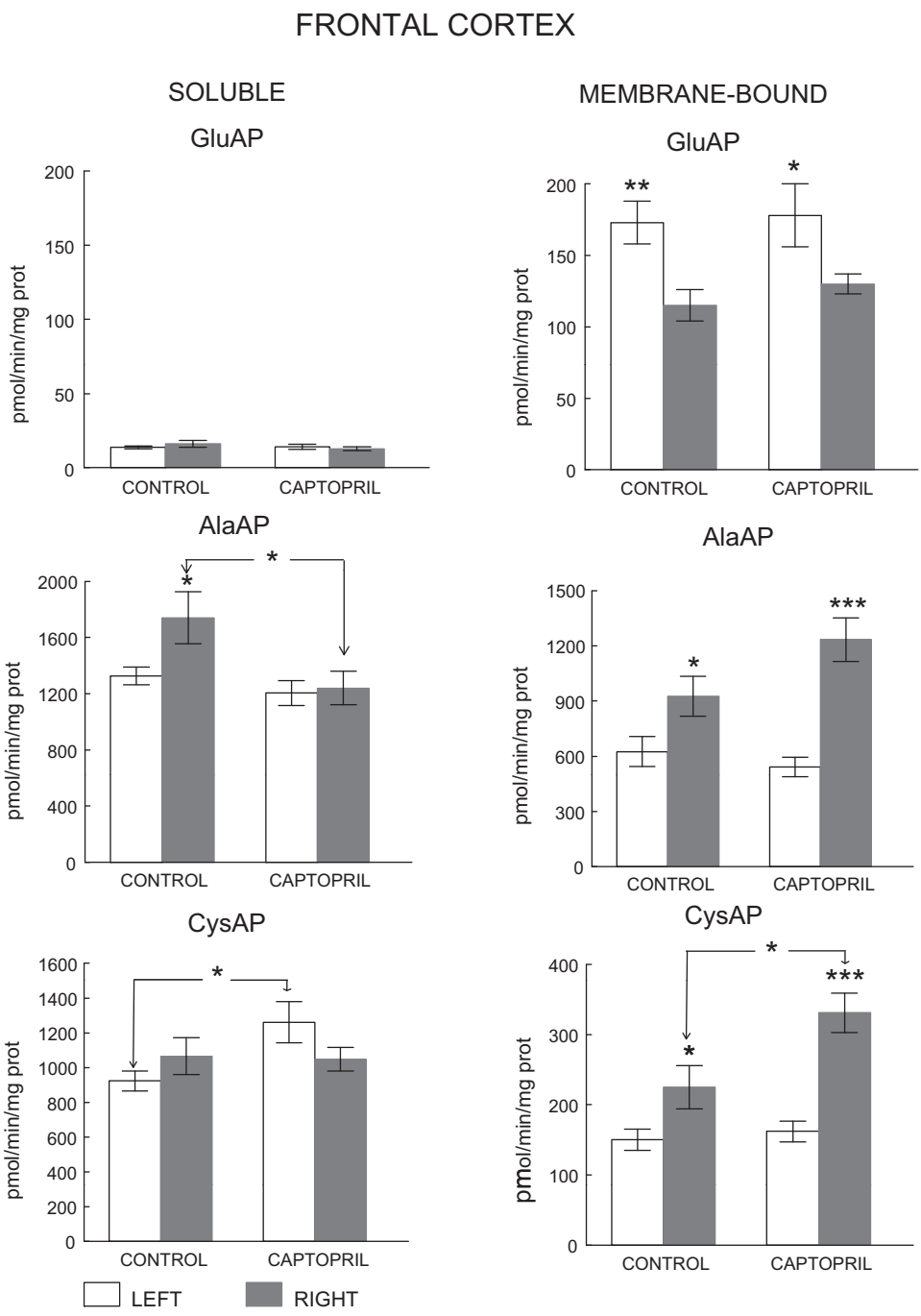

Fig. 2. GluAP, AlaAP and CysAP activities on the left and right sides of the frontal cortex of control ($n = 10$) and captopril-treated ($n = 10$) spontaneously hypertensive rats. The values represent the mean ± SEM of specific GluAP, AlaAP and CysAP activities expressed as picomoles of glutamyl-, alanyl- or cystinyl-β-naphthylamide hydrolyzed per min per mg of protein. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

greater the right FC AP activity, the greater the plasma AP activity and inversely) but only for the MB AP activities. No correlations between the left FC and plasma were observed in the controls. In contrast to the control SHRs, the correlations between the left FC and plasma AP activities were mainly negative in captopril-treated animals and were specific for SOL AP activities. Again, some correlations were positive but solely for the MB AP activities. These findings clearly indicate a difference between brain SOL AP and MB AP activities. No correlations between the right FC AP and plasma activities were observed in captopril-treated animals. These results reveal new aspects of neuroendocrine asymmetries whose functional meaning remains to be elucidated.

Neuropeptidases not only inactivate neuropeptides but also metabolize them, forming peptides with different properties from their precursors. The higher the activity of the neuropeptidase, the lower the availability of the neuropeptide substrate and the higher the availability of the newly formed neuropeptide. We have repeatedly observed differences in the responses of SOL and MB neuropeptidase activities in several experimental conditions [2,5]. In the present study, important discrepancies between the SOL and MB activities were again observed. Differences in the SOL and MB responses reveal remarkable discrepancies between both enzymatic pools in the bilateral brain pattern after captopril treatment. An intracellular RAS in neurons, whose

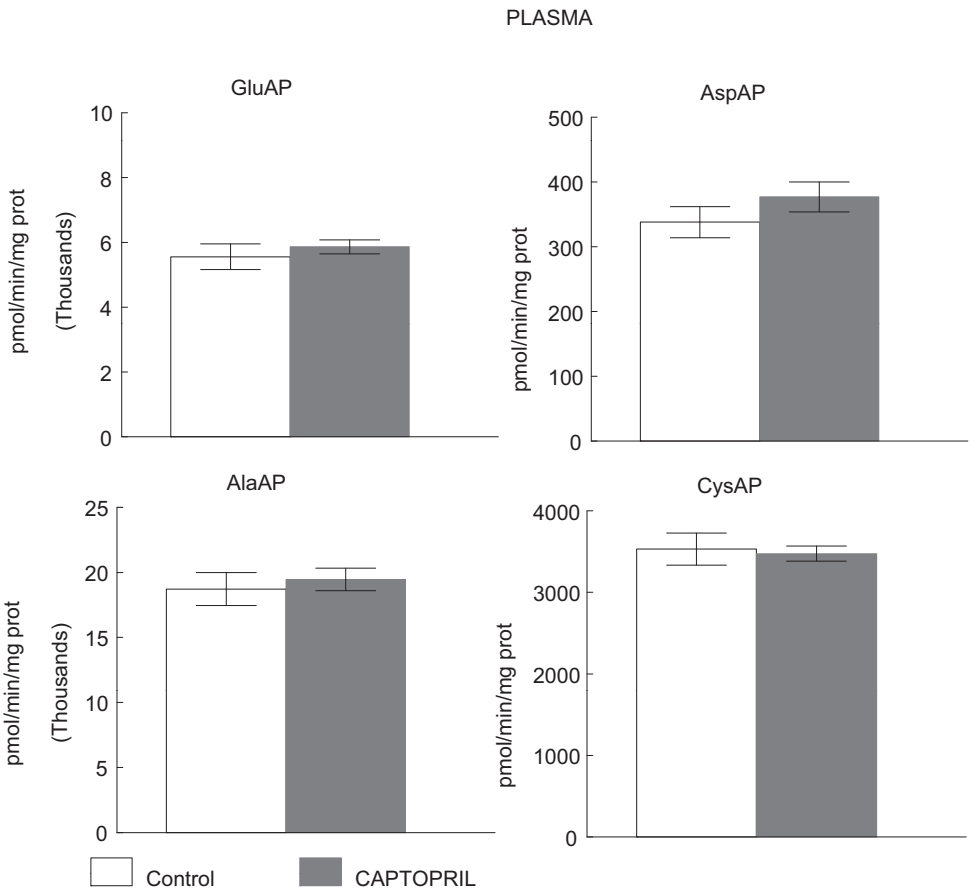

Fig. 3. GluAP, AspAP, AlaAP and CysAP activities in the plasma of control ($n = 10$) and captopril-treated ($n = 10$) spontaneously hypertensive rats. The values represent the mean ± SEM of specific GluAP, AspAP, AlaAP and CysAP activities expressed as picomoles of glutamyl-, aspartyl-, alanyl- or cystinyl-β-naphthylamide hydrolyzed per min per mg of protein. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

function is not yet fully understood, has been described recently [14]. In addition, synaptic-level uptake systems for neuropeptides such as the cholecystokinin octapeptide [16] or Met-enkephalin [26] have been reported. Therefore, although the metabolism of neuropeptides by MB neuropeptidases (presumably functionally associated with their receptors) is accepted as a major mechanism for the regulation of the neuropeptides function, these enzymes are also present in their SOL form in the cytosol or clustered into

Table 1
Frontal cortex vs. plasma.

Control					
Left frontal cortex			Right frontal cortex		
FC vs. PL	*r*	*p*	FC vs. PL	*r*	*p*
No correlations			SOL AlaAP vs. AspAP	−0.829	0.01
			SOL CysAP vs. AspAP	−0.842	0.008
			SOL GluAP vs. AspAP	−0.842	0.008
			MB GluAP vs. *GluAP*	+0.723	0.04
			MB GluAP vs. *AlaAP*	+0.704	0.05
Captopril					
Left frontal cortex			Right frontal cortex		
FC vs. PL	*r*	*p*	FC vs. PL	*r*	*p*
SOL CysAP vs. AlaAP	−0.771	0.02	No correlations		
SOL CysAP vs. GluAP	−0.836	0.009			
SOL CysAP vs. AspAP	−0.722	0.04			
SOL GluAP vs. AlaAP	−0.750	0.03			
SOL GluAP vs. GluAP	−0.890	0.003			
SOL GluAP vs. AspAP	−0.801	0.01			
MB CysAP vs. *AspAP*	+0.850	0.007			
MB CysAP vs. *AlaAP*	+0.746	0.03			

Correlations between the left or right frontal cortex (FC) soluble (SOL) or membrane-bound (MB) AP activities versus plasma (PL) AP activities in the control and captopril-treated animals. Pearson's correlation coefficients (r) and p-values are indicated and specify the significance of the differences between these correlations.

subcellular organelles [21] and may also take part in the regulation of neuropeptides at a synaptic level. Although SOL and MB neuropeptidases may hydrolyze the same substrates, the regulatory mechanisms of both enzymatic pools may be independent, leading to different functional responses in different environmental conditions.

We recently reported that plasma angiotensinase activity, nitric oxide levels and SBP were differentially affected after unilateral lesions of the nigrostriatal system, and the effect depended on the brain hemisphere injured [3,4]. Normotensive and hypertensive rats responded differently to the unilateral lesions [4]. In addition, the SBP increased dramatically, mainly in hypertensive animals, after a left hemisphere lesion. No differences were observed in the right-lesioned or sham-operated groups [3]. These results suggested that such responses were due to an asymmetry in the organization of the autonomic nervous system of the blood vessels.

The present results reveal an additional pattern of left-right behavior between the nervous and cardiovascular systems. The inverted bilateral behavior after captopril treatment suggests a systematized, lateralized neuroendocrine response, representative of a regular bilateral behavior not yet specified. Extending the *neurovisceral integration* model, first proposed by Bernard [6] and more recently developed by Thayer and Lane [25], we can speculate the existence of a bilateral brain synchronization that results in a coordinated response between the central nervous system and the peripheral tissues through the autonomic nervous system. Each bilateral brain status (symmetrical or asymmetrical) may elicit a certain peripheral autonomic response.

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