Chronic ACE inhibition by quinapril modulates central vasopressinergic system

Frank Muders a,*, Dietmar Elsner a, Karin Jandeleit b, Udo Bahner c, Eckhard P. Kromer a, Ingrid Kirst a, Günter A.J. Riegger a, Miklos Palkovits d

a Klinik und Poliklinik für Innere Medizin II, University of Regensburg, Franz-Josef-Strauss-Allee II, 93053 Regensburg, Germany
b Zentrum für Innere Medizin, Medizinische Hochschule Hannover, Hannover, Germany
c Medizinische Universitätsklinik Würzburg, Würzburg, Germany
d Laboratory of Neuromorphology, Semmelweis University Medical School, Budapest, Hungary

Received 24 July 1996; accepted 10 March 1997

Abstract

Objective: The role of the brain as a target for angiotensin converting enzyme (ACE) inhibitors in the treatment of heart failure and hypertension is unclear. To test the hypothesis that ACE inhibitors may modulate other central neuropeptide systems such as the central vasopressin system, we studied the effects of chronic treatment with the ACE inhibitor, quinapril, on ACE activity and on central vasopressin content in specific brain areas in rats.

Methods: 22 rats were chronically treated with quinapril 6 mg · kg⁻¹ BW per gavage daily for 6 weeks; untreated controls, n = 14. ACE density in various brain regions was assessed by in vitro autoradiography using the specific ACE inhibitor, [¹²⁵I]351A. Vasopressin content was determined in 19 brain areas micropunch technique known to be involved in cardiovascular regulation.

Results: Following chronic quinapril treatment ACE was significantly decreased in the thalamus (−38%), hypothalamus (−37%), hypophysis (−35%), cerebellum (−36%), choroid plexus (−20%), and locus coeruleus (−35%). Additionally, a marked reduction in serum ACE activity (−97%) was observed. Plasma levels of vasopressin were significantly decreased after quinapril treatment 0.97 ± 0.11 vs. 1.63 ± 0.24 pg · ml⁻¹ in controls, P < 0.05. Vasopressin content was significantly reduced in 9 of 19 specific brain areas. Regarding the hypothalamic vasopressin-producing nuclei, vasopressin was decreased in the paraventricular (292 ± 197 vs. 237 ± 58 pg · mg⁻¹ protein in controls; P < 0.001) and supraoptic nuclei (136 ± 197 vs. 245 ± 389 pg · mg⁻¹ protein; P < 0.05), but not in the suprachiasmatic nucleus. Vasopressin content was also significantly reduced in the median eminence (588 ± 183 vs. 283 ± 49 pg · mg⁻¹ protein, P < 0.001), where the hormone is mainly concentrated in the hypothalamo-hypophysial tract.

Conclusions: Autoradiographic studies in vitro indicate that orally administered quinapril suppresses central ACE activity after chronic treatment. ACE inhibition by quinapril strongly influences vasopressin content in important brain areas which are involved in central cardiovascular regulation. Therefore, central modulatory effects of ACE inhibitors may also contribute to overall therapeutic efficacy.

Keywords: Central cardiovascular regulation; ACE; Vasopressin; ACE inhibitors; Brain nuclei; Rat

1. Introduction

Until recently, the beneficial effects of angiotensin converting enzyme (ACE) inhibitors in hypertension and heart failure were thought to be mainly due to their effects on the circulating and local renin–angiotensin systems. However, the role of the central actions of ACE inhibitors on their therapeutic efficacy is less well studied. All the components of the renin–angiotensin system have been identified not only in tissues such as the heart, kidney and vasculature, but also in the brain, all indicating a central
neuropeptide system which appears to be regulated independently from the circulating renin–angiotensin system [1–4].

ACE in the brain has been shown to be involved in a variety of physiological functions via generation of angiotensin II. After central application, ACE inhibitors attenuate the pressor responses to centrally administered angiotensin I [5]. Similarly, ACE inhibition reduces thirst stimulated by water deprivation, hypovolaemia and central administration of renin and angiotensin I [6,7].

One of the important functions of the central renin–angiotensin system may be the modulation of vasopressin synthesis and secretion. ACE, angiotensin II receptors and renin were found to be highly concentrated in the vasopressin-synthesising hypothalamic nuclei, especially in the paraventricular and supraoptic nuclei [1]. The highest concentrations of angiotensin-II-immunoreactive cell bodies were found in magnocellular neurones of the paraventricular nucleus which are also primary sites of vasopressin synthesis. It has been demonstrated that angiotensin II influences the release of hypothalamic hormones, particularly vasopressin and oxytocin [8]. In rats, ACE inhibition by captopril has a dipsogenic effect and decreases urinary excretion of vasopressin, suggesting suppression of vasopressin release [9]. These data suggest that central ACE might be involved in controlling local angiotensin II content, thereby modulating pituitary hormone release.

In addition to its importance as a neurohormone secreted into the bloodstream, vasopressin in the brain may play also an important role in cardiovascular regulation through central mechanisms by acting as a neurotransmitter and modulator. Microapplication of vasopressin to specific brain areas, as well as intrathecal or intracerebroventricular injections, increases heart rate and blood pressure significantly. These effects are reversed by central, but not peripheral, pretreatment with specific vasopressin receptor antagonists [10–12]. However, it is not known whether these central cardiovascular effects of vasopressin are also modulated by the central renin–angiotensin system.

To test the hypothesis that ACE inhibition may modulate the central vasopressin system, we measured the vasopressin content of specific microdissected brain areas in rats chronically treated with the ACE inhibitor, quinapril. Furthermore, ACE in various brain areas was determined by in vitro autoradiography to assess the local inhibition of angiotensin converting enzyme in the brain after chronic treatment with quinapril.

2. Methods

2.1. Study protocol

Male albino Wistar rats (n = 36) with an average weight of 185 [14] g (Charles River Wiga Inc., Sulzfeld, Germany) were individually housed under standard laboratory conditions. They were allowed free access to a standard diet (Fa. Alma, Kempen, Germany) and tap water. Fourteen untreated animals served as controls, while 22 rats received quinapril (6 mg · kg⁻¹ · BW) per gavage daily for a period of 6 weeks. Body weight as well as water consumption were monitored daily. At the end of the experiment, all rats were killed by decapitation and trunk blood was immediately collected for determination of plasma vasopressin concentration as well as serum ACE activity. The brains were quickly removed from the cranium and snap-frozen in isopentane at –40°C.

2.2. Determination of vasopressin in brain areas

For determination of the vasopressin content in discrete brain areas, brains of 15 quinapril-treated rats and 10 untreated rats were cut into 300-μm-thick serial coronal sections in a cryostat at –20°C. Nineteen specific brain nuclei corresponding to functional systems involved in cardiovascular regulation and water and electrolyte balance were obtained by the micropunch technique [13]: supraoptic nucleus (SON), suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), organum vasculosum of laminae terminalis (OVLT), bed nucleus of the stria terminalis (NIST), subfornical organ, periventricular nucleus, central amygdala, median eminence, perifornical nucleus, parafascicular nucleus, subcommissural organ, central gray, dorsal raphe nucleus, locus coeruleus, parabrachial nucleus, ventrolateral medulla, and nucleus of the solitary tract (NTS). Vasopressin concentration was measured by radioimmunoassay (RIA) without extraction as described previously [14–16]. For this purpose, punched tissue was placed in 0.1 N HCl and sonicated. Duplicated aliquots were removed from the brain samples for determination of protein content based on the method of Lowry [17], then samples were stored (–80°C) until assay. Brain samples were centrifuged for 10 min at low temperatures to remove debris, and the supernatant appropriately diluted in assay buffer so that values fell on the standard curve of the vasopressin assay. Vasopressin content is expressed as pg · mg⁻¹ protein.

2.3. In vitro autoradiography

To assess the local inhibition by quinapril, ACE was determined in several brain areas of 7 quinapril-treated rats and 4 untreated rats by in vitro autoradiography as described previously [18]. Briefly, 20 μm coronal sections from the brain were cut in a cryostat at –20°C, thaw-mounted onto gelatine-coated slides, and dried in a desicator at 4°C. Sections were incubated in a buffer containing 0.3 μCi/ml 125I-351A, a radiolabeled derivative of lisinopril, for 1 h at 20°C, washed in buffer at 0°C, dried under cold air and exposed to X-ray film. Non-specific binding was determined in parallel incubations containing
1 mM EDTA and was less than 2% of specific binding. Optical density of autoradiographs was quantified by laser densitometry and calibrated by fitting curves with a computer using standards carried through the above procedure.

2.4. Hormone measurements

Serum angiotensin converting enzyme activity was determined by a radioenzymatic assay using a commercially available kit (Ventrex Lab. Inc., Portland, OR, USA), and plasma vasopressin concentration was measured by RIA after extraction with a Sep-Pak C\textsubscript{18} cartridge (Waters Associates, Milford, MA, USA) as previously described in detail [19].

2.5. Blood pressure measurement

Systolic arterial blood pressure was measured in an additional group of 5 conscious rats by tail-cuff sphygmonanometer using an automated cuff inflator–pulse detection system (BP recorder no. 8005, W + W Electronic AG, Basel, Switzerland). The blood pressure value for each rat was calculated as the average of 3 separate measurements taken before and 6 h after oral treatment with quinapril.

All procedures performed were in accordance with national animal regulations and were approved by an independent ethical committee as well as the domestic regulatory authorities.

2.6. Statistical analysis

All results are expressed as means (s.e.m.). Statistical analysis was performed using the Mann-Whitney U-Wilcoxon rank sum W-test for unpaired samples. Statistical significance was accepted at \( P < 0.05 \).

3. Results

In quinapril-treated rats, plasma vasopressin concentration and serum ACE activity were significantly reduced (Fig. 1). There was no difference in body weight or water consumption between rats treated with the ACE inhibitor and controls throughout the experimental period (data not shown). Quinapril produced a significant reduction in blood pressure measured 6 h after oral administration (114[2] vs. 129[1] mmHg in controls, \( P < 0.05 \)), whereas heart rate was unchanged (392[7] vs. 386[1] \( \text{beats min}^{-1} \)).

In control rats, ACE in the brain was widely distributed and detectable in various brain regions by in vitro autoradiography (e.g., in the plexus choroideus, in thalamic and hypothalamic areas, and in circumventricular organs [OVLT]). After chronic quinapril treatment, ACE was significantly reduced in the thalamus, hypothalamus, cerebellum, locus coeruleus, OVLT and choroid plexus (Figs. 2 and 3). There was no significant inhibition in the central gray (69.4[31.6] vs. 79.4[25.3] dpm \( \text{min}^{-1} \text{mm}^{-2} \) in controls), substantia nigra (117.4[39.5] vs. 206.1[73.6] dpm \( \text{mm}^{-2} \); \( P = 0.07 \)), dorsal raphe (40.0[4.6] vs. 54.4[5.5] dpm \( \text{mm}^{-2} \)) and the subfornical organ (291.5[28.9] vs. 322.2[39.8] dpm \( \text{mm}^{-2} \)).

Vasopressin content in specific brain areas of rats treated with quinapril is summarised in Figs. 4 and 5, and Table 1. The highest vasopressin content was found in the SON, PVN, SCN and in the median eminence. The lowest vasopressin content was measured in the frontal cortex, an area which is known for only moderate vasopressin concentration and, therefore, may serve as a control value for measurements of vasopressin in specific brain areas. Vasopressin in rats treated with quinapril was significantly reduced in 9 of the 19 brain areas investigated. Regarding the hypothalamic nuclei, vasopressin concentration was significantly reduced in the SON and PVN, but not in the SCN (Fig. 4). Vasopressin content was significantly reduced in the median eminence and in the so-called circumventricular organs (subcommisural organ and OVLT).

![Fig. 1. Plasma concentrations of vasopressin (AVP) and serum activities of angiotensin converting enzyme (nmol·ml\(^{-1}·\text{min}^{-1}\) ACE (U)) in control rats (\( n = 10 \)) and in rats treated with quinapril (6 mg/kg BW for 6 weeks per gavage; \( n = 15 \)). * \( P < 0.05 \); ** \( P < 0.001 \).](image1)

![Fig. 2. Density of angiotensin converting enzyme (ACE) in various brain regions of rats following oral treatment with quinapril for 6 weeks (\( n = 7 \)) expressed as percentage of untreated controls (\( n = 4 \)). * \( P < 0.05 \); ** \( P < 0.001 \).](image2)
Fig. 3. Pseudocolour, computer-generated images of angiotensin converting enzyme ACE in a coronal section at the level of the rostral hypothalamus of a rat brain in vitro autoradiography. Different colours represent different ACE densities. Red, 5100 dpm mm\(^{-2}\) (high density); yellow, 565 dpm mm\(^{-2}\) (moderate); green, 170 dpm mm\(^{-2}\) (low); blue, < 55 dpm mm\(^{-2}\) (undetectable). After chronic treatment with quinapril (lower panel) ACE densities are significantly lower in various brain regions as compared with control rats (upper panel). CPU = putamen; ChP = plexus choroides; POR = preoptic region including hypothalamic brain areas such as the paraventricular nucleus.

Fig. 4. Vasopressin content (pg AVP mg protein\(^{-1}\)) in discrete hypothalamic brain nuclei in control rats and in rats treated with quinapril (6 mg kg\(^{-1}\) for 6 weeks per gavage). \(^*\) P < 0.05; \(^*\)* P < 0.001. ME = median eminence; PBN = parabrachial nucleus; CG = central gray; DR = dorsal raphe; LC = locus coeruleus; SCO = subcommissural organ; OVLT = organum vasculosum laminae terminalis.

whereas the changes in the subfornical organ were not significant. Decreased vasopressin levels were also found in other brain structures which are innervated by vasopressinergic fibres from the hypothalamus. Specifically, a significant reduction in vasopressin content was observed in the central gray, dorsal raphe, and the parabrachial nucleus. In addition, vasopressin concentration was significantly reduced in the locus coeruleus, which is involved in the regulation of sympathetic activity (Fig. 5).

Vasopressin content was not significantly changed in the hypothalamic periventricular nucleus, which topographically forms the so-called anteroventral third ventricle (AV3V) region together with the OVLT and the preoptic periventricular nucleus. Vasopressin levels were unaltered in the other brain areas investigated, which include the NTS, ventrolateral medulla, central amygdaloid nucleus, parafascicular nucleus, perifornical nucleus, NIST and the frontal cortex (Table 1).

Table 1
Vasopressin content (pg AVP mg protein\(^{-1}\)) in discrete brain areas of rats treated with quinapril and untreated rats (CTRL) in which no significant changes in vasopressin content were observed

<table>
<thead>
<tr>
<th>Region</th>
<th>CTRL</th>
<th>Quinapril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subfornical organ</td>
<td>18.5 [5.5]</td>
<td>12.5 [2.1]</td>
</tr>
<tr>
<td>Periventricular nucleus</td>
<td>363.6 [88.2]</td>
<td>512.9 [117.2]</td>
</tr>
<tr>
<td>Nucleus of the solitary tract</td>
<td>14.4 [9.4]</td>
<td>7.1 [2.0]</td>
</tr>
<tr>
<td>Ventrolateral medulla</td>
<td>7.6 [3.1]</td>
<td>9.0 [3.8]</td>
</tr>
<tr>
<td>Central amygdaloid nucleus</td>
<td>119.0 [32.2]</td>
<td>55.6 [14.7]</td>
</tr>
<tr>
<td>Parafascicular nucleus</td>
<td>35.8 [9.9]</td>
<td>45.7 [11.4]</td>
</tr>
<tr>
<td>Perifornical nucleus</td>
<td>122.2 [32.7]</td>
<td>65.4 [16.4]</td>
</tr>
<tr>
<td>Bed nucleus of the stria terminalis</td>
<td>6.9 [1.5]</td>
<td>7.3 [1.6]</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>0.6 [0.2]</td>
<td>1.0 [0.3]</td>
</tr>
</tbody>
</table>

Values are means [s.e.m.].
4. Discussion

A prerequisite for the hypothesised central action of quinapril is the demonstration of an inhibition of central ACE after chronic oral administration. In agreement with previous investigations [18], in vitro autoradiography revealed an extensive distribution of angiotensin converting enzyme in the brains of normal rats. Following 6 weeks of oral treatment with quinapril, serum ACE activity was markedly reduced, demonstrating effective suppression of circulating ACE. In parallel, ACE density was significantly lowered in various brain regions. Since the choroid plexus and OVLT are deficient in the blood–brain barrier, the reduction in ACE density in these areas may be due to local inhibition of ACE, but might also reflect the suppression of circulating ACE. However, the thalamus, hypothalamus, cerebellum and locus coeruleus are all areas within the brain and the blood–brain barrier. Thus, the reduction in ACE density in these regions indicates central action of the ACE inhibitor with chronic oral treatment, suggesting penetration of the blood–brain barrier.

Central inhibition of ACE following a single dose of captopril and enalapril has been previously demonstrated in the brain cortex of spontaneously hypertensive rats (SHR) [20]. After long-term administration enalapril, ramipril, and perindopril significantly suppressed ACE in the cerebral cortex of SHR, while only the ACE inhibitor, ramipril, was able to reduce the activity of the enzyme in other brain areas [21,22]. Pre-treatment with ramipril and SA446 suppressed pressor responses to intracerebroventricular application of angiotensin I, while these responses were unchanged in captopril- and enalapril-treated animals [22,23]. The differences among the various ACE inhibitors with respect to their inhibitory action on brain ACE may, at least in part, be explained by their differences in lipid solubility, which in turn largely determines the ability to penetrate the blood–brain barrier. In comparison with 8 other ACE inhibitors, quinapril and trandolapril were shown to be the most lipophilic ACE inhibitors [24]. In a previous study using a single oral dose, quinapril (0.1 mg·kg$^{-1}$) inhibited ACE only in brain areas lacking the blood–brain barrier, but not in structures within the brain [25]. This discrepancy with our findings may be explained by the differences in dosage and duration of administration. Similarly, only high, but not low, doses of perindopril have been shown to suppress ACE in structures within the brain [26]. Therefore, while single low doses result in ACE inhibitor effects which appear to be restricted predominantly to areas outside the blood–brain barrier, high doses or chronic administration may enable at least some compounds, including quinapril, to penetrate the blood–brain barrier and to act on ACE within the brain.

The biological effectiveness of the quinapril dose chosen (6 mg·kg$^{-1}$) was demonstrated by the significant decrease in blood pressure. This effect, however, was well below maximal antihypertensive effects of very high doses (400 mg·kg$^{-1}$) as reported in normotensive rats [27], making non-specific effects unlikely.

In control rats, the relative abundance and distribution of vasopressin in the specific brain areas investigated is in agreement with previous studies using RIA [28,29]. The discrepancy of absolute values may be attributed to different dissection and tissue extraction techniques, as well as differences in the RIA procedure. The discrete brain regions in which vasopressin content was determined do not exactly correspond to the regions in which ACE density was measured. This is due to the fact that ACE was determined by in vitro autoradiography of whole brain slices. Thus, larger brain regions involving several nuclei were analysed for ACE density, rather than single nuclei, in order to demonstrate effectiveness of ACE inhibition in the brain by oral administration.

Oral treatment with quinapril for 6 weeks significantly decreased vasopressin content in specific brain regions. With respect to the hypothalamic nuclei, vasopressin was lowered in the PVN and SON, which are important sites of vasopressin synthesis [30]. In these hypothalamic brain nuclei a high concentration of ACE, angiotensin II receptors and renin has been demonstrated [31]. In addition to this anatomical association, in vivo and in vitro studies have shown a stimulation of vasopressin release by angiotensin II [32,33]. Therefore, central inhibition of vasopressin synthesis and/or release by the ACE inhibitor may be involved in the observed decrease in plasma vasopressin levels.

Vasopressin content was significantly reduced in the OVLT and the subcommissural organ. These two regions receive vasopressinergic fibres from the hypothalamus and are part of the circumventricular organs which are located outside the blood–brain barrier. Therefore, it is not clear whether the decrease in vasopressin is due to a decrease in neuronal vasopressin content or if this mainly reflects the lower vasopressin concentration in plasma. Vasopressin content was decreased in the parabrachial nucleus, central gray and the dorsal raphe nucleus, which are also part of the central vasopressin system with vasopressinergic innervation from hypothalamic nuclei. Their exact role, however, is not known. Since the density of ACE was not changed in the central gray and dorsal raphe nuclei after quinapril treatment, the decreases in vasopressin may indicate modulation due to altered input from the hypothalamic nuclei after quinapril treatment. Furthermore, we observed a significant decline in vasopressin concentration in the locus coeruleus (LC). Noradrenergic neurons in the LC receive vasopressinergic innervation from the hypothalamic paraventricular nucleus and, in turn, catecholaminergic efferents originating in the LC project to the supraoptic and paraventricular nuclei which contain vasopressinergic cell bodies [30]. Microinjection of vasopressin into the LC produces increases in arterial pressure and heart rate transmitted by enhanced sympathetic outflow [34]. This indicates the role of vasopressin in central cardiovascular
control in this region which might therefore be modulated by central ACE inhibition.

The most likely mechanism of the observed effects of ACE inhibition on central vasopressin is central blockage of angiotensin II formation. It appears that the receptors involved in the influence of angiotensin II on central vasopressin are of AT_1 subtype. This is suggested by the finding that vasopressin release is attenuated by pretreatment with intracerebroventricular losartan—a selective AT_1 receptor subtype 1 antagonist [35]. Furthermore, AT_1 receptor subtypes are associated with brain regions that control cardiovascular function, including the hypothalamic areas, median eminence and OVLT—i.e., areas in which we found a significant reduction in vasopressin after ACE inhibition. In contrast, AT_2 receptor subtypes have been detected in areas not involved in cardiovascular effects [31]. In addition to direct angiotensin II effects, a catecholaminergic pathway may be involved in central angiotensin II stimulation of vasopressin. Using the brain microdialysis technique, vasopressin release in the PVN and SON was shown to be mediated by an angiotensin-II-stimulated release of norepinephrine and activation of α₁-receptors in these nuclei [36].

Mechanisms other than blockade of angiotensin II, such as enhanced kinin effects, may have contributed to the observed effects of ACE inhibition on central vasopressin. In the SHR a hypotensive response to intracerebroventricular injection of captopril was completely blocked by simultaneous administration of a kinin receptor antagonist, suggesting that endogenous brain kinins may contribute to lowering of blood pressure by central ACE inhibition [37]. However, the influence of kinins, especially bradykinin or bradykinin antagonists, on central vasopressin has not yet been studied.

Central effects are also produced by the heptapeptide angiotensin-(1–7) (Ang(1–7)), which can be directly formed from angiotensin I independently from ACE. Ang(1–7) is as potent as angiotensin II for releasing vasopressin in hypothalmo-hypophysial explants [38]. Treatment of SHR rats with lisinopril results in an increase in plasma Ang(1–7) accompanied by a decrease in plasma vasopressin and blood pressure. However, these effects were observed in the SHR, but not in the normotensive rat [39]. Finally, effects of ACE inhibition on other neuropeptides, such as substance P, enkephalin, neurotensin, β-endorphin, dynorphin and luteinizing hormone-releasing hormone, have been reported [40]. Further studies need to clarify the exact mechanism by which ACE inhibition in the brain influences central vasopressin.

There is growing evidence that, in addition to its peripheral actions, vasopressin may also play an important role in central cardiovascular regulation via neuroregulatory and modulatory properties [41,42]. Extensive projections of vasopressinergic neurons have been shown to extend from the hypothalamus to various brain regions which participate in central cardiovascular regulation. Micropipette of picomolar quantities of vasopressin to such brain areas, as well as intrathecal or intracerebroventricular injections, produce significant increases in heart rate and blood pressure which are attenuated by central, but not peripheral, pretreatment with specific vasopressin receptor antagonists [10–12]. The results of our study support the hypothesis that chronic ACE inhibition may influence cardiovascular control not only by modulation of hypothalamic vasopressin synthesis or pituitary release of vasopressin into the bloodstream, but also by influencing the modulatory role of vasopressin in central cardiovascular regulation within the brain.

In conclusion, chronic oral treatment with quinapril in rats reduces central ACE and vasopressin content in various brain nuclei involved in central cardiovascular control. These findings are a prerequisite for a possible involvement of such a central mechanism in the therapeutic efficacy of ACE inhibitors. Further studies investigating the functional consequences of central vasopressin modulation of the antihypertensive action of ACE inhibitors are warranted based on our findings.

Acknowledgements

Research carried out in the authors’ laboratory was supported by a grant from the Deutsche Forschungsgemeinschaft (Mu 1140/4-1).

References

[9] Crofton JT, Share L, Horowitz ZP. The effects of SQ14225 on


