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Involvement of angiotensin-(1–7) in the hypothalamic hypotensive effect of captopril in sinoaortic denervated rats

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Abstract

The role of anterior hypothalamic angiotensin-(1–7) (Ang-(1–7)) on blood pressure regulation was studied in sinoaortic denervated (SAD) rats. Since angiotensin-converting enzyme inhibitors increase endogenous levels of Ang-(1–7), we addressed the involvement of Ang-(1–7) in the hypotensive effect induced by captopril in SAD rats. Wistar rats 7 days after SAD or sham operation (SO) were anaesthetized and the carotid artery was cannulated for monitoring mean arterial pressure (MAP). A needle was inserted into the anterior hypothalamus for drug administration. Intrahypothalamic administration of Ang-(1–7) (5 pmol) was without effect in SO rats but reduced MAP in SAD rats by 15.5±3.2 mm Hg and this effect was blocked by 250 pmol [D-Ala^7]-Ang-(1–7), a Mas receptor antagonist. Angiotensin II (Ang II) induced an increase in MAP in both groups being the effect greater in SAD rats (ΔMAP=15.8±1.4 mm Hg) than in SO rats (ΔMAP=9.6±1.0 mm Hg). Ang-(1–7) partially abolished the pressor response caused by Ang II in SAD rats. Whilst the captopril intrahypothalamic injection did not affect MAP in SO animals, it significantly reduced MAP in SAD rats (ΔMAP=−13.3±1.9 mm Hg). Either [D-Ala^7]-Ang-(1–7) or an anti-Ang-(1–7) polyclonal antibody partially blocked the MAP reduction caused by captopril. In conclusion, whilst Ang-(1–7) does not contribute to hypothalamic blood pressure regulation in SO normotensive animals, in SAD rats the heptapeptide induces a reduction of blood pressure mediated by Mas receptor activation. Although Ang-(1–7) is not formed in enough amount in the AHA of SAD animals to exert cardiovascular effects in normal conditions, our results suggest that enhancement of hypothalamic Ang-(1–7) levels by administration of captopril is partially involved in the hypotensive effect of the ACE inhibitor.

Keywords: Angiotensin-(1–7); Angiotensin II; Sinoaortic denervation; Anterior hypothalamus; Angiotensin-converting enzyme inhibitor; Antihypertensive effect

1. Introduction

Most forms of experimental hypertension are associated with a wide range of functional changes in the hypothalamus [1]. Hypothalamic angiotensin II (Ang II) receptors are involved in the maintenance of the hypertensive stage in different experimental models, including spontaneously hypertensive rats (SHR) [2,3], Dahl-S rats [4], aortic coarctated rats [5,6], renal hypertension [7] and DOCA-salt hypertension [8].

In the last decade, new biologically active components of the renin–angiotensin system (RAS) were found. Angiotensin-(1–7) (Ang-(1–7)), a metabolite of angiotensin I and Ang II, is considered the most pleiotropic component of RAS [9–11]. Ang-(1–7) lacks the pressor, dipsogenic and aldosterone secretory actions elicited by Ang II, and, conversely to the octapeptide, systemic administration of Ang-(1–7) induces natriuresis, diuresis and vasodilation [9–11]. However, this heptapeptide increases vasopressin release, prostaglandin synthesis and facilitates peripheral noradrenergic neurotransmission in the same manner as Ang II [9–11].

In the central nervous system, Ang-(1–7) is mainly generated in central nuclei related to blood pressure regulation, such

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as medullary areas and the hypothalamus [9,10,12]. In normotensive rats, intracerebroventricular administration of the peptide does not modify blood pressure or heart rate, although it facilitates baroreceptor reflex control of heart rate [13]. Conversely, Ang-(1–7) induces a depressor response in situations involving overactivity of Ang II. For instance, cerebroventricular infusion of Ang-(1–7) caused a significant decrease in blood pressure and injections of a specific Ang-(1–7) monoclonal antibody increased blood pressure and heart rate in [mRen-2]27 transgenic hypertensive (Tg+) rats but not in normotensive rats [14,15]. In a previous study [2], we found that Ang-(1–7) blocked the pressor response to intrahypothalamic administration of Ang II in SHR. Regarding the mechanisms involved in central actions of Ang-(1–7), we have demonstrated that the heptapeptide does not only inhibit the release of noradrenaline [16] but also the Ang II-stimulated noradrenaline outflow in hypothalamic nuclei from hypertensive rats [17].

Several reports have described that angiotensin-converting enzyme (ACE) inhibitors, such as captopril, increase endogenous levels of Ang-(1–7) [18–23]. Considering the physiological actions of Ang-(1–7), enhancement of the heptapeptide levels by ACE inhibition may participate in the beneficial effects of these therapeutic agents, including their systemic antithrombotic [18], baroreflex improvement [19] and hypotensive effect [20–23].

The sinoaortic denervated (SAD) rat is an experimental model of cardiovascular disease characterized by an increase in blood pressure variability rather than a sustained hypertensive stage [24,25]. In the last years, increased cardiovascular risk was found in patients with blood pressure variability [26]. Among the different mechanisms involved in high blood pressure variability-induced end-organ damage, activation of RAS plays an important role [27]. However, little is known regarding the role of central RAS in SAD rats. Paul et al. have found that SAD rats exhibit enhanced sensitivity to the central pressor effects of Ang II [28].

Accordingly, the aim of the present work was to investigate the role of Ang-(1–7) in hypothalamic control of blood pressure in SAD rats, and to address the involvement of this heptapeptide in the hypotensive effect of intrahypothalamic perfusion of captopril in this experimental model of cardiovascular disease.

2. Materials and methods

Male Wistar rats weighing 200–250 g were used. Animal experiments were performed in accordance with the *Principles of Laboratory Animal Care* (NIH publication no. 85–23, revised 1985).

SAD was performed following the method described by Krieger [29] in rats anaesthetized with chloral hydrate (250 mg kg^{-1}, i.p.). Control rats were sham operated (SO). Experiments were carried out 7 days after the corresponding surgery.

On the day of the experiment, rats were anaesthetized with a mix of chloralose (50 mg kg^{-1}, i.p.) and urethane (500 mg kg^{-1}, i.p.). The left carotid artery was cannulated and connected to a Statham P23ID pressure transducer (Gould Instruments, Cleveland, OH, USA) coupled to a Grass 79D polygraph (Grass Instrument Co., Quincy, MA, USA). Mean arterial pressure (MAP) was calculated according to the formula diastolic pressure + (systolic pressure − diastolic pressure)/3. The heart rate (HR) was calculated tachographically by counting the pulsatile waves of the arterial pressure recording. A stainless-steel needle (32 G) was inserted into the anterior hypothalamic area (AHA) according to the stereotaxic coordinates A/P − 1.5 mm, L/M − 0.6 mm, V/D − 8.5 mm, from the bregma [30]. All drugs were dissolved in Ringer solution and 0.5 μL solution was injected at a rate of 1 μL/min in the AHA.

Although knowing the dose–response effects of angiotensin peptides on hypothalamic blood pressure regulation is desirable, performing this task would have involved a large number of animals. Thus, since the main purpose of the present study was to determine the effect of angiotensin peptides on blood pressure regulation in SO and SAD rats and the involvement of angiotensin receptors in their actions, we carried out our study with doses of Ang II, Ang-(1–7), captopril and irbesartan reported to exert clear central cardiovascular effects [2,5,6,31,32].

A stabilization period of 30 min preceded drug administration, and changes in blood pressure were determined. As control of intrahypothalamic administration, the cardiovascular effects of Ringer solution administration ($n=5$) were recorded in SO and SAD animals. The following protocols were carried out.

2.1. Effects of intrahypothalamic administration of angiotensin-(1–7) on blood pressure

In the first set of experiments, the cardiovascular effects of intrahypothalamic administration of 1 pmol Ang-(1–7) ($n=5$), 5 pmol Ang-(1–7) ($n=5$) or 50 pmol Ang-(1–7) ($n=5$) were determined in SO and SAD rats.

In another set of SAD rats ($n=5$), the cardiovascular effects of the co-administration of 250 pmol [D-Ala^7]-Ang-(1–7) (Mas receptor antagonist) with 5 pmol Ang-(1–7) ($n=5$) or 50 pmol Ang-(1–7) (n=5) were investigated. It is important to mention that in this protocol, as described in a previous work [2], we decided to administer [D-Ala^7]-Ang-(1–7) concomitantly with Ang-(1–7) (and not prior to Ang-(1–7) administration), based on the expected rapid degradation of the antagonist due to its peptidergic nature.

The doses of [D-Ala^7]-Ang-(1–7) tested in our assay are in accordance with those reported from previous studies by us and other authors [2,14,33].

Finally, in a third experiment ($n=5$), 500 pmol irbesartan (AT_1 receptor antagonist) was administered in the AHA of SAD animals followed by the administration of 50 pmol Ang-(1–7) 5 min after the antagonist administration. Conversely to the other protocols, irbesartan was applied 5 min before 50 pmol Ang-(1–7) administration, considering that intrahypothalamic administration of irbesartan induced a hypotensive effect reaching maximal effect 5 min after drug application [2]. In addition, irbesartan shows a partial insurmountable antagonism at AT_1 receptors and therefore its hypothalamic hypotensive effect is maintained during our protocol [34].