Behavioural Brain Research 256 (2013) 537–544

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Effects of angiotensin (5-8) microinfusions into the ventrolateral periaqueductal gray on defensive behaviors in rats

Karina Genaro[a,b,c,*], Maria A. Juliano[d], Wiliam A. Prado[b], Marcus L. Brandão[c,e], Antônio R. Martins[a,b]

[a] Universidade Federal do Triângulo Mineiro, Instituto de Ciências Biológicas, Uberaba, MG, Brazil
[b] Universidade de São Paulo, Departamento de Farmacologia, Ribeirão Preto, SP, Brazil
[c] Instituto de Neurociências e Comportamento, INeC, Ribeirão Preto, SP, Brazil
[d] Universidade Federal de São Paulo – Escola Paulista de Medicina, Departamento de Biofísica, São Paulo, SP, Brazil
[e] Universidade de São Paulo, Departamento de Psicologia, Ribeirão Preto, SP, Brazil

HIGHLIGHTS

- Ang (5-8) is the smallest biologically active Ang peptide.
- Ang (5-8) modulate distinct defensive behaviors into the vlPAG.
- Ang (5-8) effects in the vlPAG seem to require the presence of aversive stimuli.

ARTICLE INFO

Article history:
Received 17 July 2013
Received in revised form 6 September 2013
Accepted 10 September 2013
Available online 13 September 2013

Keywords:
Angiotensin (5-8)
Periaqueductal gray
Defensive behavior
Contextual fear
Anxiety

ABSTRACT

Peptides of the renin–angiotensin system modulate blood pressure and hydro-electrolyte composition. Angiotensin (Ang) receptors are localized in brain areas related to the regulation of autonomic and endocrine control and involved in sensory perception, memory process and behavioral responses. Among these areas, the ventrolateral periaqueductal gray (vlPAG) is one of the most important structures of the neuronal circuitry controlling the autonomic and behavioral components of emotional states. Although Ang II metabolism in the vlPAG forms several Ang-peptides including Ang (5-8), the role of this tetrapeptide in the organization of defensive responses has not yet been described. To address this issue, the purpose of the present study was to determine the effects of intra-vlPAG injections of Ang (5-8) (0.2, 0.4 and 0.8 nmol/0.25 μL) in rats submitted to the elevated plus-maze (EPM) test. Additionally, it was evaluated the effects of intra-vlPAG Ang (5-8) on the expression of conditioned fear, assessed by the fear-potentiated startle and contextual conditioned freezing tests. The results showed that Ang (5-8) produced an intense, dose-related reduction in the entries into and time spent in the open arms of the EPM, decreased direct exploration and increased risk assessment behaviors. Moreover, intra-vlPAG injections of Ang (5-8) before the test session promoted pro-aversive effects in the FPS and enhanced contextual freezing. Taken together, these results point out to an important anxiogenic-like action for Ang (5-8) in the mediation of defensive behaviors organized in the vlPAG.

1. Introduction

The brain renin–angiotensin system (RAS) comprises all necessary precursors and enzymes required for the formation and metabolism of the biologically active forms of angiotensin (Ang). Renin converts the hormonal substrate angiotensinogen to the decapeptide Ang I, which in turn is converted into Ang II by the Ang-converting enzyme [1–4]. This cascade is far from being a simple linear system for the production of Ang II – the main effector peptide involved in the blood pressure regulation and hydroelectrolytic homeostasis. Actually, other metabolites of Ang I and Ang II also can exert their own biological activity. These peptides include Ang III [5], Ang IV [6], Ang (1-7) [7] and Ang (5-8) [8], and distinct enzymatic pathways for the synthesis of these neuropeptides have been proposed [9–12].

These angiotensins are pharmacologically active, with markedly different potencies and effects, which has indicated the existence of multiple Ang receptors. It has been proposed that the physiological actions of Ang peptides are mediated via a number of specific receptors: AT1, AT2, AT4 and Mas receptors [for review see: 13–15].

* Corresponding author at: Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14040-901 Ribeirão Preto, SP, Brazil. Tel.: +55 16 36023837; fax: +55 16 36024830.
E-mail addresses: karinagb@usp.br, karinagenaro@yahoo.com.br (K. Genaro).

0166-4328/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2013.09.023

These receptors are localized not only in structures that control blood pressure but also in brain regions involved in the sensory perception, the regulation of stress response and multiple aspects of behavior and cognition [4,16–20].

Substantial evidence indicates the involvement of the brain RAS in different anxiety-related behaviors [4,20,21]. Ang II increases the activity of the hypothalamus–pituitary–adrenal axis and enhances stress responses and anxiety [22,23]. Furthermore, Ang receptors are expressed in the stress–sensitive brain structures including the hypothalamus, amygdala and periaqueductal gray (PAG) [24–26]. These structures are classically considered as components of the brain aversion system and seem to be responsible for the organization of fear and anxiety-like behaviors [27–30].

The PAG is an important neural region for numerous physiological functions including cardiovascular regulation, pain modulation and defensive behavior [31–34]. Considering the well-defined columnar organization of the PAG, the ventrolateral PAG (vlPAG) gives rise to several major efferent pathways connecting the PAG to other nuclei in the brainstem and structures of the brain aversion system [35–39]. Ang II injected into the dorsal column of the PAG of rats increases blood pressure, an effect predominantly mediated by AT1 receptors. Additionally, renin substrate, Angs I, II and III modulate nociception in the vlPAG [40]. These and other evidence suggest that the RAS is engaged in the regulation of the functional activity of the PAG [41,42]. More interestingly, vlPAG can synthesize short chain peptides such as Ang (1-7) and Ang (5-8) from Ang II and Ang III [8,40]. A recent study demonstrated that Ang (5-8) can be synthesized by several enzymatic pathways, from several substrates, and at widely differing rates. Besides, Ang (5-8) degradation can be carried out by several peptidases, among them an amastatin-sensitive aminopeptidase [8].

Previous studies confirmed that the RAS is implicated in anxiety and cognitive processes and reported, particularly, physiological and behavioral effects for Ang (1-7) and Ang II [11,21,43–48]. However, very little is known about the central actions of Ang (5-8). This tetrapeptide has long been considered to represent an inactive degradation product of the Ang II [49]. In fact, Ang (5-8) elicits small or no changes of cardiovascular parameters compared to Ang II [8]. However, a recent report showed that Ang (5-8) is effective in the regulation of cellular proliferation [50]. Importantly, we have previously demonstrated that Ang (5-8) contains the smallest amino acid sequence that is necessary and sufficient to elicit a strong, dose-dependent and region-specific modulation of antinociceptive effect upon injection into the vlPAG [8]. The involvement of Ang receptors on pain modulation elicited by Ang (5-8) was studied using saralasin ([Sar1-Ala8]-Ang II), a non-selective Ang-receptor antagonist.

The vlPAG has been considered necessary for the acquisition of aversive information and expression of the different types of defensive behaviors. Therefore, the purpose of the present study was to evaluate the effects of Ang (5-8) injection into the vlPAG on several anxiety-related behavioral parameters, using the elevated plus maze (EPM) and two paradigms of conditioned fear response, the fear-potentiated startle (FPS) and the freezing, in rats subjected to a contextual fear conditioning.

2. Materials and methods

2.1. Animals

A total of 116 male Wistar rats from the animal house of the University of São Paulo, Ribeirão Preto *campus*, weighing 220–240 g, were used. Rats were housed in groups of four animals per cage with food and water available *ad libitum*, in a temperature-controlled room ($23 \pm 1\,^{\circ}C$) under a 12 h/12 h light/dark cycle (lights on at 7:00 AM). The rats were transported to the experimental room in their home cages and left undisturbed for 1 h prior to testing. All efforts were made to minimize animal suffering and reduce the number of rats used. The experiments reported in this article were performed in accordance with the recommendations of the Brazilian Society for Neuroscience and Behavior and complied with the United States National Institutes of Health Guide for Care and Use of Laboratory Animals. The procedures were approved by the Ethics Committees of the Federal University of Triângulo Mineiro (CEUA No. 181/2010) and Committee for Animal Care and Use, University of São Paulo (CEUA No. 5.1.1209.53.8).

2.2. Surgical procedures

The rats were anesthetized with ketamine/xylazine (100/7.5 mg/kg, i.p.) and fixed in a stereotaxic apparatus (David Kopf, Tujunga, CA, USA). The upper incisor bar was set 3.0 mm below the interaural line so that the skull was horizontal between bregma and lambda. After scalp anesthesia with 2% lidocaine, the skull was surgically exposed, and a stainless-steel guide cannula (12 mm length, 0.6 mm outer diameter, 0.4 mm inner diameter) was unilaterally implanted in the vlPAG, using lambda as the reference point (angle of 18°; coordinates: anterior/posterior, −0.2 mm; medial/lateral, ±2.6 mm; dorsal/ventral: −3.3 mm). The cannula was fixed to the skull with dental cement and two stainless-steel screws. After surgery, each guide cannula was sealed with a stainless steel wire to prevent obstruction. The rats then received an intramuscular injection of penicillin G benzathine (Pentabiotic, 600,000 IU, 0.2 mL; Fort Dodge, Campinas, SP, Brazil). After surgery, the rats were returned to their home cages in groups of four and were allowed to recover over a period of five days.

2.3. Drugs

Ang (5-8) from Peninsula Laboratories LLC, (a member of the Bachem Group, San Carlos, CA, USA) was dissolved in 0.1 M phosphate-buffered saline (PBS) to achieve a final concentrations of 0.2, 0.4 and 0.8 nmol/0.25 μL. The rats received the injections of PBS or drug before the test session. The doses and time of the injections were based on previous studies [8].

2.4. Microinjection procedure

Drug or vehicle was microinjected into the vlPAG using a glass needle (70–90 mm, outer diameter OD) protected by a system of telescoping steel tubes [51]. A thin needle attached by polyethylene tubing to a 5 μL Hamilton syringe was introduced through the guide cannula. The injection needle extended 3 mm below the ventral tip of the implanted guide cannula. The volume of microinjection was 0.25 μL, delivered at a constant rate over a period of 30 s. The displacement of an air bubble inside the polyethylene tubing that connected the syringe to the injection needle was used to monitor the microinjections. The injection needles were left in place for 30 s after the end of the infusion to allow for diffusion.

2.5. Experiment 1: elevated plus-maze

The first behavioral test was performed on EPM, a classical model of fear/anxiety, for measuring a wide range of unconditioned exploratory behaviors. The EPM was a wooden apparatus with two opposite open arms (50 cm × 10 cm) crossed at a right angle by two arms of the same dimensions enclosed by 40-cm-high walls with no roof. The maze was located 50 cm above the floor, and a raised edge made of transparent Plexiglas (0.5 cm) on the open arms provided additional grip for the rats [52]. All testing was conducted during the morning between 9:00 and 11:00 AM. The apparatus