Neuroscience 231 (2013) 315–327

ANGIOTENSIN (5–8) MODULATES NOCICEPTION AT THE RAT PERIAQUEDUCTAL GRAY VIA THE NO–sGC PATHWAY AND AN ENDOGENOUS OPIOID

L. M. GUETHE, [a,d] **A. PELEGRINI-DA-SILVA,** [c] **K. G. BORELLI,** [b,d] **M. A. JULIANO,** [e] **G. G. PELOSI,** [c,h] **J. B. PESQUERO,** [e] **C. L. M. SILVA,** [f] **F. M. A. CORRÊA,** [c] **F. MURAD,** [g] **W. A. PRADO** [c] **AND A. R. MARTINS** [c,d]*

[a] *Department of Psychology, FFCLRP University of São Paulo, Ribeirão Preto 14049-901, SP, Brazil*

[b] *Institute for Neurosciences and Behavior (INeC), University of São Paulo, Ribeirão Preto 14049-901, SP, Brazil*

[c] *Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto 14049-900, SP, Brazil*

[d] *Institute of Biological Sciences, Federal University of Triângulo Mineiro, Uberaba 38025-015, MG, Brazil*

[e] *Department of Biophysics, Escola Paulista de Medicina-UNIFESP, São Paulo 04044-020, SP, Brazil*

[f] *Cellular and Molecular Pharmacology Research Program, ICB, Federal University of Rio de Janeiro, Rio de Janeiro 21941-599, RJ, Brazil*

[g] *Department of Biochemistry and Molecular Biology, George Washington University, Washington, DC 20037, USA*

[h] *Department of Physiological Sciences, Center of Biological Sciences, State University of Londrina, Londrina 86055-900, PR, Brazil*

Abstract—Angiotensins (Angs) modulate blood pressure, hydro-electrolyte composition, and antinociception. Although Ang (5–8) has generally been considered to be inactive, we show here that Ang (5–8) was the smallest Ang to elicit dose-dependent responses and receptor-mediated antinociception in the rat ventrolateral periaqueductal gray matter (vlPAG). Ang (5–8) antinociception seems to be selective, because it did not alter blood pressure or act on vascular or intestinal smooth muscle cells. The non-selective Ang-receptor (Ang-R) antagonist saralasin blocked Ang (5–8) antinociception, but selective antagonists of Ang-R types I, II, IV, and Mas did not, suggesting that Ang (5–8) may act via an unknown receptor. Endopeptidase EP 24.11 and amastatin-sensitive aminopeptidase from the vlPAG catalyzed the synthesis (from Ang II or Ang III) and inactivation of Ang (5–8), respectively. Selective inhibitors of neuronal-nitric oxide (NO) synthase, soluble guanylyl cyclase (sGC) and a non-selective opioid receptor (opioid-R) inhibitor blocked Ang (5–8)-induced antinociception. In conclusion, Ang (5–8) is a new member of the Ang family that selectively and strongly modulates antinociception via NO–sGC and endogenous opioid in the vlPAG.

Key words: angiotensin (5–8), antinociception, pain, periaqueductal gray matter, incision allodynia model, tail-flick test.

INTRODUCTION

The brain renin–angiotensin (Ang) system (RAS) participates in cardiovascular and body fluid homeostasis and in neuronal plasticity (Antunes-Rodrigues et al., 2004; von Bohlen und Halbach and Albrecht, 2006; Bader, 2010). The brain RAS can also be involved in metabolic disorders and obesity (Baltatu et al., 2011) as well as in Alzheimer's disease (Wright and Harding, 2010). Evidence for the participation of the RAS in nociception has been based on the administration of Angs II, III and Ang receptor (Ang-R) antagonists into brain ventricles or medullary nuclei (Haulica et al., 1986; Shimamura et al., 1987; Yang et al., 1996; Georgieva and Georgiev, 1999; Marques-Lopes et al., 2009).

Prado et al. (2003) identified the ventrolateral periaqueductal gray matter (vlPAG) as a mesencephalic region where the injection of the tetradecapeptide renin substrate, Angs I, II and III, elicited antinociception in the rat tail-flick model. The injection of Ang II or Ang III into the vlPAG elicits Ang type 1 (AT_1) and type 2 (AT_2) receptor (R)-mediated antinociception that was detected using the tail-flick test. Both Losartan and CGP 42,112A, which are specific AT_1R and AT_2R antagonists (De Gasparo et al., 2000), respectively, blocked the antinociceptive activity (AA) of Ang II and Ang III injected into the vlPAG (Pelegrini-da-Silva et al., 2005, 2009). Each of these antagonists elicits an increase of post-incision allodynia when injected alone into the rat vlPAG, indicating that Ang II and/or Ang III is an endogenous modulator of vlPAG-mediated antinociception. The AA elicited by the injection of Ang II into the vlPAG has been ascribed to a dominant effect of Ang III synthesized from Ang II (Pelegrini-da-Silva et al., 2009).

*Correspondence to: A. R. Martins, Institute of Biological Sciences, Federal University of Triângulo Mineiro (UFTM), Praça Manoel Terra, 330 – Abadia, Uberaba 38025-015, MG, Brazil. Tel: +55-1681122411.

E-mail address: armartin@fmrp.usp.br (A. R. Martins).

Abbreviations: AA, antinociceptive activity; Angs, angiotensins; Ang-R, Ang receptor; ANOVA, analysis of variance; AT, Ang type; ATR, Ang type receptor; DMSO, dimethylsulfoxide; HR, heart rate; MAP, mean arterial pressure; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NPLA, N^{ω}-propyl-L-arginine; ODQ, 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one; opioid-R, opioid receptor; PBS, phosphate-buffered saline; RAS, renin–angiotensin system; sGC, soluble guanylyl cyclase; vlPAG, ventrolateral periaqueductal gray matter.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.11.048

The vlPAG can synthesize Ang III, Ang (1–7), Ang IV and Ang (1–4) from Ang II (Pelegrini-da-Silva et al., 2009); however, except for Ang III, the AA of these Ang II derivatives is not yet known. Here, we report a screen for AA of Ang peptides carried out by injecting Ang peptides into the vlPAG and testing antinociception using the tail-flick test. Among the antinociceptive Angs identified, we further investigated Ang (5–8), which has generally been considered to be inactive in biological systems tested to date, except for inhibiting cell proliferation and viability in lactosomatotrophic (GH3) cell culture (Ptasinska-Wnuk et al., 2012). The antinociceptive effect of Ang (5–8) was approached using the tail flick and incision allodynia models, combined with an HPLC time-course of Ang (5–8) metabolism, with and without peptidase, neuronal nitric oxide (nNO) synthase (nNOS) and soluble guanylyl cyclase (sGC) inhibitors, and antagonists of Ang-R and opioid receptor (opioid-R), to study its synthesis, inactivation and mechanism of antinociceptive effect. The selectivity of the antinociceptive effect of Ang (5–8) was studied by injecting Ang (5–8) into the vlPAG or intravascularly in normotensive rats, and by observing its effects on the isolated rat aorta and guinea-pig ileum.

EXPERIMENTAL PROCEDURES

Materials

Angs II, III, IV, (4–8) and (5–8), saralasin, Losartan and CGP 42,112A, were from Peninsula Laboratories (San Carlos, CA, USA). Divalinal-Ang IV, phenylephrine, acetylcholine, propranolol, sodium nitroprusside and N^G-nitro-L-arginine were from Sigma (St. Louis, MO, USA). N^{ω}-propyl-L-arginine (NPLA) and 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) were from Tocris (Bristol, UK).

Subjects

Male albino Wistar rats weighing 140–160 g or 240–260 g were used for antinociception or blood pressure studies, respectively. All experiments were conducted according to our Institutions' guidelines for the use of laboratory animals. All protocols involving experiments with animals were approved by the Ethics Committees of the Federal University of Triângulo Mineiro (CEUA No. 181/2010), the Federal University of Rio de Janeiro (CEUA No. DFBCICB 011), and the Faculty of Medicine of Ribeirão Preto USP (CEUA No. 5.1.1209.53.8).

Surgery

Rats were anesthetized with tribromoethanol (Sigma), 250 mg/kg body weight i.p. A 12-mm length of a 23-gauge stainless steel guide cannula was implanted into the skull to lie 3.0 mm above the target site in the PAG. The stereotaxic coordinates (in mm) used were the following: AP, 0.7 from the ear bars; *L*, 0.5 from the midline; and *H*, −3.1 from the skull surface, all taken from Paxinos and Watson's (1986) Atlas. The guide cannula was then fixed to the skull with two steel screws and dental cement. After receiving penicillin (50 mg/kg i.m.), the animal was allowed to recover for 5–7 days before the experiment. Intracerebral injection was carried out according to Azami et al. (1980). The volume injected was 0.25 μL for all rats, delivered at a constant rate over a period of 40 s. The localization of the injection site was performed according to Pelegrini-da-Silva et al. (2005).

I.c.v. injection was carried out using a stainless steel guide cannula of 0.7-mm outer diameter implanted into the lateral ventricle at the coordinates (in mm) AP = −1.5 from bregma, *L* = +1.8 from the medial suture, *V* = −2.9 from the skull and the incisive bar positioned at +2.5 mm from the interaural line. Two microliters of Ang II or Ang (5–8) in phosphate buffered saline (PBS) was injected using a 10 μL syringe (705-N, Hamilton (Reno, Navada, USA)) connected to a dental injection needle (200 μm o.d.) by PE-10 tubing. The microinjection needle was 1 mm longer than the guide cannula.

Tail-flick test

The rat was introduced into a glass tube for up to 20 s, with the tail laid across a nichrome wire coil at room temperature (23 ± 2 °C). The coil temperature was then raised by an electric current until a tail withdrawal reflex occurred within 2.5–3.5 s. A cutoff time of 6 s was used. Tail-flick latencies were measured at 5-min intervals until a stable baseline was obtained over three consecutive trials (Pelegrini-da-Silva et al., 2005). After baseline determination, antagonist, enzyme inhibitor (Losartan, CGP 42,112A, divalinal-Ang IV, compound A-779, NPLA, ODQ or naloxone) or vehicle was injected into vlPAG, and tail-flick latencies were measured at 5-min intervals over 15 min. Five minutes later, vehicle or an Ang was injected intracerebrally and latencies were measured at 5-min intervals over a further 40-min period.

Incision allodynia model

Rats were anesthetized with 1.5% halothane in oxygen. A 1-cm longitudinal incision was made with a surgical blade through the skin and fascia of the plantar region, starting 0.5 cm from the proximal edge of the heel (Pelegrini-da-Silva et al., 2009). The plantaris muscle was elevated, but its origin and insertion were left intact. This procedure is similar to that reported by Brennan et al. (1996) and Vandermeulen and Brennan (2000). After hemostasis, the wound was sutured with two 5-0-nylon stitches. Rats were placed in an elevated clear plastic chamber with a wire mesh grid floor, which allowed easy access to the paw plantar surface. The threshold to mechanical stimulation was measured with an automated electronic von Frey apparatus (Digital Analgesiometer, Insight, Ribeirão Preto, SP, Brazil), consisting of a hand-held probe unit to which a rigid plastic tip (tip area 0.44 mm^2) was connected. The experimenter applied the plastic tip with an increasing force in an upward direction against sites near the heel, 1–2 mm adjacent to the medial border of the wound. A single trial consisted of three applications of the stimulus, delivered at 10-s intervals. The mechanical threshold was tested 5 min before paw incision, 5 min before and after vehicle or Ang (5–8) (0.1, 0.2 and 0.4 nmol/0.25 μL) injection into vlPAG, and then at 5-min intervals for up to 50 min. The response was defined as withdrawal of the stimulated paw, followed by flinching movements.

Arterial pressure and heart rate (HR) preparation

Male Wistar rats weighing 240–260 g were anesthetized with tribromoethanol (250 mg/kg i.p.). A polyethylene catheter was implanted into the left femoral artery for recording blood pressure. A similar catheter was introduced into the left femoral vein for intravenous drug injection or into carotid artery for intra-arterial administration in urethane-anesthetized rats and experiments were initiated 1 h after anesthesia onset. On the day of the experiment, the animals were allowed a 15-min period to adapt to the conditions of the experimental room, before starting blood pressure and HR recording. The experimental room was acoustically isolated and had constant