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A NEW ROLE FOR THE RENIN–ANGIOTENSIN SYSTEM IN THE RAT PERIAQUEDUCTAL GRAY MATTER: ANGIOTENSIN RECEPTOR-MEDIATED MODULATION OF NOCICEPTION

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Abstract—Renin–angiotensin (Ang) system (RAS) peptides injected into the periaqueductal gray matter (PAG) elicit antinociception. Saralasin blocks Ang II-elicited antinociception. Thus, it is possible that endogenous RAS peptides could participate on the modulation of nociception in the PAG. This possibility was tested here injecting, in the PAG, the specific Ang type 1 and type 2 receptor (AT_1 receptor and AT_2 receptor) antagonists losartan and CGP42,112A, respectively, either alone or before Ang II. The effects of Ang II, losartan and CGP42,112A on nociception were measured using the tail flick test and the model of incision allodynia. Ang II increased tail-flick latency, an effect inhibited by both losartan and CGP42,112A. Ang II reduced incisional allodynia. Either losartan or CGP42,112A alone increased incision allodynia, suggesting that endogenous Ang II and/or an Ang-peptide participates in the control of allodynia by the PAG. AT_1 and AT_2 receptors were immunolocalized in neuronal cell bodies and processes in the ventrolateral PAG. Taken together, the antinociceptive effect of Ang II injection into the ventrolateral PAG, the increase of allodynia elicited by injecting either losartan or CGP42,112A alone in the PAG, and the presence of AT_1 and AT_2 receptors in neurons and neuronal processes in the same region, represent the first evidence that part of the tonic nociceptive control mediated by the PAG is carried out locally by endogenous Ang II and/or an Ang-peptide acting on AT_1 and AT_2 receptors.

Key words: angiotensin, angiotensin receptor antagonists, periaqueductal gray matter, nociceptive control, tail flick test, incision allodynia model.

Angiotensin (Ang) II, a peptide of the renin-Ang system (RAS), can play several roles in the CNS, such as cardiovascular and body fluid homeostasis (Phillips, 1987; McKinley et al., 2003). RAS peptides also seem to be involved in the processing of nociceptive information. Antinociception following intracerebroventricular (i.c.v.) renin substrate (RS), Ang II (Haulica et al., 1986; Georgieva and Georgiev, 1999; Raghavendra et al., 1999), and Ang III (Shimamura et al., 1987) has been demonstrated in several rodent pain models. Administration of Ang III into the rat nucleus reticularis gigantocelullaris also evokes antinociception (Yien et al., 1993) and reduces the response of local cells to peripheral noxious stimulation (Chan et al., 1994). Additional evidence for the involvement of RAS peptides in nociception includes the reduction of the stress-induced analgesia in mice by i.c.v. saralasin, a non-selective Ang receptor antagonist (Haulica et al., 1986), and by losartan, an AT_1-antagonist (Raghavendra et al., 1999).

Microinjection of RS, Ang I, Ang II, or Ang III into the ventral or ventrolateral periaqueductal gray matter (PAG) produces a dose-dependent antinociceptive effect in the rat-tail flick test, i.e. increases the tail flick latency (Prado et al., 2003). The antinociceptive effect of RAS peptides injected in the PAG seems to be receptor mediated at least for Ang II, since it was inhibited by the previous local administration of saralasin (Prado et al., 2003). In addition to exhibiting an antinociceptive effect, Ang II has been immunolocalized in cells and nerve terminals in the mesencephalic PAG (Lind et al., 1985). Regarding the PAG, its ventral and ventrolateral regions are widely known as key stations in descending pathways that act to control nociceptive inputs in the dorsal horn of the spinal cord (Millan, 1999). The PAG is also involved in nociceptive processing, since neural blockade of ventral and ventrolateral PAG reduces tail flick latency (Rosenfeld and Xia, 1993). Also, peripheral persistent noxious stimulation increases the electrical activity, 2-deoxyglucose uptake, and c-fos expression in ventral PAG neurons (Wang and Nakai, 1994). Taken together, the above considerations suggest that some RAS peptides might be involved in the activation of descending pain control mechanisms in the PAG. However, there is no evidence that an endogenous RAS peptide mediates nociception in the PAG acting via an Ang receptor.

To address this issue, the specific Ang receptor antagonists type I (AT_1, losartan) and II (AT_2, CGP42,112A; Timmermans et al., 1993; Häuser et al., 1998) were injected in the ventrolateral PAG either alone or before Ang II. The effects of Ang II, losartan and CGP42,112A were determined using the tail flick test and the incision allodynia model. Moreover, the immunolocalization of AT_1 and AT_2 receptors was studied to ascertain whether or not the ventrolateral PAG region does contain Ang receptors.

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Abbreviations: Ang, angiotensin; ANOVA, analysis of variance; AT_1 receptor, angiotensin type 1 receptor; AT_2 receptor, angiotensin type 2 receptor; BL, baseline; IR, immunoreactive-like; PAG, periaqueductal gray matter; PBS, phosphate-buffered saline; RAS, renin–angiotensin system; RS, renin substrate; TL, tail-flick latency.

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EXPERIMENTAL PROCEDURES

Materials

Ang II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), losartan and CGP42, 112A were from Peninsula Laboratories (Belmont, CA, USA), and were diluted in phosphate-buffered saline [PBS; 20 mM sodium phosphate buffer, pH 7.4, containing 0.9% (weight/volume) sodium chloride]. The concentration of Ang II solutions was determined by amino acid analysis after acid hydrolysis. Rabbit anti-AT_1 and goat anti-AT_2 polyclonal antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Biotinylated swine anti-rabbit and biotinylated rabbit anti-goat antibodies were from Dako (Carpinteria, CA, USA). Lidocaine chloride (2%; Xylocaine) was purchased from AstraZeneca (São Paulo, Brazil). All other reagents were analytical grade or equivalent.

Subjects and surgery

Male albino Wistar rats weighing 140–160 g were used, and the experiments conducted in accordance to the IASP guidelines on using laboratory animals (Zimmermann, 1983). All animal experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee at the Facility of Medicine of Ribeirão Preto-USP. The animal suffering and number of animals per group were kept at a minimum. Each animal was anesthetized with sodium thiopentone (50 mg/kg, 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 ventrolateral PAG. The stereotaxic coordinates (in mm) used were 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 7–10 days before the experiment.

Microinjection procedure

Drug or vehicle was microinjected intracerebrally using a glass needle (70–90 μm, o.d.) protected by a system of telescoping steel tubes (Azami et al., 1980). The assembly was inserted into the guide cannula and the needle advanced to protrude 3 mm beyond the guide cannula tip. The volume of microinjection was 0.25 μl, delivered at a constant rate over a period of 3 min. The needle was removed 20 s after completion of the injection.

Localization of the injection site

At the end of each behavioral experiment Fast Green dye (0.5 μl) was injected to label the injection site. Dye spots were identified on 50 μm serial coronal sections stained with Neutral Red, and were localized using the atlas by Paxinos and Watson (1986). Only rats showing injection sites localized in the ventrolateral caudal PAG were used here. All behavioral tests were conducted on separate groups of animals.

Tail flick test

The animal was introduced in a ventilated 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. The heat stimulus was always applied to the ventral surface of the tail between 4 and 6 cm from the tip. A cutoff time of 6 s was established to minimize the probability of skin damage. Tail-flick latencies were measured at 5 min intervals until a stable baseline (BL) was obtained over three consecutive trials. Only rats showing a stable BL after six trials were used in each experiment. Each tail-flick latency (TL) was normalized using the equation $IA = (TL - \text{average } BL)/(6 - \text{average } BL)$, where IA is the index of antinociception (Azami et al., 1982). After BL determination, antagonist or vehicle was injected intracerebrally, and the TL was determined at 5 min intervals over 15 min. Five minutes later, vehicle or AII was injected intracerebrally and the TL determined at 5-min intervals over a further period of 40 min.

Incisional allodynia

On the day of the experiment each animal was anesthetized with ether via a loose-fitting, cone-shaped mask. 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. 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). After hemostasis, the wound was closed with two 5-0-nylon sutures and the animal allowed to recover in the home cage for a period of 1.5–1.75 h. The tactile threshold was measured with von Frey filaments (Stöelting, Wood Dale, IL, USA). Rats were placed in an elevated clear plastic cage with a nylon mesh bottom, which allowed easy access to the paw plantar surface. Before the injection, the animals remained in the cage for approximately 15 min for behavioral accommodation. The area tested was the mid-plantar right hind paw bordering the incision wound near the heel. The paw withdraw threshold was recorded touching the paw with one of a series of filaments with logarithmically incremental stiffness, in the range 0.0045–28.84 g. Each filament was applied from underneath the nylon mesh floor through the mesh, vertically to the plantar surface with sufficient force to bend the filament. A single trial consisted of six applications of a particular filament, applied once every 3–4 s. Testing was initiated with the 2.04 g filament, which corresponds to the middle of the filament series. A response was defined as the withdrawal of the stimulated paw. In the absence of a response to a particular filament, the next stronger filament was used. If a response was obtained, the next weaker filament was tested. The up-down method was used to record the threshold (Chaplan et al., 1994). The upper limit value (28.84 g) was recorded even if there was no withdrawal response to this force. In the experiments on the effects of different doses of Ang II on the incisional allodynia, the basal threshold was measured 1.75 h after the incision. Fifteen minutes later Ang II or vehicle was injected in the PAG, and the threshold was measured soon after this procedure, and then at 5 min intervals for up to 60 min. In the experiments on the effects of lidocaine or antagonists of Ang receptors on incisional allodynia, the basal threshold was measured 90 min after the incision, and vehicle, lidocaine or antagonists were injected 15 min later. Threshold measurement was started 15 min after finishing the injection procedure, and proceeded for the next 60 min at 5 min intervals. As additional controls, lidocaine and PBS were injected in the PAG of rats without paw incision ($n=6$), and thresholds were measured as above.

Brain fixation and processing

Animals were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and perfused through the left ventricle of the heart for 1–2 min with PBS, followed by perfusion with a freshly prepared fixative: 50 mM sodium phosphate buffer, pH 7.4, containing 1% (w/v) paraformaldehyde, 0.075 M lysine and 0,01 M sodium metaperiodate (McLean and Nakane, 1974), during 30 min. The brain was then removed, dehydrated, cleared with xylene and paraffin-embedded. Five micrometer sections were obtained from the interaural plane 0.7 mm up to 1.7 mm. Five sections differing in AP position by 100 μm were mounted sequentially on the same gelatin-coated glass slide. The next slide also contained five sections, each of them adjacent to each one of the sections in the previous slide, and so on.