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Angiotensin-(1-7) Inhibits Neuronal Activity of Dorsolateral Periaqueductal Gray via a Nitric Oxide Pathway

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Abstract

The midbrain periaqueductal gray (PAG) is a neural site for several physiological functions related to cardiovascular regulation, pain modulation and behavioral reactions. Recently, angiotensin-(1-7) [Ang-(1-7)] has been considered as an important biologically active component of the renin-angiotensin system in the CNS. The purpose of this study was to determine 1) existence of Ang-(1-7) receptor, Mas-R, within the dorsolateral PAG (dl-PAG), 2) the role for Ang-(1-7) in modulating activity of dl-PAG neurons, and 3) the mechanisms by which Ang-(1-7) plays a regulatory role. Western blot analysis showed that Mas-R appears within the dl-PAG. Whole cell patch-clamp recording demonstrated that the discharge rates of dl-PAG neurons were decreased from 4.35 ± 0.32 Hz of control to 1.06 ± 0.34 Hz ($P < 0.05$, vs. control) by 100 nM of Ang-(1-7). With pretreatment of A-779, a Mas-R inhibitor, the discharge rate was 4.66 ± 0.62 Hz ($P > 0.05$, vs. control) during infusion of Ang-(1-7). Additionally, neuronal nitric oxide synthase (nNOS) was largely localized within the dl-PAG among the three isoforms. The effects of Ang-(1-7) on neuronal activity of the PAG were attenuated in the presence of S-methyl-L-thiocitrulline (SMTC), a nNOS inhibitor. The discharge rates were 4.21 ± 0.39 Hz in control and 4.09 ± 0.47 Hz ($P > 0.05$, vs. control) when Ang-(1-7) was applied with pretreatment of SMTC. Those findings suggest that Ang-(1-7) plays an inhibitory role in the dl-PAG via a NO dependent signaling pathway. This offers the basis for the physiological role of Ang-(1-7) and Mas R in the regulation of various functions in the CNS.

INTRODUCTION

The midbrain periaqueductal gray (PAG) is an important neural site for numerous physiological functions including cardiovascular regulation, pain modulation and behavioral activities [1, 3, 19]. Among regions of the PAG, the dorsolateral (dl) region receives abundant afferent inputs from the spinal cord [9, 14] and sends descending neuronal projections to the medulla in regulating cardiovascular activity and pain [21, 29]. Activation of the dl-PAG contributes to an increase in blood pressure (BP) and antinociception [1, 3]. Moreover, the dl-PAG plays a functional role in regulating adaptive behavior, emotion, and

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anxiety etc. as a key relay area that receives numerous neuronal projections from other brain regions [19].

The brain renin angiotensin system (RAS) plays an essential role in control of sympathetic nerve activity, BP and balance of hydromineral and fluid volume [4, 23]. Also, the RAS contributes to the development of hypertension and cardiac hypertrophy [5, 27]. In the RAS, angiotensin II (Ang II) has been widely studied and findings suggest that brain Ang II represents the most important effective hormone of this system. Ang II injected into the PAG of rats increases BP via AT1 receptors [10], suggesting that the RAS is engaged in regulation of BP in the PAG. Additionally, the role played by Ang II of the PAG in modulating nociceptive and behavioral responses has been previously reported [24, 26].

The heptapeptide angiotensin-(1-7) [Ang-(1-7)] was traditionally considered as an inactive metabolic breakdown product of Ang II. Because angiotensin converting enzyme 2 (ACE2) is identified to cleave directly Ang II to Ang-(1-7) and the G-protein coupled receptor Mas (Mas-R) is recognized as the first binding site for Ang-(1-7) [11, 25, 32], many studies demonstrated that this peptide is involved in cardiovascular actions. Opposed to Ang II, the effects of Ang-(1-7) are primarily beneficial via counter-regulating Ang II actions [13, 25]. In the brain, Ang-(1-7) and Mas-R are expressed in cardiovascular related-regions [2]. The role for Ang-(1-7) in central regulation of cardiovascular activities and in the pathogenesis of neurogenic hypertension has been reported [6, 12]. However, presence of Mas-R and its effects on neuronal activities in the dl-PAG have not specifically been studied. Also, evidence showed that Ang-(1-7) exerts its actions via a nitric oxide (NO) dependent mechanism [8, 33].

Thus, the purposes of this study were to examine (1) existence of Ang-(1-7) receptor, Mas-R, within the dl-PAG, (2) the role for Ang-(1-7) in modulating neuronal activity of the dl-PAG and (3) if Ang-(1-7) plays a role via a NO signaling pathway. It was hypothesized that Mas-R appears within the dl-PAG and Ang-(1-7) decreases the discharge rate of dl-PAG neurons via Mas-R, and neuronal NO synthase (nNOS) is present within the dl-PAG and the inhibitory effects of Ang-(1-7) on PAG activities were blocked after an inhibition of nNOS.

METHODS

All procedures were approved by the *Institutional Animal Care and Use Committee* of Penn State College of Medicine and complied with the NIH guidelines.

Western Blot Analysis

Sprague Dawley rats of either gender (4-6 weeks old) were anesthetized by inhalation of isoflurane oxygen mixture, and were decapitated. The brain was then removed and regions of the PAG were dissected under an anatomical microscope. The tissues were processed using a standard western blot procedure to determine Mas-R and NOS. Briefly, total protein was extracted by homogenizing dl-PAG sample in ice-cold radioimmunoprecipitation assay buffer. The lysates were centrifuged and the supernatants were collected for measurements of protein concentrations. After being denatured by heating at 95°C for 5 min in buffer, the supernatant samples containing 20 µg of protein were loaded onto 4-20% Mini-PROTEAN TGX gels and electrically transferred to a polyvinylidene fluoride membrane. The membrane was blocked in 5% nonfat milk in 0.1% Tween-TBS buffer and was incubated overnight with primary antibodies (rabbit anti-Mas at 1:200; mouse anti-nNOS and anti-iNOS at 1:1000; rabbit anti-eNOS at 1:1000). Next, the membranes were washed and incubated with an alkaline phosphatase conjugated anti-rabbit secondary antibody (1:1000), and anti-mouse secondary antibody (1:1000). The immunoreactive proteins were detected by enhanced chemiluminescence. The bands recognized by the primary antibody were

visualized by exposure of the membrane onto an x-ray film. The membrane was stripped and incubated with mouse anti- β -actin to show equal loading of the protein. Then, the film was scanned and the optical density of Mas-R, NOS and β -actin bands was analyzed using the NIH Scion Image software.

Electrophysiology

The rats were anesthetized by inhalation of isoflurane oxygen mixture and decapitated. Briefly, the brain was quickly removed and placed in ice-cold artificial cerebral spinal fluid (aCSF) solution as described previously [30, 31]. A tissue block containing the PAG was cut from the brain and glued onto the stage of the vibratome. Coronal slices (300 μ m) containing the PAG were obtained from the tissue block in ice-cold aCSF solution. An equilibrium period of 60 min was required to incubate the slices in the aCSF at 34°C before being transferred to the recording chamber. During those procedures, aCSF were saturated with 95% O₂ - 5% CO₂.

A whole cell current-clamp technique was used to record the spontaneous firing activity of dl-PAG neurons. Borosilicate glass capillaries (1.2 mm OD, 0.69 mm ID) were pulled to make the recording pipettes using a puller. The resistance of the pipette was 4–6 M Ω when it was filled with the internal solution [30, 31]. The slice was placed in a recording chamber and the aCSF saturated with 95% O₂ - 5% CO₂ was perfused into the chamber at 3.0 ml/min. The temperature of the perfusion solution was maintained at 34°C by an in-line solution heater. Whole cell recordings from dl-PAG neurons were performed visually using differential interference contrast (DIC) optics on an upright microscope (BX50WI, Olympus). The tissue image was captured and enhanced through a camera and displayed on a video monitor. A tight giga-ohm seal was subsequently obtained in dl-PAG neuron viewed using DIC optics. Signals were recorded and saved in a computer using a MultiClamp 700B amplifier digitized with a DigiData 1440A, and pClamp 10.1 software. A liquid junction potential of -15.0 mV was corrected during off-line analysis [15, 16]. An equilibration period of 5-10 min was allowed after the recording reached a steady state. The recording was abandoned if the monitored input resistance changed greater than 15%.

Ang-(1-7), A-779 (Mas-R antagonist) and S-methyl-L-thiocitrulline (SMTC, nNOS inhibitor) were obtained from Sigma-Aldrich. All drugs were dissolved in the aCSF solution immediately before being used. The drugs were delivered into the recording chamber at final concentrations using syringe pumps during the experiment [30]. Based on prior studies [8, 22, 33], Ang-(1-7) (10, 100 nM and 1 μ M), A-779 (10 μ M) and SMTC (1 μ M), were chosen in this experiment. The responses of action potentials (AP) of dl-PAG neurons to drugs were recorded after control data were collected.

At the end of experiments, location of the recording pipette in the PAG slice was visualized and identified under a microscope using the DIC. All cells included for data analysis in this experiment located in the dl-PAG based on the identification with a microscopy and rat brain maps [28]. A prior report illustrated representative locations of recorded neurons with anterior-posterior coordinates of the sections [20] using the same methods.

The discharge rates of PAG neurons were analyzed off-line with a peak detection program (MiniAnalysis). The firing rate of dl-PAG neurons was analyzed with one-way ANOVA to determine differences between groups. All values were expressed mean $\pm$ SE. For all analyses, differences were considered significant at $P < 0.05$. All statistical analyses were performed using SPSS for windows version 17.0.

RESULTS

Expression of Mas-R and NOS within dl-PAG

The protein levels of Mas-R and three isoforms of NOS in the dl-PAG were analysed (number of rats=3). Mas-R was localized and relatively high levels of nNOS were observed among all isoforms. Figure 1 illustrates that Mas-R appears within the dl-PAG and as compared with eNOS and iNOS, nNOS was mainly present in this region using the same amount of protein samples.

Ang-(1-7) Inhibits Neuronal Activities of dl-PAG via Mas-R and nNOS Pathways

Data of the whole cell patch-clamp experiments were collected from a total of 60 dl-PAG neurons obtained from 21 rats. The resting membrane potential was -65.7 ± 1.1 mV and amplitude of AP was >60 mV. The input resistance was 646.9 ± 32.1 M Ω .

The effects of Ang-(1-7) on the discharge of dl-PAG neurons were examined. 10 and 100 nM, and 1 μ M of Ang-(1-7) were applied to the perfusion chamber to obtain a dose-relationship effect ($n=33$). Figure 2 showed that Ang-(1-7) significantly decreased the discharge rate of dl-PAG neurons. The discharge rate was decreased from 4.35 ± 0.32 Hz of control to 1.06 ± 0.34 Hz ($P < 0.05$, vs. control) in 15 dl-PAG neurons tested after 100 nM of Ang-(1-7). Application of Ang-(1-7) did not significantly alter the resting membrane potential of dl-PAG neurons (-65.5 ± 1.2 mV vs. 66.2 ± 1.3 mV, $P > 0.05$). Note that the resting membrane potential was measured from the cells without the firing activity. The responses of AP to Ang-(1-7) developed at a latency of 14 to 60 s. Then, the responses recovered during washout of the perfusion solution and completely returned to the levels of control without Ang-(1-7) at 5-10 min.

Additionally, the role for Mas-R in Ang-(1-7) attenuation of dl-PAG neurons was examined (Figure 3). The firing activities of dl-PAG neurons were first observed following application of 100 nM of Ang-(1-7), and a recovery was allowed. Then, the firing activities were examined during Ang-(1-7) in the presence of Mas-R antagonist, A-779. The spontaneous discharge rate of PAG neurons was not significantly altered following perfusion of 10 μ M of A-779 (4.22 ± 0.41 Hz in control vs. 4.64 ± 0.60 Hz after A-779, $P > 0.05$, $n=19$). However, subsequent application of 100 nM of Ang-(1-7) failed to decrease the spontaneous neuronal activities in the presence of A-779. The discharge rate was 4.66 ± 0.62 Hz ($P > 0.05$, vs. control) during infusion of Ang-(1-7) as Mas-R was blocked.

In additional experiment, the role for nNOS in Ang-(1-7) attenuation of dl-PAG neurons was further determined (Figure 4). The firing activities of dl-PAG neurons were examined in the presence of nNOS inhibitor, SMTC, following application of 100 nM of Ang-(1-7). The discharge rates of PAG neurons were not significantly altered following perfusion of 1 μ M of SMTC (4.21 ± 0.38 vs. 4.11 ± 0.36 Hz, $P > 0.05$, $n=8$). A decrease in the discharge rates induced by 100 nM of Ang-(1-7) was not observed in the presence of SMTC. The discharge rates were 4.21 ± 0.38 Hz in control and 4.09 ± 0.47 Hz ($P > 0.05$, vs. control) when Ang-(1-7) was applied with pretreatment of SMTC.

DISCUSSION

Results of the present study showed that Mas-R was present in the dl-PAG and nNOS was largely localized in this region compared with other two isoforms. Furthermore, regulatory effects of Ang-(1-7) on activities of dl-PAG neurons were determined. The results demonstrated that Ang-(1-7) significantly attenuated the discharge rates of AP in the dl-PAG. These effects were abolished in the presence of Mas-R antagonist. Additionally, a nNOS inhibitor SMTC significantly attenuated the effects of Ang-(1-7) on discharges of dl-

PAG neurons. These data suggest that stimulation of Mas-R inhibits neuronal activities of the dl-PAG via a NO dependent signaling pathway.

Increased NO by S-nitroso-N-acetyl-penicillamine significantly decreases the discharge rate of spontaneous AP in dl-PAG neurons and the effect is reduced in the presence of the GABA_A receptor antagonist, bicuculline [30]. This suggests that NO has an inhibitory effect on neuronal activity of the dl-PAG via a potentiation of GABAergic synaptic inputs. Thus, it is well reasoned that activation of Mas-R inhibits neuronal activities of the dl-PAG via NO mechanisms. Since nNOS is found to be the majority of NOS expression in the dl-PAG, in the present study the role for nNOS in Ang-(1-7) modulating the discharge of dl-PAG neurons was examined. Ang-(1-7) was reported to increase nNOS-derived NO levels and then increase potassium currents in catecholaminergic neurons [33].

Ang-(1-7) can exert its actions via PTEN (phosphatase and tensin homologue deleted on chromosome ten) and AT2 receptor [8, 18, 22]. For example, incubation of hypothalamic neurons obtained from control and prehypertensive spontaneously hypertensive rats (SHR) with Ang-(1-7) increases PTEN activity via Mas-R and abolishes the enhanced chronotropic effect of Ang II in the SHR neurons [22]. An increase in NOS activity and eNOS phosphorylation are observed in isolated ventricle slices incubated with Ang-(1-7), and this effect is blocked by an AT2 antagonist, suggesting that Ang-(1-7) can upregulate cardiac NOS expression and activity through an AT2 dependent mechanism [8]. Another study has demonstrated that Ang-(1-7) can decrease activity of tyrosine hydroxylase in hypothalamus of normotensive and SHR rats and the effects are blocked by an AT2 receptor antagonist [18]. Thus, the roles of PTEN and AT2 receptor in Ang-(1-7) regulating discharges of dl-PAG neurons can't be ruled out in the present study. Also, it should be noted that non-specificity of Ang-(1-7) and Mas-R and nNOS inhibitors can't be excluded.

Ang II is representative of RAS and plays an important role in control of BP, and balance of hydromineral and fluid volume [4, 23], and contributes to the development of hypertension and cardiac hypertrophy [5, 27]. The effects of Ang II on the inhibitory GABAergic inputs to dl-PAG neurons were previously examined [31], showing that Ang II has an inhibitory effect on GABAergic synaptic inputs to the dl-PAG through stimulation of the presynaptic AT1 receptors. Other studies have also reported that Ang II attenuates synaptic GABA release and excites activities of the hypothalamic paraventricular nucleus-rostral ventrolateral medulla and -presympathetic neurons via AT1 receptor [15, 16]. Results of the present study evidently showed that Ang-(1-7) has an opposed effect to Ang II in regulation of neuronal activities of the PAG. In opposition to Ang II, the effects of Ang-(1-7) have been considered to be beneficial via counter-regulating Ang II actions [13, 25].

Besides the autonomic regulation, Ang II plays a role in modulating nociceptive and behavioral responses in the PAG [24, 26]. The effects of Ang II are through presynaptic AT1 inhibition of GABAergic inputs based on data of the prior studies [15, 16, 31]. Although Ang II acts as a neurotransmitter/modulator in several regions of the brain including the PAG [10, 24, 26], the specific sources of RAS-Ang II in the PAG are not clear. A major source of the angiotensinergic inputs to the PAG is possibly circumventricular organs like the source of Ang II to other brain regions [17]. There is likely a neuronal connection between the subfornical organ and PAG because the pressor response induced by Ang II microinjected into the subfornical organ is attenuated by Ang II antagonists injected into the PAG [7]. Nevertheless, the present study showed that Ang-(1-7) inhibits neuronal activities within the dl-PAG and this effect is opposed to Ang II in the CNS. Thus, the data offer the basis for the physiological role of Ang-(1-7) and Mas R in the regulation of cardiovascular functions in the CNS.

In summary, results of this study demonstrated that Mas-R is present within the dl-PAG and Ang-(1-7) has an inhibitory effect on neuronal activities of the dl-PAG via Mas-R. Also, a blockade of nNOS can significantly attenuate the effects of Ang-(1-7) on discharges of dl-PAG neurons. These data suggest that stimulation of Mas-R inhibits neuronal activities of the dl-PAG via a NO dependent signaling pathway, which provides new information for understanding the role played by Ang-(1-7) in modulating activities of dl-PAG neurons.

Acknowledgments

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Literature References

1. Bandler R, Carrive P, Zhang SH. Integration of somatic and autonomic reactions within the midbrain periaqueductal gray: Viscerotopic, somatotopic and functional organization. *Prog. In Brain Res.* 1991; 87:269–305. [PubMed: 1678189]
2. Becker LK, Etelvino GM, Walther T, Santos RAS, Campagnole-Santos MJ. Immunofluorescence localization of the receptor Mas in cardiovascular-related areas of the rat brain. *Am J Physiol.* 2007; 293:H1416–H1424.
3. Behbehani MM. Functional characteristics of the midbrain periaqueductal gray. *Prog. Neurobiol.* 1995; 46:575–605. [PubMed: 8545545]
4. Brooks VL. Interactions between angiotensin II and the sympathetic nervous system in the long-term control of arterial pressure. *Clin Exp Pharmacol Physiol.* 1997; 24:83–90. [PubMed: 9043811]
5. Bruner CA, Kuslikis BI, Fink GD. Effect of inhibition of central angiotensin pressor mechanisms on blood pressure in spontaneously hypertensive rats. *J Cardiovascular Pharmacol.* 1987; 9:298–304.
6. Campagnole-Santos MJ, Heringer SB, Batista EN, Khosla MC, Santos RA. Differential baroreceptor reflex modulation by centrally infused angiotensin peptides. *Am J Physiol.* 1992; 263:R89–R94. [PubMed: 1636797]
7. Chang YZ, Gu YH. Role of brain angiotensin II system in subfornical organ-pressor responses. *Acta Physiol Sinica.* 1999; 51:38–44. [PubMed: 11972173]
8. Costa MA, Verrilli MA, Gomez KA, Nakagawa P, Peña C, Arranz C, Gironacci MM. Angiotensin-(1–7) upregulates cardiac nitric oxide synthase in spontaneously hypertensive rats. *Am J Physiol.* 2010; 299:H1205–H1211.
9. Craig AD. Distribution of brainstem projections from spinal lamina I neurons in the cat and the monkey. *J. Comp. Neurol.* 1995; 361:225–248. [PubMed: 8543660]
10. D'Amico M, Di Filippo C, Berrino L, Rossi F. AT1 receptors mediate pressor responses induced by angiotensin II in the periaqueductal gray area of rats. *Life Sci.* 1997; 61:PL17–PL20. [PubMed: 9200674]
11. Doobay MF, Talman LS, Obr TD, Tian X, Davisson RL, Lazartigues E. Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin angiotensin system. *Am J Physiol.* 2007; 292:R373–R381.
12. Feng Y, Xia H, Cai Y, Halabi CM, Becker LK, Santos RA, Speth RC, Sigmund CD, Lazartigues E. Brain-selective overexpression of human Angiotensin-converting enzyme type 2 attenuates neurogenic hypertension. *Circ Res.* 2010; 106:373–382. [PubMed: 19926873]
13. Ferrario CM, Trask AJ, Jessup JA. Advances in the biochemical and functional roles of angiotensin converting enzyme 2 and angiotensin-(1–7) in the regulation of cardiovascular function. *Am. J. Physiol.* 2005; 289:H2281–H2290.
14. Keay KA, Feil K, Gordon BD, Herbert H, Bandler R. Spinal afferents to functionally distinct periaqueductal gray columns in the rat - An anterograde and retrograde tracing study. *J. Comp. Neurol.* 1997; 385:207–229. [PubMed: 9268124]
15. Li D-P, Pan H-L. Angiotensin II attenuates synaptic GABA release and excites paraventricular-rostral ventrolateral medulla output neurons. *J Pharmacol & Exp Ther.* 2005; 313:1035–1045. [PubMed: 15681656]

16. Li DP, Chen SR, Pan HL. Angiotensin II stimulates spinally projecting paraventricular neurons through presynaptic disinhibition. *J Neurosci*. 2003; 23:5041–5049.
17. Li Z, Ferguson AV. Subfornical organ efferents to paraventricular nucleus utilize angiotensin as a neurotransmitter. *Am J Physiol*. 1993; 265:R302–R309. [PubMed: 8103642]
18. Lopez Verrilli MA, Pirola CJ, Pascual MM, Dominici FP, Turyn D, Gironacci MM. Angiotensin-(1-7) through AT2 receptors mediates tyrosine hydroxylase degradation via the ubiquitin-proteasome pathway. *J. Neurochem*. 2009; 109:326–335. [PubMed: 19183250]
19. Lovick TA, Adamec R. The Periaqueductal Gray (PAG). *Neural Plasticity*. 2009
20. Lu J, Xing J, Li J. Prostaglandin E2 (PGE2) inhibits glutamatergic synaptic transmission in dorsolateral periaqueductal gray (dl-PAG). *Brain Res*. 2007; 1162:38–47. [PubMed: 17612511]
21. McGaraughty S, Chu KL, Bitner RS, Martino B, El Kouhen R, Han P, Nikkel AL, Burgard EC, Faltynek CR, Jarvis F. Capsaicin infused into the PAG affects rat tail flick responses to noxious heat and alters neuronal firing in the RVM. *J. Neurophysiol*. 2003; 90:2702–2710. [PubMed: 12815018]
22. Modgil A, Zhang Q, Pingili A, Singh N, Yao F, Ge J, Guo L, Xuan C, O'Rourke ST, Sun C. Angiotensin-(1–7) attenuates the chronotropic response to angiotensin II via stimulation of PTEN in the spontaneously hypertensive rat neurons. *Am J Physiol*. 2012; 302:H1116–H1122.
23. Peach MJ. Renin-angiotensin system: biochemistry and mechanisms of action. *Physiol Rev*. 1977; 57:313–370. [PubMed: 191856]
24. Prado WA, Pelegrini-da-Silva A, Martins AR. Microinjection of renin-angiotensin system peptides in discrete sites within the rat periaqueductal gray matter elicits antinociception. *Brain Res*. 2003; 972:207–215. [PubMed: 12711094]
25. Santos RAS, Silva ACS, Maric C. Angiotensin-(1–7) is an endogenous ligand for the G 425 protein-coupled receptor Mas. *PNAS*. 2003; 100:8258–8263. [PubMed: 12829792]
26. Sowards TV, Sowards MA. The awareness of thirst: proposed neural correlates. *Consciousness & Cognition*. 2000; 9:463–487. [PubMed: 11150217]
27. Steckelings UM, Obermuller N, Bottari SP, Qadri F, Veltmar A, Unger T. Brain angiotensin: receptors, actions and possible role in hypertension. *Pharmacol Toxicol*. 1992; 70:S23–S27. [PubMed: 1508845]
28. Swanson, LW. *Brain Maps: Structure of the rat brain*. Elsevier; New York: 1998.
29. Verberne AJM, Guyenet PG. Midbrain central gray: Influence on medullary sympathoexcitatory neurons and the baroreflex in rats. *Am J. Physiol*. 1992; 263:R24–R33. [PubMed: 1636792]
30. Xing J, Li D-P, Li J. Role of GABA receptors in nitric oxide inhibition of dorsolateral periaqueductal gray neurons. *Neuropharmacology*. 2008; 54:734–744. [PubMed: 18222497]
31. Xing J, Lu J, Li J. Angiotensin II inhibits GABAergic synaptic transmission in dorsolateral periaqueductal gray neurons. *Neurosci Lett*. 2009; 455:8–13. [PubMed: 19429096]
32. Yamazato M, Yamazato Y, Sun C, Diez-Freire C, Raizada MK. Overexpression of angiotensin-converting enzyme 2 in the rostral ventrolateral medulla causes long-term decrease in blood pressure in the spontaneously hypertensive rats. *Hypertension*. 2007; 49:926–931. [PubMed: 17325232]
33. Yang R, Yin J, Li Y, Zimmerman MC, Schultz HD. Angiotensin-(1–7) increases neuronal potassium current via a nitric oxide-dependent mechanism. *Am J Physiol*. 2011; 300:C58–C64.

Highlights

- The midbrain PAG is a neural site for several physiological functions related to cardiovascular regulation
- Ang-(1-7) is considered as an important biologically active component of the renin-angiotensin system in the CNS
- Ang-(1-7) plays an inhibitory role in the periaqueductal gray via a NO dependent signaling pathway

Figure 1.

Dual representative bands illustrate Mas-R and three isoforms of NOS expression. Mas-R is apparently present in the dl-PAG. Among the three isoforms, expression of nNOS is greater than eNOS and iNOS. Note that the same amount of protein was sampled to examine their individual expression. Beta-actin was used as control for equal loading of protein.

Figure 2. Ang-(1-7) had an inhibitory effect on the firing activity of dl-PAG neurons. **A:** Original tracings from a dl-PAG neuron show the spontaneous discharge during control, Ang-(1-7) (10 nM -1 μ M) perfusion and washout for a recovery. **B:** Average data (n=33) for three dosages of Ang-(1-7). * P < 0.05, vs. control and recovery.

Figure 3.

A prior application of A-779 to block Mas-R antagonized the effects of Ang-(1-7) on the firing activity of dl-PAG neurons. **A:** Original tracings from a dl-PAG neuron. **B:** average data (n=19) show the spontaneous discharge during control, Ang-(1-7), recovery, A-779 (10 μ M), and Ang-(1-7) perfusion in the presence of A-779. The effect of Ang-(1-7) was abolished after application of Mas-R antagonist. * $P < 0.05$, vs. control and recovery. There were no significant differences among control, recovery, A-779 and A-779 plus Ang-(1-7).

Figure 4.

An inhibitory effect of Ang-(1-7) on the firing activity dl-PAG neurons was abolished with prior application of SMTC to attenuate nNOS. **A:** Original tracings from a dl-PAG neuron show the spontaneous discharge during control, Ang-(1-7) perfusion (100 nM), washout, SMTC (1 μ M) and Ang-(1-7) perfusion in the presence of nNOS blocker. **B:** Average data (n=8). * $P < 0.05$, vs. control and recovery. No significant differences were observed among control, recovery, SMTC and SMTC plus Ang-(1-7).