Effects of Angiotensin II and Angiotensin-(1-7) on the Release of \([^3H]Norepinephrine\) From Rat Atria

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Abstract  We examined the effects of angiotensin II (Ang II) and Ang-(1-7) on the release of \([^3H]Norepinephrine\) elicited by nerve stimulation (2 Hz, 0.5 millisecond, for 2 minutes) in rat atria isolated with their cardioaccelerans nerves. The stimulation-induced release of \([^3H]Norepinephrine\) was increased 50% by \(3 \times 10^{-4}\) mol/L of either peptide. No further increase in \([^3H]Norepinephrine\) release was observed with peptide concentrations up to \(3 \times 10^{-7}\) mol/L. This effect was completely blocked by the nonselective angiotensin receptor antagonist saralasin \((1 \times 10^{-5}\) mol/L). The type 1 angiotensin receptor antagonist DuP 753 \((1 \times 10^{-6}\) mol/L) entirely prevented the increases in \([^3H]Norepinephrine\) caused by Ang II and Ang-(1-7). On the other hand, the type 2 angiotensin receptor antagonist PD 123319 \((1 \times 10^{-6}\) mol/L) prevented the increase in \([^3H]Norepinephrine\) release elicited by Ang-(1-7) but not by Ang II. These results suggest that Ang-(1-7), like Ang II, could have a neuromodulatory function in rat atria via activation of specific angiotensin receptor subtypes, which could be the subtype 1 angiotensin receptor for Ang II and subtypes 1 and 2 for Ang-(1-7). (Hypertension. 1994;24:457-460.)

Key Words  • angiotensins  • norepinephrine  • receptors, angiotensin  • angiotensin II

Angiotensin-(1-7) [Ang-(1-7)] is an active component of the renin-angiotensin system that lacks the phenylalanine present in position 8 of Ang II. It has been shown that although both peptides are products of Ang I metabolism, they result from separate enzymatic pathways. Although angiotensin-converting enzyme promotes Ang II formation, a prolyl endopeptidase converts either Ang I or II into Ang-(1-7). This heptapeptide was found to be present in central and peripheral tissues of rats and dogs and to excite neurons in the central nervous system. Although some of the responses elicited by Ang-(1-7) were similar to those of Ang II, different actions for both peptides were also reported; ie, the heptapeptide is not a dipso- gen, a constrictor agent of blood vessels, or an aldosterone secretagogue. Moreover, different actions of both peptides in the regulation of cell function were also reported; ie, the heptapeptide is a dipso- gen, a constrictor agent of blood vessels, or an aldosterone secretagogue. Although some of the responses elicited by Ang-(1-7) were similar to those of Ang II, different actions for both peptides were also reported; ie, the heptapeptide is not a dipso- gen, a constrictor agent of blood vessels, or an aldosterone secretagogue. Moreover, different actions of both peptides in the regulation of cell function were also reported; ie, the heptapeptide is a dipso- gen, a constrictor agent of blood vessels, or an aldosterone secretagogue.

Methods

Synthesis of Ang-(1-7)

The Merrifield solid-phase procedure was employed, using Boc-amino acid derivatives. The crude peptide was purified by high-performance liquid chromatography (HPLC) in a C18 column eluted with a 0 to 40% acetonitrile gradient at a flow rate of 1.8 mL/min. The purified product was characterized as a single component by HPLC and thin-layer chromatography. It showed aspartic acid as the amino-terminal residue as well as the correct amino acid composition and sequence.

Tissue Preparation

Female Wistar rats (180 to 200 g) were anesthetized with ether, and the heart was rapidly removed. Both atria were dissected with their cardioaccelerans nerves in modified Krebs' solution of the following composition \((10^{-3}\) mol/L): NaCl 118.0, KCl 4.7, CaCl₂ 2.6, MgCl₂ 1.2, NaHCO₃ 25.0, glucose 11.1, EDTA 0.004, and ascorbic acid 0.11. Atropine \((1.4 \times 10^{-4}\) mol/L) was added to the Krebs' solution to exclude any influence of muscarinic receptors on the release of norepinephrine. The atria were set up in a 5-mL isolated organ bath equipped with platinum electrodes for nerve stimulation. Incubations were carried out in the modified Krebs' solution at 37°C with continuous bubbling of 95% O₂/5% CO₂.

The spontaneous contractions of the preparation were recorded through a Grass FT03C transducer connected to a Grass polygraph. An equilibration period was allowed to elapse until the basal resting rate did not differ by more than 10 beats per minute during a 10-minute interval.

[^3H]NE Overflow Measurement

Endogenous norepinephrine stores were labeled by incubation of the tissue at 37°C for 30 minutes with \(5 \mu\text{Ci/mL of (±)-7-[^3H]NE}\) (specific activity, 14.3 Ci/mmol; New England Nuclear Corp) as described by Adler-Graschinsky et al. After
1.0

3.10 1.10 3.10

CONTROL ANG II ANG (1-7)

1.0

3.10 1.10 3.10

CONTROL ANG II ANG (1-7)

3.10 1.10 3.10

CONTROL SARALASIN ANG II plus ANG (1-7) plus SARALASIN

The spontaneous outflow of [3H]noradrenaline in rat atria labeled with [3H]norepinephrine was calculated by subtracting the spontaneous outflow assumed to have occurred in each sample during and after the stimulation period from the total amount of radioactivity lost during the successive washes to that measured in the tissue at the end of the experiment. The spontaneous outflow was the basal resting release obtained in the period immediately before the stimulation.

**Statistical Analysis**

All values are mean ± SEM. Data were submitted to one-way ANOVA. Post hoc analysis with the Scheffé test was carried out in every case, and probability values less than .05 were considered significant.

**Drugs**

Ang II was purchased from Sigma Chemical Co. DuP 753 was obtained from the Du Pont Co. PD 123319 was a gift from Dr Jack Hodges at Parke-Davis.

**Results**

**Effects of Angiotensin Peptides on [3H]NE Outflow**

The spontaneous outflow of [3H]NE 2 minutes before S1 expressed as a fraction of the tissue content was 7.0 ± 1.2 × 10^-3 (n = 5) in the control group. This value was not modified in the presence of either 3 × 10^-8 to 3 × 10^-7 mol/L Ang-(1-7) or 3 × 10^-8 to 3 × 10^-7 mol/L Ang II (Fig 1). On the contrary, the overflow of tritium per shock, which in controls was 15.2 ± 4.8 × 10^-6 during S2 (n = 5), was significantly increased by either 3 × 10^-8 to 3 × 10^-7 mol/L Ang II or 3 × 10^-8 to 3 × 10^-7 mol/L Ang-(1-7) (Fig 2); i.e., the ratio between S2 and S1, which under control conditions was 0.88 ± 0.02 (n = 6), was increased up to 50% by concentrations as low as 3 × 10^-8 mol/L of either peptide. No further increases in [3H]NE release in response to nerve stimulation were observed with concentrations of both peptides as high as 3 × 10^-7 mol/L.

**Effects of Angiotensin Receptor Antagonists on the Increase in [3H]NE Release Elicited by Ang II and Ang-(1-7)**

As shown in Fig 3, the nonselective antagonist of angiotensin receptors saralasin, at 1 × 10^-7 mol/L, abolished the facilitatory effect on [3H]NE release by nerve stimulation elicited by both Ang II and Ang-(1-7).
When the effects of selective antagonists for AT₁ and AT₂ receptor subtypes were studied, it was found that the AT₁ receptor antagonist DuP 753 (1 × 10⁻⁶ mol/L) prevented by 70% the increase in [³H]NE release elicited by Ang II and by Ang-(1-7). The reductions in [³H]NE release were 70% in both cases. The antagonist per se was without effect on [³H]NE release in response to nerve stimulation.

Unlike what was observed with the AT₁ receptor antagonist, the AT₂ receptor antagonist PD 123319 (1 × 10⁻⁶ mol/L) prevented by 70% the increase in [³H]NE release elicited by 1 × 10⁻⁷ mol/L Ang-(1-7) but did not alter the release of the [³H] transmitter in the presence of 1 × 10⁻⁷ mol/L Ang II (Fig 5). The antagonist per se did not modify the [³H]NE release elicited by nerve stimulation. Note that the nerve stimulated-induced release of [³H]NE in the presence of Ang-(1-7) was prevented even by a 10 times lower concentration of the AT₂ receptor antagonist (1 × 10⁻⁷ mol/L), whereas the effects of Ang II were not prevented by a concentration of this antagonist as high as 1 × 10⁻⁵ mol/L (data not shown).

Discussion

The present study shows that in isolated rat atria Ang II and Ang-(1-7) were equally potent in increasing the [³H]NE release evoked in response to nerve stimulation. The observation that neither peptide modified the basal efflux of [³H]NE probably indicates that they exert their action at a presynaptic site. Moreover, because the effects of the peptides were prevented by different angiotensin receptor antagonists, it appears that this presynaptic site could be an angiotensin receptor.

Despite the fact that Ang II has a high affinity for AT₂ receptors,¹⁰ no AT₂ receptors are involved for this agonist but only for Ang-(1-7). The increase in [³H]NE release by nerve stimulation caused by Ang II was selectively prevented by the AT₁ receptor antagonist DuP 753, suggesting that AT₁ receptors are involved. Increases in norepinephrine release by nerve stimulation caused by Ang II through the specific interaction with the AT₁ receptors have been reported in both in vivo and in vitro studies. Among the in vivo effects of Ang II are the results reported by Wong et al.¹⁹ and Suzuki et al.,¹² who found in anesthetized dogs that the Ang II–induced enhancement of canine renal sympathetic nerve function results from an increase in norepinephrine release through the activation of AT₁ receptors. Similar results were also obtained in pithed rats under spinal cord stimulation²¹ as well as in the paraventricular nucleus of conscious rats submitted to intracerebroventricular injections of pressor doses of Ang II.²² Among the in vitro assays, a presynaptic modulation by AT₁ receptors of norepinephrine release elicited by field stimulation was found in superfused slices of rat intercerebral fat²³ and in atria isolated from guinea pigs.²⁴

Despite the fact that, as reported for Ang II, Ang-(1-7) is devoid of dipsogenic, pressor, or direct myotropic effects in rats and humans,³,¹⁰ the observation that it is as potent as Ang II in increasing the release of norepinephrine caused by nerve stimulation of the rat atria (present results) is coincident with the findings that the heptapeptide is as potent as Ang II in releasing vasopressin from the rat hypothalamo-neurohypophysial system,⁷ producing neuronal excitation in rat paraventricular neurons,⁵,⁶ eliciting cardiovascular effects injected into the dorsal medulla of rats,⁸ and stimulating prostaglandin release from rabbit vas deferens¹¹ and C₅ glioma cells,¹³ human astrocytes,¹⁶ and porcine aortic endothelial cells.²⁷ Nevertheless, our results do not agree with those reported by Trachte et al.,¹⁴ who found that the noradrenergic neurogenic contractions of the rabbit vas deferens were potentiated by Ang II but not by the heptapeptide.

The facilitatory action on the sympathetic neurotransmission elicited in the rat atria by both Ang II and Ang-(1-7) appears to be mediated by stimulation of AT₁ receptors. Moreover, the effect of Ang-(1-7) seems to additionally involve the activation of AT₂ receptors. Identical biological activity caused by both peptides but
coupled to the activation of different angiotensin receptor subtypes has also been reported by Jaiswal et al. for the increase of prostaglandin synthesis caused by the peptides in cultured human astrocytes, which was mediated through both AT₁ and AT₂ receptors in the case of Ang II and solely through AT₂ receptors in the case of Ang-(1-7). Hence, it appears that tissue and species differences could be critical in determining either similarities or differences between the actions of both peptides.

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References

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