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Angiotensin-(1–7) Does Not Affect Norepinephrine Neuronal Uptake or Catabolism in Rat Hypothalamus and Atria

Mariela M. Gironacci,[1] Martín Rodríguez-Fermepín,[2] Marcelo Vatta,[2] Belisario E. Fernandez,[2] Modesto Rubio,[3] and Clara Peña[1,4]

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SUMMARY

1. Since we previously reported that angiotensin-(1–7) [Ang-(1–7)] increases or inhibits its norepinephrine (NE) release in rat atria or hypothalamus, respectively, the present work was undertaken to investigate the effect of the heptapeptide on NE neuronal uptake and metabolism in atria and hypothalamus isolated from rats.

2. Ang II (1–10 μM) caused a decrease in neuronal NE uptake in both atria and hypothalami isolated from rats. On the contrary, tissues incubated with [^{3}H]NE in the presence of 0.1–10 μM Ang-(1–7) showed no modification in [^{3}H]NE content with respect to the control group, suggesting that the heptapeptide did not modify [^{3}H]NE neuronal uptake.

3. To study the effect of the heptapeptide on NE catabolism, monoamine-oxidase (MAO) and catechol-*O*-methyltransferase (COMT) activities were determined. Pretreatment of the tissue with Ang-(1–7) (0.1–1.0 μM) showed a tendency to diminish MAO activity in rat atria, while no significant changes were observed in hypothalamic MAO activity. Moreover, the heptapeptide (0.1–1.0 μM) did not affect central COMT activity with respect to the control group.

4. Present results allow us to conclude that Ang-(1–7) interacts with noradrenergic neurotransmission by increasing or inhibiting NE release at the peripheral and central levels, respectively, without affecting either the neurotransmitter neuronal uptake or catabolism.

KEY WORDS: angiotensin-(1–7); norepinephrine neuronal uptake; norepinephrine catabolism; monoamine-oxidase activity; cathecol-*O*-methyltransferase activity.

INTRODUCTION

Accumulating evidence suggests that angiotensin (Ang)-(1–7) is an important active component of the renin–angiotensin system which elicits some biological responses

[1] Departamento de Química Biológica and Instituto de Química y Fisicoquímica Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

[2] Cátedra de Fisiopatología, Departamento de Biología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

[3] Instituto Nacional de Investigaciones Farmacológicas (ININFA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

[4] To whom correspondence should be addressed at Departamento de Química Biológica e Instituto de Química y Fisicoquímica Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina. e-mail: clara@qb.ffyb.uba.ar

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in common with Ang II (Ardaillou and Chansel, 1998). At the same time, it displays depressor, vasodilator, and antihypertensive actions, thus lacking the vasoconstrictor, pressor, or dipsogenic effects associated with Ang II (Ferrario *et al.*, 1997; Ardaillou and Chansel, 1998).

It is well known that facilitation of noradrenergic neurotransmission has been considered one of several mechanisms by which Ang II may influence cardiovascular function (Story and Ziogas, 1987). Ang II has been reported to increase the rate of norepinephrine (NE) synthesis, to enhance nerve impulse-evoked NE release, and to inhibit neuronal uptake of the transmitter (Story and Ziogas, 1987). In fact, we have previously shown that Ang-(1–7) also interacts with noradrenergic neuroeffector transmission, but its actions depend on the tissue involved. Thus, at the peripheral level, in rat atria, Ang-(1–7) increases stimulated NE release from presynaptic nerve endings (Gironacci *et al.*, 1994), while at the central level, in the rat hypothalamus, it displays the opposite effect (Gironacci *et al.*, 2000).

Previous studies reported that Ang II and Ang III altered NE neuronal uptake, release, synthesis, turnover, and MAO activity (Vatta *et al.*, 1990; Fernández and Domínguez, 1991; Papouchado *et al.*, 1995; Sumners *et al.*, 1987). Since we previously demonstrated that Ang-(1–7) modified NE release (Gironacci *et al.*, 1994, 2000) we considered it of interest to investigate the heptapeptide effect on NE neuronal uptake as well as on the transmitter catabolism. To this end, we examined Ang-(1–7) actions on monoamine-oxidase (MAO) and catechol-*O*-methyltransferase (COMT) activities in atria and hypothalami isolated from rat.

METHODS

Tissue Preparation

Female Wistar rats weighing 200–240 g were sacrificed by decapitation, and hypothalami and atria were rapidly dissected, cooled, and weighed. Tissues were placed into a glass tube with a mesh of nylon fitted at the bottom to permit free interchange with the medium. Tissues were preincubated at 37°C during 15 min in Krebs solution (pH 7.4) of the following composition (m*M*): NaCl, 118; KCl, 4.7; $MgCl_2$, 1.2; NaH_2PO_4, 1.0; $CaCl_2$, 2.5; EDTA-Na, 0.004; dextrose, 11.1; $NaHCO_3$, 25.0; and ascorbic acid, 0.11; bubbled with a 95% O_2 and 5% CO_2 mixture.

Neuronal [^{3}H]NE Uptake

Tissues were incubated with 0.1 μM dl-[7,8-^{3}H]NE (specific activity 13.5 Ci/mmol) in the absence (control group) or in the presence of Ang-(1–7) or Ang II during 15 min. During this period, MAO activity was inhibited with 100 μM pargyline. After a 5-min washing with Krebs solution, tissues were homogenized and centrifuged and tritium activity was determined in supernatants.

MAO and COMT Activity Assays

Tissues were incubated during 30 min in the absence (control group) or in the presence of Ang-(1–7). MAO and COMT activities were measured according to the methods described by Holt *et al.* (1997) and Jarrot (1971), respectively.

Statistical Analysis

All values are expressed as the mean ± SEM. Data were submitted to one-way analysis of variance (ANOVA) followed by the Bonferroni *t* test. Probability values lower than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Effects of Ang-(1–7) on NE neuronal uptake at both peripheral and central levels were analyzed, using as a positive control Ang II action on this mechanism. As shown in Fig. 1, Ang II (1–10 μM) decreased neuronal NE uptake in both atria (Fig. 1A) and hypothalami (Fig. 1B) isolated from rats, whereas Ang-(1–7) (0.1–10 μM) did not modify this mechanism.

In untreated atria and hypothalamus, MAO activity was linearly related to incubation time up to 50–60 min (data not shown). Due to this fact, all further analyses were performed using a 30-min incubation period. Tissue pretreatment with Ang-(1–7) (0.1–1.0 μM) for 30 min showed a tendency to diminish MAO activity in rat atria (Fig. 2A), while no significant changes were observed in hypothalamic MAO activity (Fig. 2B). As a positive control we consider the stimulatory effect of Ang II on MAO activity previously reported by our group (Fernández and Domínguez, 1991).

Moreover, the heptapeptide (100 nM–1 μM) did not affect central COMT activity with respect to the control group (Fig. 3).

Ang-(1–7) has been suggested to mimic or oppose some of the actions of Ang II (Ardaillou and Chansel, 1998). Contrary to what has been found for the effect of Ang II on neuronal NE uptake (present results), our study shows that Ang-(1–7) has no effect on that mechanism at both central and peripheral levels. In fact, Ang II displays a biphasic effect on central NE neuronal uptake, being stimulatory at short periods and inhibitory with long-term incubations (Sumners and Raizada, 1986). On the other hand, it was unexpected to find no effect of Ang-(1–7) on central NE neuronal uptake since we previously found that the heptapeptide stimulates brain synaptosomal membrane Na^+,K^+-ATPase (López Ordieres *et al.*, 1998), an enzyme known to be involved in the NE neuronal uptake process (Trendelenburg, 1991).

Furthermore, the heptapeptide tends to diminish MAO activity, opposing or lacking the Ang II stimulatory effect observed on MAO activity (Sumners *et al.*, 1987; Fernández and Domínguez, 1991). MAO-A and MAO-B have been considered mitochondrial enzymes involved in the neuronal catecholamine metabolism (Kopin, 1994). MAO deaminated a fraction of catecholamines undergoing presynap-

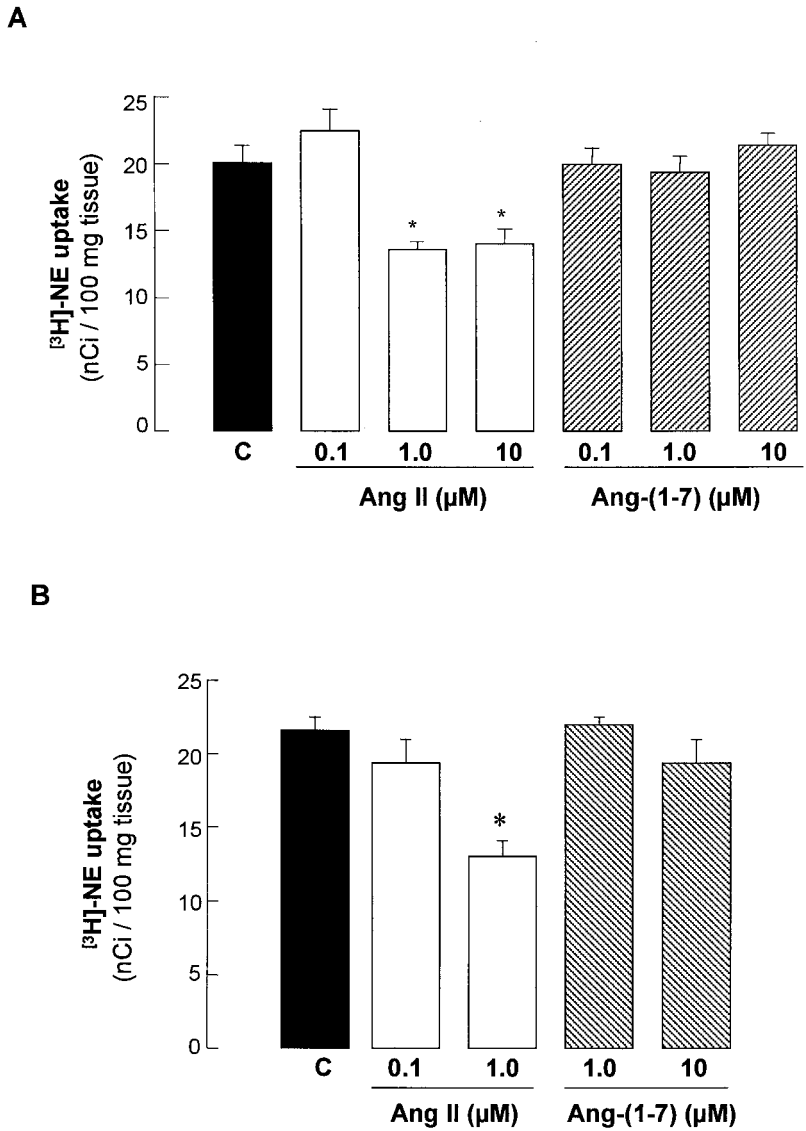

Fig. 1. Effect of Ang II and Ang-(1–7) on [^{3}H]norepinephrine neuronal uptake in atria (A) and hypothalamus (B) isolated from rats. Tissues were incubated during 15 min with 0.1 μM [^{3}H]norepinephrine in the absence (control) or in the presence of Ang II or Ang-(1–7). Black bar represents control group. Data are means ± SEM of six to eight experiments.

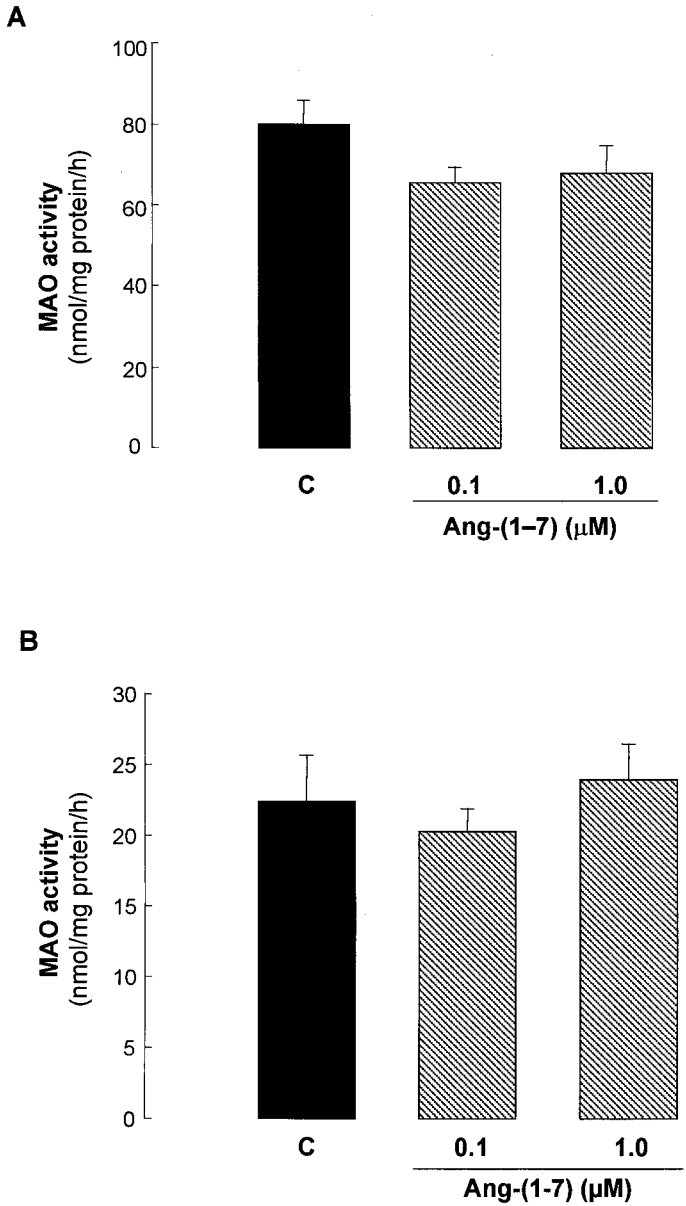

Fig. 2. Effect of Ang-(1–7) on monoamine-oxidase (MAO) activity in atria (A) and hypothalamus (B) isolated from rats. Black bar represents control group. Data are means ± SEM of six to eight experiments.

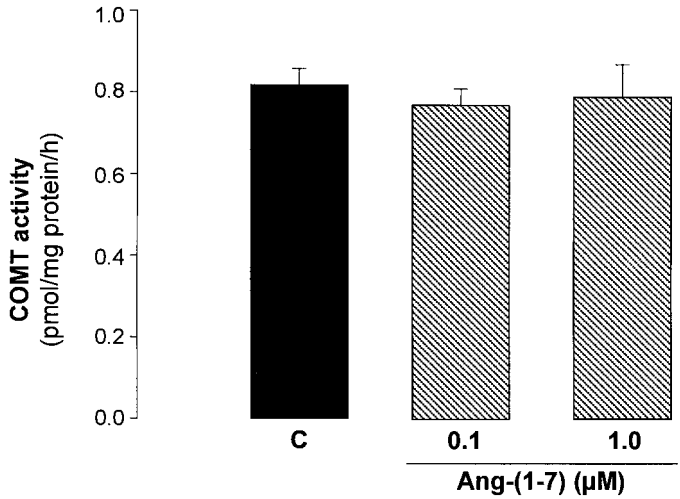

Fig. 3. Effect of Ang-(1–7) on cathecol-*O*-methyltransferase (COMT) activity in rat hypothalamus. Black bar represents control group. Data are means ± SEM of six to eight experiments.

tic uptake, with the rest being taken up into the soluble pool through the amine vesicular transport system (Kopin, 1994). Moreover, COMT is a cytosol-localized enzyme responsible for cathecolamine uptake by extraneuronal tissues (Kopin, 1994). MAO inhibition appears to prolong the effects of sympathetic nerve stimulation (Kopin, 1994). The slight decrease in MAO activity caused by Ang-(1–7) in rat atria could explain the enhancement of NE release by the heptapeptide from presynaptic nerve terminals, although it does not justify by itself the observed NE changes. It must be pointed out that by decreasing MAO activity, more uptaken NE is available to restore the NE content of synaptic vesicles, which in turn increases noradrenergic activity.

Studies about the role of Ang-(1–7) in the control of blood pressure suggest that the renin–angiotensin system possesses the ability to limit the pressor and proliferative actions of Ang II through a mechanism that relies on the alternative generation of Ang-(1–7) (Ferrario *et al.*, 1997). Since our present results show that Ang-(1–7) lacks or oposses Ang II actions on NE uptake or MAO activity, respectively, we could hypothesize that the peptide is limiting Ang II effects. The fact that Ang-(1–7) oposses the central facilitatory effect of Ang II on NE release from presynaptic nerve endings (Gironacci *et al.*, 2000) supports this view. In addition, Ang-(1–7) had no effect on the resting blood pressure in the anesthetized rat by itself, but inhibited Ang II-induced pressor responses (Mahon *et al.*, 1994). Similar inhibition could occur in our experiments, that is, the heptapeptide lacks any effect on NE uptake by itself, but possibly inhibits Ang II effect on AT_1-mediated NE uptake. In fact, it has been suggested that Ang-(1–7) acts as a specific noncompetitive AT_1-receptor antagonist (Mahon *et al.*, 1994).

Present results allow us to conclude that Ang-(1–7) interacts with noradrener-

gic neurotransmission by increasing or inhibiting NE release at the peripheral (Gironacci *et al.*, 1994) and central levels (Gironacci *et al.*, 2000), respectively, without affecting either the neurotransmitter neuronal uptake or catabolism. Therefore, our findings indicate that Ang-(1–7) and Ang II act diversely on sympathetic neurotransmission.

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