Angiotensin-(1-7) Reduces Norepinephrine Release Through a Nitric Oxide Mechanism in Rat Hypothalamus

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Abstract—Angiotensin (Ang)-(1-7) elicits a facilitatory presynaptic effect on peripheral noradrenergic neurotransmission, and because biological responses to the heptapeptide on occasion are tissue specific, the present investigation was undertaken to study its action on noradrenergic neurotransmission at the central level. In rat hypothalamus labeled with [^{3}H]-norepinephrine, 100 to 600 nmol/L Ang-(1-7) diminished norepinephrine released by 25 mmol/L KCl. This effect was blocked by the selective angiotensin type 2 receptor antagonist PD 123319 (1 μmol/L) and by the specific Ang-(1-7) receptor antagonist [D-Ala^7]Ang-(1-7) (1 μmol/L) but not by losartan (10 nmol/L to 1 μmol/L), a selective angiotensin type 1 receptor antagonist. The inhibitory effect on noradrenergic neurotransmission caused by Ang-(1-7) was prevented by 10 μmol/L N^ω-nitro-L-arginine methylester, an inhibitor of nitric oxide synthase activity, and was restored by 100 μmol/L L-arginine, precursor of nitric oxide synthesis. Methylene blue (10 μmol/L), an inhibitor of guanylate cyclase considered as the target of nitric oxide action, as well as Hoe 140 (10 μmol/L), a bradykinin B$_2$-receptor antagonist, prevented the inhibitory effect of the heptapeptide on neuronal norepinephrine release, whereas no modification was observed in the presence of 0.1 to 10 μmol/L indomethacin, a cyclooxygenase inhibitor. Our results indicate that Ang-(1-7) has a tissue-specific neuromodulatory effect on noradrenergic neurotransmission, being inhibitory at the central nervous system by a nitric oxide–dependent mechanism that involves angiotensin type 2 receptors and local bradykinin production. (*Hypertension*. 2000;35:1248-1252.)

Key Words: angiotensin ■ norepinephrine ■ nitric oxide ■ angiotensin antagonist ■ bradykinin ■ prostaglandins

Angiotensin (Ang)-(1-7) is considered a bioactive end product of the renin-angiotensin system formed from Ang I metabolism through an enzymatic pathway independent of the angiotensin-converting enzyme.[1] It can be formed either from Ang I or Ang II by a prolyl-endopeptidase that cleaves the Pro-Phe bond.[1,2]

Ferrario and coworkers[2] demonstrated the presence of Ang-(1-7) in several regions of the rat brain such as the hypothalamus, amygdala, and medulla oblongata but not in the cerebral cortex and cerebellum. In addition, type 1 (AT$_1$) and type 2 (AT$_2$) Ang receptors were described in several regions and nucleus of the central nervous system, including the hypothalamus.[3,4]

Although Ang-(1-7) is not an agonist in terms of activating vasoconstriction,[5] stimulating thirst,[6] or promoting aldosterone secretion,[5] the heptapeptide causes neuronal excitation in the paraventricular nucleus of hypothalamus and dorsal vagal complex of the medulla oblongata,[7] facilitates the noradrenergic neurotransmission,[8] and stimulates prostaglandin[9–11] and vasopressin release[12] with potency comparable to that of Ang II. Conversely, some of the effects of Ang-(1-7) are opposite to those elicited by Ang II, that is, it displays an antiproliferative action on vascular smooth muscle cells,[13]

produces natriuresis[14] and diuresis[15] as well as vasodilation,[16,17] and facilitates the baroreflex activity.[7,18]

Several studies indicated that Ang-(1-7) effects are tissue-specific, that is, the heptapeptide activates Na$^+$, K$^+$-ATPase in rat brain synaptosomal membranes, whereas a biphasic effect on this enzymatic system is observed in rat renal membranes.[19] Furthermore, Gironacci et al[8] have reported a facilitatory effect of the heptapeptide on the sympathetic neurotransmission in rat atria. Conversely, this stimulatory action was not detected in the rabbit vas deferens.[11]

Because of the suggested tissue-specific activity and the presynaptic effect of Ang-(1-7) on peripheral noradrenergic neurotransmission,[8] the present study was performed to assess the effect of Ang-(1-7) on K$^+$-evoked neuronal release of norepinephrine (NE) in rat hypothalamus.

Methods

Animals and Chemicals

Male Sprague-Dawley rats weighing 250 to 300 g were used. DL-[7,8-^{3}H] norepinephrine (specific activity 32 Ci/mmol) was purchased from Amersham Life Science; cocaine hydrochloride was kindly supplied by Dr Edda Villaamil (Cátedra de Toxicología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires);
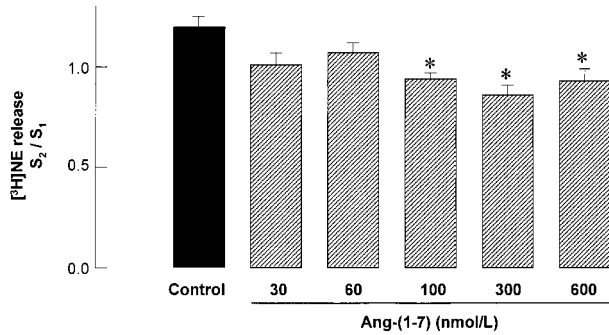
Figure 1. Effect of Ang-(1-7) on K$^+$-evoked [^{3}H]NE release in rat hypothalamus. S$_2$/S$_1$ represents ratio between tritium overflow in response to second (S$_2$) and first (S$_1$) 25 mmol/L KCl stimulation periods. Ang-(1-7) was added to medium during S$_2$. Values are mean±SEM (n=10). *$P<0.05$ as compared with control.

Hoe 140 was kindly supplied by Dr Martiarena (Cátedra de Control de Medicamentos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires); hydrocortisone, pargyline, indomethacin, methylene blue, L-arginine, and N^ω-nitro-L-arginine methylester were from Sigma Chemical Co. Ang-(1-7) and [D-Ala^7]Ang-(1-7) were synthesized in our laboratory by the Merrifield solid-phase procedure, as previously described.[8]

Experimental Protocol

[^{3}H]NE release was measured according to the technique described by Vatta et al,[20] with slight modification. Briefly, minced rat hypothalami were incubated at 37°C for 30 minutes in 2 mL of standard Krebs solution. Monoamine-oxidase activity and extraneuronal NE uptake were inhibited by the addition of 0.1 mmol/L pargyline and 0.1 mmol/L hydrocortisone, respectively. NE stores were labeled with 2.5 μCi/mL [^{3}H]NE (100 nmol/L) during a 30-minute incubation period. After 8 consecutive 5-minute washes with Krebs solution, tissues were then incubated during a period of 4 minutes in high-potassium Krebs solution containing 0.1 mmol/L cocaine to inhibit neuronal NE uptake, 0.1 mmol/L hydrocortisone, and 0.1 mmol/L pargyline. Two consecutive samples of incubation medium were collected every 2 minutes (S$_1$ and S$_2$), and [^{3}H]NE release was measured in each period as the amount of radioactivity present in the incubation medium. Ang-(1-7) and losartan were present during the second 2-minute period. PD 123319 and [D-Ala^7]Ang-(1-7) were added during S$_1$ and S$_2$. Methylene blue was present in the medium 15 minutes before sample collection. Results are expressed as the ratio of the radioactivity released by the second and first potassium-stimulation periods (S$_2$/S$_1$).

Statistical Analysis

All values are mean±SEM. Data were submitted to 1-way ANOVA followed by the Bonferroni test. Probability values <0.05 were considered statistically significant.

Results

To determine Ang-(1-7) effect on K$^+$-evoked neuronal NE release in hypothalamus, different concentrations of the heptapeptide were tested. Data given in Figure 1 show that 100, 300, and 600 nmol/L Ang-(1-7) diminished neuronal NE release evoked by K$^+$.

To study the Ang-receptor subtypes coupled to the inhibitory activity of Ang-(1-7) on neuronal NE release, the effects of selective antagonists for AT$_1$- and AT$_2$-receptor subtypes were assessed. Results showed that losartan (10 nmol/L to 1 μmol/L), a selective AT$_1$-receptor antagonist, diminished neuronal NE release evoked by 25 mmol/L KCl (data not shown). In addition, the reduction of evoked NE release
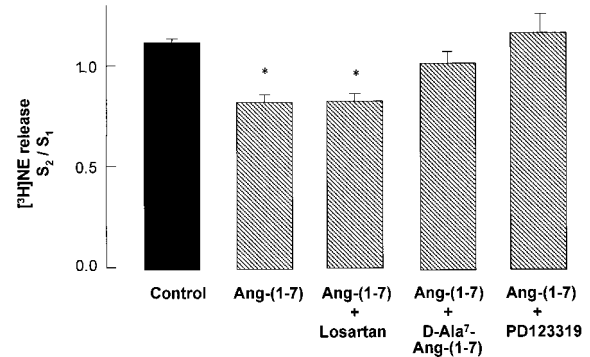

Figure 2. Effect of losartan, [D-Ala^7]Ang-(1-7), and PD 123319 on decreased [^{3}H]NE release induced by Ang-(1-7) in rat hypothalamus. S$_2$/S$_1$ represents ratio between tritium overflow in response to second (S$_2$) and first (S$_1$) 25 mmol/L KCl stimulation periods. Ang-(1-7) (100 nmol/L) was added alone or with 1 μmol/L of each antagonist during S$_2$. [D-Ala7]Ang-(1-7) and PD 123319 preceded the addition of Ang-(1-7) by 2 minutes. Values are mean±SEM (n=10). *$P<0.05$ as compared with control.

produced by 100 nmol/L Ang-(1-7) was not blocked by losartan (10 nmol/L to 1 μmol/L) (Figure 2). On the other hand, the role of AT$_2$ receptor on Ang-(1-7) reduction of evoked NE release in hypothalamus was studied in the presence of PD 123319, a selective AT$_2$-receptor blocker, which, at levels >1 μmol/L, abolished Ang-(1-7)–inhibitory effects (Figure 2). Simultaneous addition of losartan and PD 123319 did not modify the stimulated NE release (data not shown).

The selective Ang-(1-7) antagonist [D-Ala^7]Ang-(1-7) (1 μmol/L) partially blocked the effect of Ang-(1-7) on NE release (Figure 2). PD 123319 and D-Ala^7-Ang-(1-7) each had no effect by itself on NE release (data not shown).

It has been demonstrated that Ang-(1-7) exerts several effects through the NO pathway.[16,21–23] To assess the role of NO as possible mediator of Ang-(1-7) effect on neuronal NE release evoked by K$^+$ in rat hypothalamus, we investigated the effect of Ang-(1-7) in the presence of N^ω-nitro-L-arginine methylester (L-NAME), an inhibitor of NO synthase activity. The reduction of evoked NE release produced by 100 nmol/L Ang-(1-7) was prevented by the addition of 10 μmol/L L-NAME and was restored when L-arginine (100 μmol/L), the precursor of NO synthesis, was simultaneously present (Figure 3). Moreover, 10 μmol/L methylene blue, an inhibitor of guanylate cyclase considered as the target of NO action, prevented the inhibitory effect of Ang-(1-7) on NE release (Figure 3). L-NAME and methylene blue did not alter K$^+$-evoked neuronal NE output by itself (data not shown).

It has been reported that NO formation after AT$_2$-receptor stimulation is due to the activation of local bradykinin (BK) production.[24,25] Therefore, we investigated the effect of Hoe 140, a kinin B$_2$-receptor antagonist, on the inhibitory effect of Ang-(1-7) on NE release. As shown in Figure 4, 10 μmol/L Hoe 140 completely blocked the induced reduction of K$^+$-evoked neuronal NE release caused by the heptapeptide in rat hypothalamus. The antagonist had no direct effect.

To assess if the prostaglandin pathway is involved in Ang-(1-7) effects, experiments were performed in the presence of 0.1 to 10 μmol/L indomethacin, a cyclooxygenase
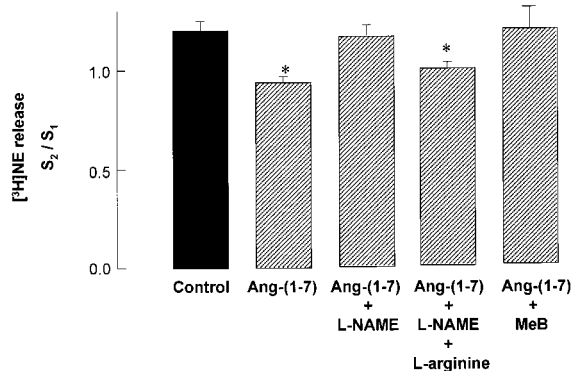
Figure 3. Effects of L-NAME and methylene blue (MeB) on decreased [^{3}H]NE release induced by Ang-(1-7) in rat hypothalamus. S_2/S_1 represents ratio between tritium overflow in response to second (S_2) and first (S_1) 25 mmol/L KCl stimulation periods. Ang-(1-7) (100 nmol/L) was added alone or with 10 μmol/L L-NAME or 10 μmol/L L-NAME plus 100 μmol/L L-arginine or 10 μmol/L MeB during S_2. L-Arginine and L-NAME preceded addition of peptide by 2 minutes, whereas MeB was present in medium 15 minutes before Ang-(1-7) was added. Values are mean±SEM (n=10). *$P<0.05$ as compared with control.

inhibitor. No differences in the effect elicited by 100 nmol/L Ang-(1-7) were observed (Figure 5). Indomethacin (0.1 to 10 μmol/L) failed to modify by itself the K$^+$-evoked neuronal NE release (data not shown).

Discussion

In agreement with previous studies in rat medulla,[26] the present findings indicate that Ang-(1-7) attenuates the K$^+$-evoked neuronal [^{3}H]NE release from rat hypothalamus. The inhibitory effect induced in rat hypothalamus differs from that produced in the peripheral nervous system, in which the heptapeptide acts presynaptically, increasing NE released by nerve stimulation.[8]

It has been reported that Ang-(1-7) elicits a tissue-specific neuromodulatory action. In this regard, a facilitatory effect on noradrenergic neurotransmission in rat atria was observed,[8] but no effect in rabbit vas deferens was described.[11]

Biological activity of Ang-(1-7) is distinguishable from that of Ang II, and frequently contrasting effects were
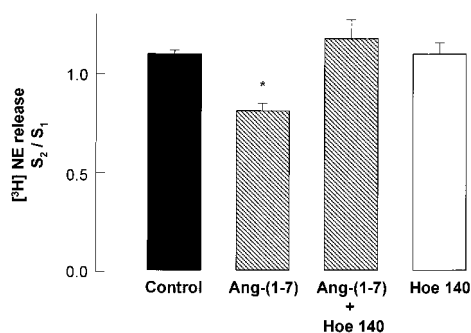

Figure 4. Effect of Hoe 140 on decreased [^{3}H]NE release induced by Ang-(1-7) in rat hypothalamus. S_2/S_1 represents ratio between tritium overflow in response to second (S_2) and first (S_1) 25 mmol/L KCl stimulation periods. Ang-(1-7) (100 nmol/L) was added during S_2. Hoe 140 (10 μmol/L) was present in medium 2 minutes before the addition of Ang-(1-7). Values are mean±SEM (n=7). *$P<0.05$ as compared with control.
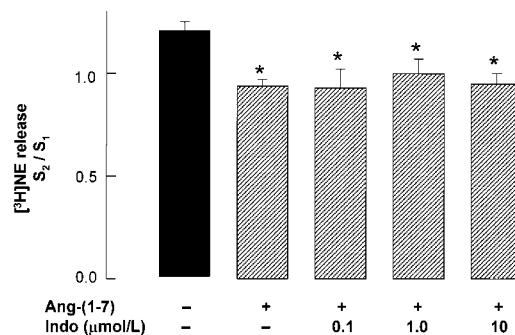

Figure 5. Effect of indomethacin (Indo) on decreased [^{3}H]NE release induced by Ang-(1-7) in rat hypothalamus. S_2/S_1 represents ratio between tritium overflow in response to second (S_2) and first (S_1) 25 mmol/L stimulation periods. Ang-(1-7) (100 nmol/L) was added during S_2. Indo (0.1 to 10 μmol/L) was present in medium 2 minutes before the addition of the peptide. Values are mean±SEM (n=8) *$P<0.05$ as compared with control.

observed[27]: For example, Ang-(1-7) facilitates the baroreflex[7,18] as well as induces vasodilation,[16,17,21] effects not produced by Ang II. There is evidence that the renin-angiotensin system regulates several functions through multiple-level feedback mechanisms.[28,29] Contrasting activities of Ang-(1-7) and Ang II would further confirm the hypothesis that the renin-angiotensin system may limit Ang II effects through generation of the heptapeptide.[27] In agreement, the present results show that Ang-(1-7) opposes the enhancement on K$^+$-induced [^{3}H]-NE release from rat hypothalamus caused by Ang II.[30,31]

The decreased NE release caused by Ang-(1-7) was blocked by the AT$_2$-receptor antagonist PD 123319 and not by the AT$_1$-receptor antagonist losartan, suggesting that AT$_2$ receptors are involved in such response. In fact, central responses of Ang-(1-7) appear to be more sensitive to inhibition by AT$_2$-receptor antagonists. For example, prostaglandin synthesis in human astrocytes[9] and substance P release in rat hypothalamus[32] as well as neuronal excitation in the paraventricular nucleus[7] induced by Ang-(1-7) were blocked by AT$_2$-receptor antagonists.

Interestingly, both AT$_1$ and AT$_2$ receptors appear to have antagonistic roles on central noradrenergic neurotransmission, since Ang II–facilitated NE release is mediated by AT$_1$ receptors[4] whereas Ang-(1-7) inhibitory action on NE release is coupled to AT$_2$ receptors (present results). Several reports have suggested that these receptors mediate opposite physiological effects.[24]

Furthermore, the inhibitory action of Ang-(1-7) on NE release was partially prevented by [D-Ala^7]Ang-(1-7), suggesting that Ang-(1-7) receptors are also involved. Likewise, the stimulation of specific Ang-(1-7) receptors accounts for the Ang-(1-7)–induced excitation of paraventricular neurones in rat[33] and [^{3}H]arachidonic acid release in rabbit aortic smooth muscle cells.[34] In this latter case, both PD 123319 and [D-Ala^7]Ang-(1-7) were required to fully block the response.

Because Ang II increases NE release through AT$_1$-receptor activation and not through AT$_2$ receptors, the opposite response induced by losartan itself may result from blockade of

endogenous Ang II binding to AT_1 receptors, as it was previously suggested.[35,36] We disregarded this possibility in our results because the addition of losartan plus PD 123319 did not modify the stimulated NE release, suggesting that losartan may unmask the binding of endogenous either Ang-(1-7) or Ang II to AT_2 sites.

Ang-(1-7) is a potent stimulator of prostaglandin release in neural and vascular cells.[9–11] Furthermore, vasodilation of cerebral arteries as well as the natriuresis and depressor activities produced by Ang-(1-7) could be abolished by indomethacin, suggesting that these effects are mediated by prostaglandins.[37] Despite their inhibitory role on NE release, the attenuation on sympathetic neurotransmission caused by Ang-(1-7) in the rat hypothalamus (present results) was not prevented by indomethacin, excluding prostaglandin involvement.

Because the neuromodulatory effect of Ang-(1-7) on NE release was blocked by L-NAME and methylene blue, an NO-mediated mechanism is suggested in accordance with previous reports that demonstrated NO dependence in various Ang-(1-7) effects.[16,21–23] Moreover, NO formation induced by Ang-(1-7) (present data) after AT_2-receptor stimulation appears to be due to the activation of local BK production, since the inhibitory effect of the heptapeptide on NE release disappeared in the presence of Hoe 140, a B_2-receptor antagonist. Accordingly, Seyedi et al[38] have shown that the increased aortic NO production induced by Ang-(1-7) resulted from the activation of AT_2 receptors and also involved local BK production. In fact, it recently has been shown that mice lacking AT_2 receptors have low renal BK and NO production,[25] suggesting that these receptors mediate BK and NO formation. It should be pointed out that Ang-(1-7) interaction with kinins is a receptor-mediated event and not simply attributable to angiotensin-converting enzyme inhibition, which may possibly prevent BK degradation[39] or down-regulation of the B_2 receptor.[40]

In conclusion, Ang-(1-7) has a tissue-specific neuromodulatory effect on noradrenergic neurotransmission, being inhibitory at the central level by a NO-dependent mechanism that involves AT_2 receptors and local BK generation.

References
1. Ferrario CM, Brosnihan KB, Diz DI, Jaiswal N, Khosla MC, Milsted A, Tallan EA. Angiotensin-(1-7): a new hormone of the angiotensin system. *Hypertension*. 1991;18(suppl III):III-126-III-133.
2. Ferrario CM, Barnes KL, Block CH, Brosnihan KB, Diz DI, Khosla MC, Santos RAS. Pathways of angiotensin formation and function in the brain. *Hypertension*. 1990;15(suppl I):I-13-I-19.
3. Bottari SP, de Gasparo M, Steckelings M, Leven NR. Angiotensin II receptors subtypes: characterization, signalling mechanisms, and possible physiological implications. *Front Neuroendocrinol*. 1993;14:123–171.
4. Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JAM, Smith RD. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev*. 1993; 45:205–251.
5. Kono T, Taniguchi A, Imura H, Oseko F, Khosla MC. Biological activities of angiotensin II-(1-6)-hexapeptide and angiotensin II-(1-7)-heptapeptide in man. *Life Sci*. 1986;38:1515–1519.
6. Fitzsimons JT. The effect on drinking of peptide precursors and of shorter chain peptide fragments of angiotensin II injected into the rat's diencephalon. *J Physiol*. 1971;214:295–303.
7. Santos RAS, Campagnole-Santos MJ. Central and peripheral actions of angiotensin-(1-7). *Braz J Med Biol Res*. 1994;27:1033–1047.
8. Gironacci MM, Adler-Graschinsky E, Peña C, Enero MA. Effects of angiotensin II and angiotensin (1-7) on the release of [³H]-norepinephrine from rat atria. *Hypertension*. 1994;24:457–460.
9. Jaiswal N, Tallant EA, Diz DI, Khosla MC, Ferrario CM. Subtype 2 angiotensin receptors mediate prostaglandin synthesis in human astrocytes. *Hypertension*. 1991;17:1115–1120.
10. Jaiswal N, Diz DI, Chappell MC, Khosla MC, Ferrario CM. Stimulation of endothelial cell prostaglandin production by angiotensin peptides. *Hypertension*. 1992;19(suppl II):II-49-II-55.
11. Trachte GJ, Meixner K, Ferrario CM, Khosla MC. Prostaglandin production in response to angiotensin-(1-7) in rabbit isolated vasa deferentia. *Prostaglandins*. 1990;39:385–394.
12. Schiavone MT, Santos RAS, Brosnihan KB, Khosla MC, Ferrario CM. Release of vasopressin from the rat hypothalamo-neurohypophysial system by angiotensin-(1-7) heptapeptide. *Proc Natl Acad Sci U S A*. 1988;85:4095–4098.
13. Freeman EJ, Chisolm GM, Ferrario CM, Tallant EA. Angiotensin-(1-7) inhibits vascular smooth muscle cell growth. *Hypertension*. 1996;28: 104–108.
14. DelliPizzi AM, Hilchey SD, Bell-Quilley CP. Natriuretic action of angiotensin-(1-7). *Br J Pharmacol*. 1994;111:1–3.
15. Handa RK, Ferrario CM, Strandhoy JW. Renal actions of angiotensin-(1-7): in vivo and in vitro studies. *Am J Physiol*. 1996;270:F141–F147.
16. Osei SY, Ahima RS, Minkes RK, Weaver JP, Khosla MC, Kadowitz PJ. Differential responses to angiotensin-(1-7) in the feline mesenteric and hindquarters vascular beds. *Eur J Pharmacol*. 1993;234:35–42.
17. Pörsti I, Bara AT, Busse R, Hecker M. Release of nitric oxide by angiotensin-(1-7) from porcine coronary endothelium: implications for a novel angiotensin receptor. *Br J Pharmacol*. 1994;111:652–654.
18. Britto RR, Santos RAS, Fagundes-Moura CR, Khosla MC, Campagnole-Santos MJ. Role of angiotensin-(1-7) in the modulation of the baroreflex in renovascular hypertensive rats. *Hypertension*. 1997;30(part 2):549–556.
19. López Ordieres MG, Gironacci MM, Rodríguez de Lores Arnaiz G, Peña C. Effect of angiotensin-(1-7) on ATPase activities in several tissues. *Regul Peptides*. 1998;77:135–139.
20. Vatta M, Peña C, Fernandez BE, Rodriguez de Lores Arnaiz G. A brain Na^+, K^+- ATPase inhibitor (Endobain E) enhances norepinephrine release in rat hypothalamus. *Neuroscience*. 1999;90:573–579.
21. Brosnihan KB, Li P, Ferrario CM. Angiotensin-(1-7) dilates canine coronary arteries through kinins and nitric oxide. *Hypertension*. 1996;27(part 2):523–528.
22. Gironacci MM, Lorenzo PS, Adler-Graschinsky E. Possible participation of nitric oxide in the increase of norepinephrine release caused by angiotensin peptides in rat atria. *Hypertension*. 1997;29:1344–1350.
23. Nakamoto H, Ferrario CM, Fuller SB, Robaczewski DL, Winicov E, Dean RH. Angiotensin-(1-7) and nitric oxide interaction in renovascular hypertension. *Hypertension*. 1995;25:796–802.
24. Inagami T. Molecular biology and signaling of angiotensin receptors: an overview. *J Am Soc Nephrol*. 1999;10:S2–S7.
25. Siragy HM, Inagami T, Ichiki T, Carey RM. Sustained hypersensitivity to angiotensin II and its mechanism in mice lacking the subtype-2 (AT_2) angiotensin receptor. *Proc Natl Acad Sci U S A*. 1999;96:6506–6510.
26. Diz DI, Pirro NT. Differential actions of angiotensin II and angiotensin-(1-7) on transmitter release. *Hypertension*. 1992;19(suppl II):II-41–II-48.
27. Ferrario CM, Chappell MC, Tallant EA, Brosnihan KB, Diz DI. Counterregulatory actions of angiotensin-(1-7). *Hypertension*. 1997;30(part 2):535–541.
28. Schunker H, Ingelfinger JR, Jacob H, Jackson B, Bouyounes B, Dzau VJ. Reciprocal feedback regulation of kidney angiotensinogen and renin mRNA expression by angiotensin II. *Am J Physiol*. 1992;263: E863–E869.
29. Timmermans PBMWM, Benfield P, Chiu AT, Herblin WF, Wong PC, Smith RD. Angiotensin II receptors and functional correlates. *Am J Hypertens*. 1992;5:221S–235S.

30. Vatta MS, Bianciotti LG, Papouchado ML, Locatelli AS, Polidoro-Arena J, Fernandez BE. Modulation of noradrenergic neurotransmission by angiotensin II and angiotensin III. *Pharmacodynamics Therapeutics (Life Sci Adv)*. 1990;9:177–185.

31. Vatta MS, Papouchado ML, Bianciotti LG, Fernandez BE. Atrial natriuretic factor inhibits noradrenaline release in the presence of angiotensin II and III in the rat hypothalamus. *Comp Biochem Physiol*. 1993;106C:545–548.

32. Diz DI, Bosch SM, Westwood B. Identification of angiotensin receptor subtypes mediating substance P release in brain slices of hypothalamus and medulla. *Hypertension*. 1998;32:594. Abstract.

33. Ambühl P, Felix D, Khosla MC. [7-D-Ala]-angiotensin-(1-7): selective antagonism of angiotensin-(1-7) in the rat paraventricular nucleus. *Brain Res Bull*. 1994;35:289–291.

34. Muthalif MM, Benter IF, Uddin MR, Harper JL, Malik KU. Signal transduction mechanisms involved in angiotensin-(1-7)-stimulated arachidonic acid release and prostanoid synthesis in rabbit aortic smooth muscle cells. *J Pharmacol Exp Ther*. 1998;284:388–398.

35. Kumagai K, Reid IA. Losartan inhibits sympathetic and cardiovascular responses to carotid occlusion. *Hypertension*. 1994;23(part 2):827–831.

36. Suzuki Y, Matsumura Y, Egi Y, Morimoto S. Effects of losartan, a nonpeptide angiotensin II receptor antagonist on norepinephrine overflow and antidiuresis induced by stimulation of renal nerves in anesthetized dogs. *J Pharmacol Exp Ther*. 1992;263:956–963.

37. Ardaillou R, Chansel D. Synthesis and effects of active fragments of angiotensin II. *Kidney Int*. 1998;52:1458–1468.

38. Seyedi N, Xu X, Nasjletti A, Hintze TH. Coronary kinin generation mediates nitric oxide release after angiotensin receptor stimulation. *Hypertension*. 1995;26:164–170.

39. Li P, Chappell MC, Ferrario CM, Brosnihan KB. Angiotensin-(1-7) augments bradykinin-induced vasodilation by competing with ACE and releasing nitric oxide. *Hypertension*. 1997;29(part 2):394–400.

40. Deddish PA, Marcic B, Jackman HL, Wang H, Skidgel RA, Erdös EG. N-Domain-specific substrate and C-domain inhibitors of angiotensin-converting enzyme. *Hypertension*. 1998;31:912–917.