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Role of Central α_1- and α_2-Adrenoceptors on the Dipsogenic and Cardiovascular Effect of Angiotensin II

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COLOMBARI, E., W. A. SAAD, L. A. A. CAMARGO, A. RENZI, L. A. DE LUCA, JR. AND J. V. MENANI. *Role of central α_1- and α_2-adrenoceptors on the dipsogenic and cardiovascular effect of angiotensin II.* PHARMACOL BIOCHEM BEHAV **36**(4) 893–896, 1990.—We investigated the effects of previous central treatment with prazosin (an α_1-adrenoceptor antagonist) or clonidine (an α_2-adrenoceptor agonist) on the dipsogenic, pressor and tachycardic responses produced by intracerebroventricular (ICV) injection of angiotensin II (AII) in conscious rats. Holtzman rats with a chronic cannula implanted in the lateral ventricle were tested for dipsogenic and cardiovascular (arterial pressure and heart rate) responses in separate experiments. Previous ICV treatment with clonidine (20, 40, 80 and 120 nmol) abolished the pressor, tachycardic and dipsogenic effects of ICV AII. After all doses of prazosin (40, 80 and 120 nmol), AII induced bradycardic responses, but only the 80 and 120 nmol doses of prazosin reduced the pressor responses to AII. Prazosin produced no alteration in the dipsogenic effect of AII. The results show that the periventricular α_1-adrenoceptors are involved only in the cardiovascular responses produced by central AII, whereas clonidine acting through α_2-adrenergic and/or imidazole receptors can modulate all actions of AII.

Arterial pressure Thirst α-Adrenoceptors Angiotensin II

INCREASED mean arterial pressure (MAP), heart rate (HR) and water intake are well-known effects of central angiotensin II (AII) in rats (4, 7, 14, 25). Central catecholamine-containing neurons participate in the regulation of arterial pressure and the control of drinking behavior. The integrity of the central nervous system (CNS) catecholamines is required for the development of one-kidney renal hypertension and the increased drinking which accompanies it. The acute pressor responses produced by intracerebroventricular (ICV) injections of AII or carbachol are abolished by central catecholamine depletion with 6-hydroxydopamine (6-OHDA). The drinking produced by central cholinergic stimulation remains intact while AII drinking is significantly reduced (16). Forebrain dopamine (DA) is essential for the mediation of sensorimotor integration or general arousal mechanisms involved in responses to acute homeostatic stressors, but only the noradrenergic system has a specific role in the mediation of AII-induced thirst and blood pressure responses (1–3, 17).

ICV injection of phentolamine (an α-adrenergic antagonist) prevented angiotensin-induced drinking, sympathetic stimulation and vasopressin release (26). An interaction between central AII effects on drinking behavior or pressor responses and the adrenergic receptors was also shown by Jones (19), who suggested that α_1-, but not α_2-adrenergic receptors, in the rostral hypothalamus are involved in the control of both the drinking and pressor responses elicited by ICV injections of AII. The β-adrenergic receptors altered only the drinking response in a nonspecific manner. Camacho and Phillips (8) reported that the previous ICV administration of phentolamine blocked the pressor, but not the dipsogenic response produced by ICV injection of AII.

In the present study we investigated the effect of previous ICV treatment with prazosin (an α_1-adrenergic antagonist) or clonidine (an α_2-adrenergic agonist) on the cardiovascular and dipsogenic responses induced by central AII.

METHOD

Animals

Male albino Holtzman rats weighing 250 to 300 g were used. The animals were maintained in individual cages with free access to pellet chow and tap water.

Brain Surgery

The rats were anesthetized with ether and submitted to stereotaxic implant of a unilateral stainless steel cannula (0.7 mm

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o.d.) directed to a point 2 mm above the left lateral ventricle (LV). The cannula was positioned at the bregma level, 1.5 mm lateral to the midline to a depth of 3.0 mm from the skull (10). The cannula was fixed to the skull with screws and acrylic resin.

Arterial Pressure and Heart Rate Recording

Direct mean arterial pressure and heart rate were measured in unanesthetized and unrestrained rats by means of polyethylene (PE) tubing (PE-10 connected to a PE-50) inserted into the abdominal aorta through the femoral artery under ether anesthesia on the day before the experiment. The cannula was tunneled subcutaneously to the back of the rat and connected, through a 30-cm length of PE-90, to a Statham (P23-Db) pressure transducer (Statham-Gould, USA) coupled to a multichannel recorder (Physiograph 4A, Narco Bio-Systems, USA). The heart rate was recorded using a biotachometer (Narco Bio-Systems) activated with signals from the arterial pressure pulse. No water was offered to the rats during arterial pressure and heart rate recordings.

Water Ingestion Recording

Water intake was tested in a separate experiment in satiated animals. Each rat was tested in its individual cage. The volume of water consumed during 1 hr after the drug was measured using a burette with 0.1 ml marks fitted with a metal spout for water ingestion.

Drug Injection Into the Brain

Angiotensin II (Sigma Lab.), prazosin hydrochloride (Pfizer) and clonidine hydrochloride (Boheringer-Ingelheim) were used. The drugs were dissolved in saline solution (0.15 M NaCl) and injected into the LV using a Hamilton (10 μl) microsyringe connected by PE-10 tubing (20 cm) to a dental needle (0.3 mm o.d.), 2 mm longer than the guide cannula fixed in the animal's head. The volume injected was 1 μl.

Histology

At the end of the experiments the animals were anesthetized with ether and submitted to cerebral perfusion with 10% formalin through the heart. The brains were removed, fixed in 10% formalin, frozen, cut into 20-μm sections, stained with hematoxylin-eosin and analyzed under light microscopy to confirm the position of the cannula in the LV.

Statistical Analysis

The results are reported as mean ± SEM. The Student *t*-test, Mann-Whitney U-test or analysis of variance were used for the comparisons. Differences were considered significant when $p<0.05$.

Experimental Protocol

The effects of prazosin and clonidine on the cardiovascular and dipsogenic actions of AII were studied in different groups of animals: 0.15 M NaCl (control); 0.15 M NaCl + AII (12 ng); prazosin (40, 80 and 120 nmol) + AII (12 ng); clonidine (20, 40, 80 and 120 nmol) + AII (12 ng). Prazosin or clonidine was injected into the LV 25 minutes before the injection of AII into the same site.

The arterial pressure and heart rate recording was started 30 minutes before the first drug injection and terminated 60 minutes after AII injection.

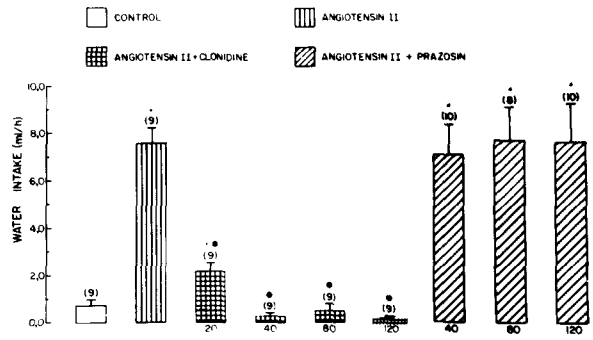

FIG. 1. Water intake produced by ICV injection of angiotensin II (12 ng) in rats previously injected ICV with prazosin or clonidine. The results are expressed as mean ± SEM. The number of rats is given at the top and the doses of prazosin or clonidine are given at the bottom of each column. *Different from control ($p<0.05$); ● different from angiotensin II only.

During the water ingestion experiments, rats had free access to water. The water ingestion measurement was done for 1 hr after the second injection.

RESULTS

Effect of Previous Treatment With Prazosin on the Central Cardiovascular and Dipsogenic Action of AII

ICV injection of AII (12 ng) in normotensive rats (MAP = 120 ± 6 mmHg, HR = 356 ± 13 bpm) produced pressor (32 ± 3 mmHg), tachycardic (41 ± 8 bpm) and dipsogenic (7.6 ± 0.6 ml/hr) responses. When only 0.15 M NaCl (control) was injected ICV, water ingestion was 0.7 ± 0.2 ml/hr, but no significant alteration was observed in arterial pressure or heart rate.

Previous ICV injection of the α_1-adrenergic antagonist prazosin reduced the pressor and tachycardic, but not the dipsogenic responses produced by central AII (Figs. 1, 2 and 3). After prazosin (80 and 120 nmol), the pressor responses produced by AII (12 ng) were reduced to 7 ± 3 and 8 ± 2 mmHg, respectively. The tachycardic response was abolished and a discrete bradycardic response of −9 ± 12, −3 ± 3 and −17 ± 16 bpm, respectively, was observed with previous prazosin treatment (40, 80 and 120 nmol).

Only ICV prazosin (40, 80 and 120 nmol) injection produced a depressor response (−9 ± 2, −14 ± 4 and −21 ± 4 mmHg, respectively) and tachycardia (51 ± 8, 31 ± 4 and 64 ± 9 bpm, respectively). The greatest depressor and tachycardic responses were observed 15 minutes after prazosin injection and were still present when AII was injected.

Effect of Previous Treatment With Clonidine on the Central Cardiovascular and Dipsogenic Action of AII

Previous ICV injection of the α_2-adrenergic agonist clonidine reduced all the central effects of AII studied (Figs. 1, 2 and 3). After clonidine (20, 40, 80 and 120 nmol), the pressor responses produced by ICV AII (12 ng) were reduced to 16 ± 5, 9 ± 4, 3 ± 2 and 5 ± 2 mmHg, respectively. The increase in heart rate (1 ± 5, 1 ± 1, 3 ± 3 and 4 ± 4 bpm, respectively) and water intake (2.2 ± 0.4, 0.3 ± 0.1, 0.5 ± 0.3 and 0.1 ± 0.1 ml/hr, respectively) showed that the tachycardic and dipsogenic actions of central AII were also abolished after previous treatment with clonidine (20, 40, 80 and 120 nmol).

Only ICV clonidine (20, 40, 80 and 120 nmol) injection

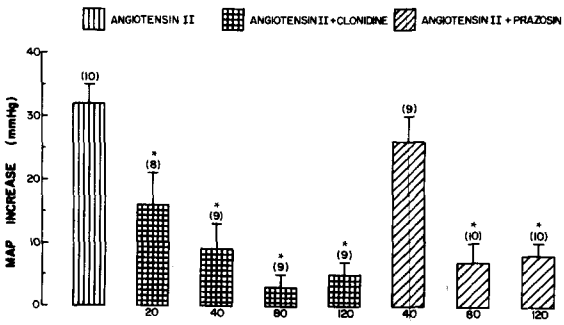

FIG. 2. Mean arterial pressure (MAP) increase produced by ICV injection of angiotensin II (12 ng) in rats previously injected ICV with prazosin or clonidine. The results are expressed as mean ± SEM. The number of rats is given at the top and the doses of prazosin or clonidine are given at the bottom of each column. *Different from angiotensin II ($p<0.05$).

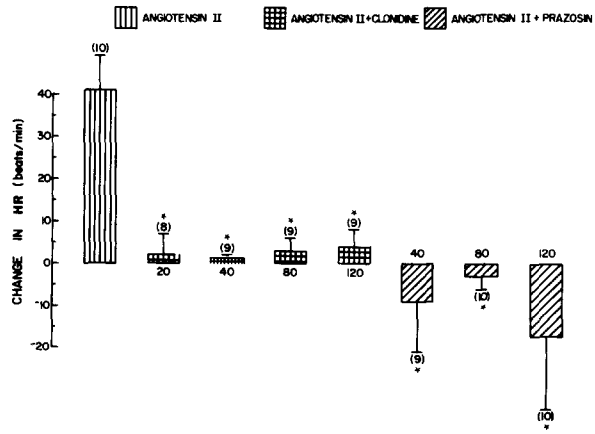

FIG. 3. Changes in heart rate (HR) produced after ICV injection of angiotensin II (12 ng) in rats previously injected ICV with prazosin or clonidine. The results are expressed as mean ± SEM. The number of rats is given at the top and the doses of prazosin or clonidine are given at the bottom of each column. *Different from angiotensin II ($p<0.05$).

produced a transitory pressor response lasting 15 to 20 minutes (43 ± 4, 40 ± 5, 41 ± 4 and 44 ± 3 mmHg, respectively). The bradycardic responses (-76 ± 7, -65 ± 16, -77 ± 11 and -150 ± 19 bpm, respectively) were still present at the time of AII injection (25 minutes after clonidine injection).

DISCUSSION

Previous reports have shown the importance of central catecholaminergic neurons and adrenergic receptors for the central pressor and dipsogenic actions of AII (1–3, 19, 26). In the present study we showed that previous ICV injection of clonidine blocked all central actions of AII. The previous injection of prazosin blocked the pressor and tachycardic effect of central AII, but not the dipsogenic response.

Severs *et al.* (26) showed that previous ICV injection of phentolamine prevents angiotensin-induced drinking, sympathetic stimulation and vasopressin release. Camacho and Phillips (8) reported that the previous ICV administration of phentolamine blocked the pressor, but not the dipsogenic response produced by the injection of AII into the same site. The present results showing that prazosin blocked the pressor, but not the dipsogenic response to central AII is in accordance with those reported by Camacho and Phillips (8) for phentolamine. These results show a dissociation in the central pathways involved in the pressor and dipsogenic responses to AII. The present results show that specifically the periventricular α_1-adrenergic receptors are involved in the pressor and tachycardic responses produced by central AII, but not in the dipsogenic response. Otherwise, Jones (19) reported that the previous injection of the α_1-adrenergic antagonist prazosin but not α_2-adrenergic antagonist yohimbine into the rostral hypothalamus reduces both the dipsogenic and pressor response induced by ICV injection of AII. This contradictory effect of prazosin on the actions of central AII is probably related to the site of injections. The α_1-adrenergic receptor of the rostral hypothalamus is important for all actions of central AII, but the periventricular α_1-adrenergic receptor is involved only in the pressor response. The effect observed after previous ICV injection of phentolamine confirms the importance of the periventricular α-adrenergic receptors only for the pressor response (8). Recent data from our laboratory (unpublished results) showed that the injection of prazosin into the lateral hypothalamus blocked the dipsogenic action to central AII similar to that showed by Jones (8) for rostral hypothalamus.

The antidipsogenic effect of peripherally or centrally injected clonidine on the thirst induced by different stimuli and by AII has been described [for references, see (15)]. Recent work from our laboratory (13) has shown that injection of clonidine into the lateral hypothalamus blocks the thirst induced by water deprivation or central AII in rats. The present data confirm the effect of clonidine on the thirst induced by central AII and show that clonidine can also block all cardiovascular responses to central AII. Although it is known as a hypotensive drug, we may consider that central (ICV and in other prosencephalic areas) injection of clonidine in unanesthetized rats produces immediate and transitory, but potent, pressor and bradycardic responses (9,20), an effect that was also observed in the present study. AII was always injected when mean arterial pressure had returned to the level before clonidine, and in this situation clonidine blocked the pressor and tachycardic responses of AII.

Although it is widely assumed that clonidine acts centrally as an α_2-adrenergic agonist (21,27), the central action of clonidine may be mediated, at least in part, by putative imidazole receptors which selectively bind imidazolines (6). The existence of imidazole binding sites in the ventrolateral medulla in rats (11) has recently been established. The endogenous ligand which acts at imidazole binding sites is unknown. A potential candidate is the clonidine-displacing substance (CDS), a low-molecular weight substance isolated from bovine brain which potently displaces the binding of [^{3}H] clonidine or p-[^{3}H] amino-clonidine to brain membranes (12, 22, 23). Microinjection of low doses of clonidine or a preparation of CDS into the ventrolateral medulla induces vasodepressor responses (6,18). The inhibitory effect produced by clonidine on the responses to central AII could be due to the action of clonidine in one or more cerebral areas which produce inhibitory effects similar to that observed when clonidine or CDS are injected directly into the ventrolateral medulla. Thus, the interaction of clonidine with central α_2-adrenergic and/or imidazole receptors could block all pressor and dipsogenic response to central AII. The antidipsogenic central action of clonidine certainly is not related to the α_2-adrenergic receptors alone, since the previous treatment with yohimbine (a specific α_2-adrenergic antagonist) only partially reduced the antidipsogenic effect of clonidine (13).

It was also reported (5) that the activation of central α_1-adrenergic receptors produces pressor response, whereas the

activation of the α_2-adrenergic receptors produces opposite effects. This is in accordance with the present results where the blockade of the central α_1-adrenergic receptors with prazosin or the activation of the α_2-adrenergic receptors with clonidine inhibited the pressor response to central AII. The present results do not explain exactly how clonidine acts to inhibit the central effect of AII, but we can suggest that the activation of central α_2-adrenergic and/or imidazole receptors blocks the pressor and dipsogenic actions to central AII.

The participation of the central noradrenergic system in the mediation of AII-induced dipsogenic and cardiovascular responses has been demonstrated (1, 3, 17). In this work we showed that specifically the α_1-adrenergic receptor is important for the pressor, but not for the dipsogenic response to central AII. Probably other adrenergic receptors are involved in the mediation of the dipsogenic response to AII. Saad (24) showed that previous treatment with propranolol (a β-adrenoceptor antagonist) blocks the water intake produced by AII injection into the subfornical organ, one of the most important circumventricular organs mediating the actions of blood-borne AII. Experiments from our laboratory (unpublished data) and those performed by Severs (26) have also shown that ICV administration of β-adrenergic-blocking drugs prevents AII-induced drinking.

In summary, the present results show that ICV injection of clonidine blocks the pressor and dipsogenic responses to central AII, whereas prazosin blocks only the pressor response. Thus, the activation of the central α_2-adrenergic and/or imidazole receptors inhibit the cardiovascular and dipsogenic responses to central AII, while periventricular α_1-adrenergic receptors participate only in the cardiovascular response.

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REFERENCES

1. Bellin, S. J.; Bhatnagar, R. K.; Johnson, A. K. Periventricular noradrenergic system are critical for angiotensin-induced drinking and blood pressure responses. Brain Res. 403:105–112; 1987.
2. Bellin, S. J.; Landas, S. K.; Johnson, A. K. Localized injections of 6-hydroxydopamine into lamina terminalis-associated structures: effects on experimentally induced drinking and pressor responses. Brain Res. 416:75–83; 1987.
3. Bellin, S. J.; Landas, S. K.; Johnson, A. K. Selective catecholamine depletion of structures along the ventral lamina terminalis: effects on experimentally-induced drinking and pressor responses. Brain Res. 456:9–16; 1988.
4. Bickerton, R. K.; Buckley, J. P. Evidence for a central mechanism of angiotensin induced hypertension. Proc. Soc. Exp. Biol. Med. 106: 834–836; 1961.
5. Bousquet, P.; Rouot, B.; Schwartz, J. The central α-adrenoceptors: some new aspects. Trends Pharmacol. Sci. 4:206–208; 1983.
6. Bousquet, P.; Feldman, J.; Schwartz, J. Central cardiovascular effects of alpha adrenergic drugs: differences between catecholamines and imidazolines. J. Pharmacol. Exp. Ther. 230:232–236; 1984.
7. Buckley, J. P.; Ferrario, C. M. Central action of angiotensin and related hormones. New York: Pergamon Press; 1977.
8. Camacho, A.; Phillips, M. I. Separation of drinking and pressor responses to central angiotensin by monoamines. Am. J. Physiol. 240:R106–R113; 1981.
9. Colombari, E.; Saad, W. A.; Camargo, L. A. A.; Renzi, A.; De Luca, L. A., Jr.;Marcelino, A. C.; Menani, J. V.; Pressor responses of clonidine injected into some prosencephalic areas of conscious rats. Braz. J. Med. Biol. Res. 22:769–771; 1989.
10. De Groot, J. The rat forebrain in stereotaxic coordinates. Verh. Kon. Nederl. Akad. Wetensch. 52:1–40; 1959.
11. Ernsberger, P.; Meeley, M. P.; Mann, J. J.; Reis, D. J. Clonidine binds to imidazole binding sites as well as α_2-adrenoceptors in the ventrolateral medulla. Eur. J. Pharmacol. 134:1–13; 1987.
12. Ernsberger, P.; Meeley, M. P.; Reis, D. J. An endogenous substance with clonidine-like properties: selective binding to imidazole sites in the ventrolateral medulla. Brain Res. 441:309–318; 1988.
13. Ferrari, A. C.; Camargo, L. A. A.; Saad, W. A.; Renzi, A.; De Luca, L. A., Jr.; Menani, J. V. Clonidine and phenylephrine injected into the lateral hypothalamus inhibits water intake in rats. Brain Res., in press; 1990.
14. Ferrario, C. M.; Gildenberg, P. L.; McCubbin, J. W. Cardiovascular effects of angiotensin mediated by the central nervous system. Circ. Res. 30:257–262; 1972.
15. Fregly, M. J.; Rowland, N. E.; Greenleaf, J. E. A role for presynaptic α_2-adrenoceptors in angiotensin II-induced drinking in rats. Brain Res. Bull. 12:393–398; 1984.
16. Gordon, F. J.; Brody, M. J.; Fink, G. D.; Buggy, J.; Johnson, A. K. Role of central catecholamines in the control of blood pressure and drinking behavior. Brain Res. 178:161–173; 1979.
17. Gordon, F. J.; Brody, M. J.; Johnson, A. K. Regional depletion of central nervous system catecholamines: effects on blood pressure and drinking behavior. Brain Res. 345:285–297; 1985.
18. Granata, A. R.; Numao, Y.; Kumada, M.; Reis, D. J. A_1-noradrenergic neurons tonically inhibit sympathoexcitatory neurons of the C_1-area in rat brainstem. Brain Res. 377:127–146; 1986.
19. Jones, D. L. Hypothalamic α-adrenergic blockade modifies drinking and blood pressure responses to central angiotensin II in conscious rats. Can. J. Physiol. Pharmacol. 66:1270–1277; 1988.
20. Kawasaki, H.; Takasaki, K. Effect of water deprivation on the centrally-mediated hypertensive response to clonidine in freely moving, normotensive rats. Life Sci. 40:1929–1934; 1987.
21. Kobinger, W. Central alpha-adrenergic systems as targets for hypertensive drugs. Rev. Physiol. Biochem. Pharmacol. 81:39–100; 1978.
22. Meeley, M. P.; Ernsberger, P.; Granata, A. R.; Reis, D. J. An endogenous clonidine-displacing substance from bovine brain: receptor binding and hypotensive actions in the ventrolateral medulla. Life Sci. 38:1119–1126; 1986.
23. Meeley, M. P.; Towle, A. C.; Ernsberger, P.; Char, L. K.; McCanley, P. M.; Reis, D. J. Clonidine-specific antisera recognize an endogenous clonidine-displacing substance in brain. Hypertension 13:341–351; 1989.
24. Saad, W. A.; Menani, J. V.; Camargo, L. A. A.; Saad, W. A. Interaction between cholinergic and adrenergic synapses of rat subfornical organ and the thirst-inducing effect of angiotensin II. Braz. J. Med. Biol. Res. 18:37–46; 1985.
25. Severs, W. B.; Daniels, A. E.; Buckley, J. P. On the central hypertensive effect of angiotensin II. Int. J. Neuropharmacol. 6: 199–205; 1967.
26. Severs, W. B.; Summy-Long, J.; Daniels-Severs, A.; Connor, J. D. Influence of adrenergic blocking drugs on central angiotensin effects. Pharmacology 5:205–214; 1971.
27. Timmermans, P. B. M. W. M.; Van Zwieten, P. A. Alpha$_2$-adrenoceptors classification, localization, mechanisms and targets for drugs. J. Med. Chem. 25:1389–1401; 1982.