Vol. 117, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 16, 1983 Pages 420-428

ANGIOTENSIN II RECEPTORS NEGATIVELY COUPLED TO ADENYLATE CYCLASE IN RAT AORTA[1]

Madhu B. Anand-Srivastava[2]

Clinical Research Institute of Montréal,
110 Pine Ave West, Montréal, Québec, Canada

Received October 27, 1983

SUMMARY: Angiotensin II (AII) inhibited adenylate cyclase from rat aorta in a concentration dependent manner. The maximal inhibition (∿20%) was observed at 10 μM. The inhibitory effect of angiotensin II was dependent on monovalent cations such as Na^+ or Li^+ and was blocked by saralasin, an antagonist of angiotensin. Guanine nucleotides such as GTP or GMP-P(NH)P were also required to elicit the inhibition by angiotensin II. In addition, angiotensin II also inhibited the stimulation exerted by catecholamines. These data suggest that angiotensin receptors are present in aorta which are negatively coupled to adenylate cyclase.

Angiotensin II, a vasoactive peptide has been shown to produce vasoconstriction of vascular and nonvascular smooth muscle and exhibit positive inotropic and chronotropic effects on mammalian heart muscle (1,2). Various studies have demonstrated that the effect of angiotensin II on target tissues is mediated by its interaction with the receptor sites on the membranes (3). The existence of angiotensin receptors has been shown in several tissues (4-9). An inhibition of adenylate cyclase by angiotensin II has been recently demonstrated in kidney, adrenal cortex, and liver (7-9). Very recently, an evidence was presented which demonstrates that the effect of angiotensin II on carbohydrate metabolism was mediated through angiotensin II receptors in the liver (10). Although there is considerable evidence that vasoactive drugs produce their effects on vascular smooth muscle and heart contraction through the changes in cAMP (11) no study was performed in aorta or heart which could demonstrate that the effects of angiotensin in these tissues are mediated

[1] This work was supported by grants from the Quebec Heart Foundation and the Fonds de la Recherche en Santé du Québec.

[2] Canadian Heart Foundation Scholar.

Abbreviations used: GMP-P(NH)P, guanyl-5'-yl(β-γ-imino)diphosphate; AII, angiotensin II.

0006-291X/83 $1.50

through adenylate cyclase/cAMP system. The present studies were therefore undertaken to investigate if angiotensin II receptors demonstrated in aorta (3,12,13) by receptor binding studies are coupled to adenylate cyclase system.

MATERIALS AND METHODS

Preparation of particulate fraction Aorta were dissected out and quickly frozen in liquid nitrogen. The frozen aorta were pulverized to a fine powder using a percussion mortar cooled in liquid nitrogen. The powdered aorta were stored at -70° until assayed. Aorta were homogenized using motor-driven teflon-glass homogenizer in a buffer containing 10 mM Tris-HCl, 1 mM EDTA and 1 mM DTT, pH 7.5. The homogenate was centrifuged at 16,000 x g for 10 min. The supernatant was discarded and the pellet was finally suspended in 10 mM Tris-HCl, 1 mM EDTA and 1 mM DTT, pH 7.5 and used for the determination of adenylate cyclase activity.

Adenylate cyclase activity determination. Adenylate cyclase activity was determined by measuring [^{32}P] cAMP formation from [α-^{32}P] ATP as described previously (14). Typical assay medium contained 50 mM glycylglycine, pH 7.5, 0.5 mM Mg ATP, [α^{32}P] ATP (1-1.5 x 10^6 CPM), 5 mM $MgCl_2$ (in excess of the ATP concentration), 100 mM NaCl, 0.5 mM cAMP, 5 U adenosine deaminase per ml, 1 mM DTT, 10 μM GTP, and ATP regenerating system consisting of 2 mM creatine phosphate, 0.1 mg creatine kinase per ml, and 0.1 mg myokinase per ml in a final volume of 200 μl. Incubations were initiated by the addition of the particulate fraction (50-100 μg) to the reaction mixture which had been thermally equilibrated for 2 min at 37°C. Reactions were conducted in triplicate for 10 min at 37°C. Reactions were terminated by the addition of 0.6 ml of 120 mM zinc acetate. cAMP was purified by co-precipitation of other nucleotides with $ZnCO_3$ by the addition of 0.5 ml of 144 mM Na_2CO_3 and subsequent chromatography by the double column system as described by Salomon et al (15). Under the assay conditions used adenylate cyclase activity was linear with respect to protein concentration and time of incubation.

Protein was determined essentially as described by Lowry et al (16), with crystalline bovine serum albumin as standard.

Materials. Adenosine deaminase (EC 3.5.4.5), GTP, GMP-P(NH)P (guanyl-5'-yl-β-γ-imino diphosphate), ATP, cyclic AMP were purchased from Sigma, St.Louis, Missouri. Creatine kinase (EC 2.7.3.2) and myokinase (EC 2.7.4.3) were purchased from Boehringer Mannheim, Canada. [α^{32}P]ATP was purchased from Amersham and angiotensin II and saralasin were from Peninsula Laboratories, Inc., California.

RESULTS

Effect of Angiotensin II on adenylate cyclase. In order to determine the presence of angiotensin receptors coupled to adenylate cyclase in rat aorta, the effect of various concentrations of angiotensin II on adenylate cyclase was studied and the results are shown in Fig. 1. Angiotensin II inhibited adenylate cyclase activity in a concentration dependent manner, the maximal inhibition ($\sim$ 20%) was obtained at 10^{-5} M. The inhibitory effect of AII on adenylate cyclase was reversed by about 90% by saralasin (10^{-5} M), an antagonist of angiotensin, as shown in Table I. These data suggest the presence of angiotensin receptors in rat aorta which are negatively coupled to adenylate cyclase.

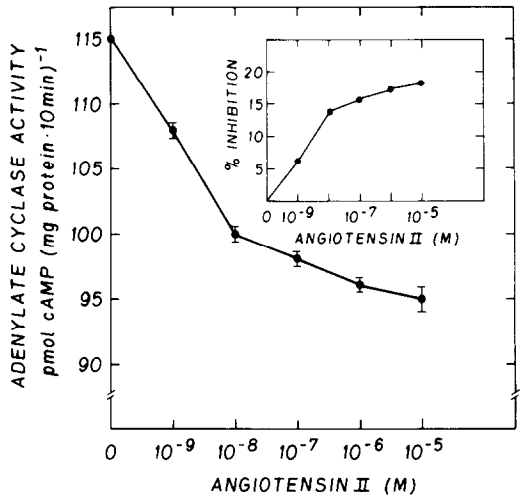

Figure 1 Effect of various concentrations of angiotensin II on adenylate cyclase activity in rat aorta. Adenylate cyclase activity was determined in the presence of 100 mM NaCl and 10 μM GTP as under "Methods". Inset shows the percent inhibition of adenylate cyclase activity by various concentrations of angiotensin II. Values are the means ± SEM of triplicate determinations from one of three experiments.

Effect of sodium chloride on angiotensin II induced inhibition of adenylate cyclase.

The requirement of sodium has been demonstrated for the inhibitory response of adeny late cyclase to α-adrenergic agents in renal cortex (17) and liver (7),and adenosine in myocardial sarcolemma (18). It was of interest to determine if the inhibition of adenylate cyclase by angiotensin II was also dependent on the presence of sodium.

Table I Effect of saralasin [Sar^{1} Ala^{8}]-angiotensin II on angiotensin induced inhibition of adenylate cyclase in rat aorta

Additions	Adenylate cyclase activity pmol cAMP (mg protein. 10 min)-1
None	117 ± 1
Angiotensin II (AII) (10^{-5} M)	96 ± 2
AII + saralasin (10^{-5} M)	114 ± 2

Adenylate cyclase activity was determined in the presence of 100 mM NaCl and 10 μM GTP as given under "Methods". The values represent the mean ± SEM of triplicate determinations from one of two experiments.

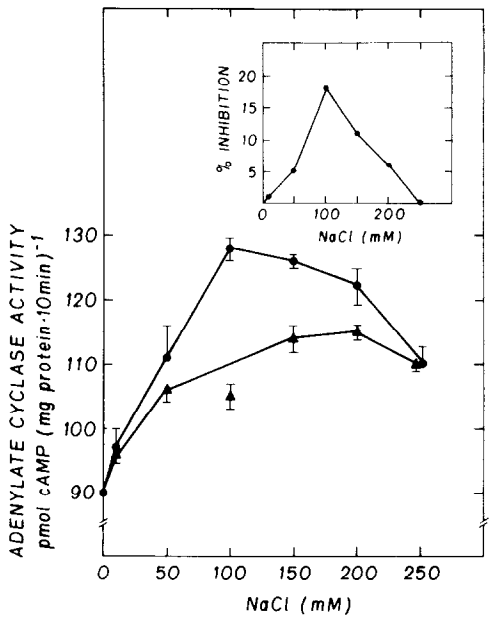

Figure 2 Effect of various concentrations of NaCl on adenylate cyclase activity in rat aorta. Adenylate cyclase activity was determined in the absence (●-●) or presence (▲-▲) of 10 μM angiotensin II as under "Methods". 10 μM GTP was present in the assay mixture. Inset shows the dependence on NaCl of inhibition of adenylate cyclase by angiotensin II. Values are the mean SEM of triplicate determinations from one of two experiments.

Fig. 2 shows the effect of various concentrations of NaCl on adenylate cyclase activity in the presence and absence of 10^{-5} M angiotensin. Basal adenylate cyclase activity was increased up to 100 mM NaCl and then declined. However the percentage inhibition by angiotensin was maximum at 100 mM NaCl and decreased at higher concentrations as is shown in the inset. The inhibitory effect of AII on adenylate cyclase was also observed in the presence of LiCl, however it was less potent than NaCl (data not shown).

Guanine nucleotide requirement for inhibition of adenylate cyclase by AII. Fig. 3 shows the effect of various concentrations of GTP or GMP-P(NH)P on AII responsive adenylate cyclase activity. A small inhibition (∿ 5%) of adenylate cyclase by AII (10^{-5} M) observed in the absence of guanine nucleotides was increased in a concentration dependent manner by GTP or GMP-P(NH)P. Maximum inhibition (∿20%) was observed at 10 μM of GTP or GMP-P(NH)P. These data suggest that angiotensin II receptors like other hormone receptors are coupled to adenylate cyclase by guanine nucleotide binding protein. A small inhibition of adenylate cyclase by angiotensin in the

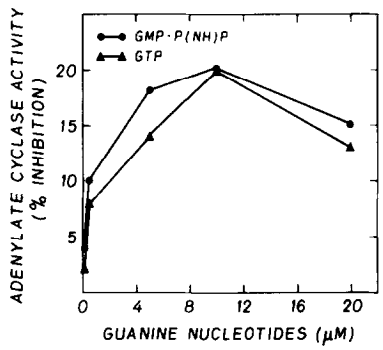

Figure 3 Dependence on guanine nucleotides of inhibition of adenylate cyclase by angiotensin II in rat aorta. Adenylate cyclase activity was determined in the presence of 10 μM angiotensin II, 100 mM NaCl and increasing concentration of GTP (▲-▲) or GMP-P(NH)P (●-●). Values are the means of triplicate determinations from one of three experiments.

absence of guanine nucleotides might be due to the presence of endogenous GTP in aorta washed particles.

Effect of some agonists on adenylate cyclase and their interaction with angiotensin II

In order to determine the presence of some other hormone receptors in addition to angiotensin receptors in rat aorta, the effect of various catecholamines such as isoproterenol, epinephrine and dopamine on adenylate cyclase was studied and the results are shown in Table II. These experiments were carried out in the presence of 100 mM

Table II Interaction of angiotensin II (AII) with some agonists

Additions	Adenylate cyclase activity pmol cAMP (mg protein. 10 min)-1	Percentage inhibition by angiotensin II
None	96 ± 7.1	
AII (10 μM)	79 ± 3.2	18
Isoproterenol (50 μM)	229 ± 9.1	
Isoproterenol + AII (10 μM)	206 ± 5.5	10.1
Epinephrine (50 μM)	207 ±11.6	
Epinephrine + AII (10 μM)	187 ±10.3	11.2
Dopamine (100 μM)	152 ±10.5	
Dopamine + AII (10 μM)	134 ±14.6	11.9

Adenylate cyclase activity was determined in the presence of 100 mM NaCl and 10 μM GTP as given under "Methods". The values represent the mean ± SEM of triplicate determinations from one of two experiments.

Table III Effect of some antagonists on adenylate cyclase activity stimulated by epinephrine, norepinephrine and dopamine in rat aorta

Additions	Adenylate cyclase activity pmol cAMP (mg protein. 10 min)-1			
	Basal	Epinephrine 50 μM	Norepinephrine 50 μM	Dopamine 100 μM
None	76±2	263±18	224±9	126±1
Propranolol (10 μM)	76±1	93± 5	86±4	62±4
Phentolamine (10 aM)	75±3	212± 8	179±5	87±4
Flupentixol (10 μM)	65±2	186± 9	159±2	70±4

Adenylate cyclase activity was determined as given under "Methods". NaCl was omitted from the assay mixture. The values represent the mean ± SEM of triplicate determinations from one of two experiments.

NaCl. Isopreterenol (50 μM), epinephrine (50 μM) and dopamine (100 μM) all stimulated adenylate cyclase activity by about 140, 115 and 40% respectively. This stimulation was decreased in the presence of 10^{-5} M angiotensin II. These data suggest (1) the presence of adrenergic and dopaminergic receptors in rat aorta and (2) that angiotensin was also able to inhibit the hormone-stimulated adenylate cyclase activities in vascular smooth muscle as has been reported in other nonvascular tissues (7-9).

To investigate the nature of catecholamine receptors in aorta, the effect of some antagonists on catecholamine-stimulated adenylate cyclase activity was studied and the results are shown in Table III. Propranolol inhibited epinephrine and norepinephrine-stimulated enzyme activities by about 90-95% whereas a small inhibition (∿20-25%) was observed by phenotolamine. On the other hand dopamine-stimulated adenylate cyclase activity was inhibited by all three antagonists. The higher stimulation by epinephrine and dopamine (∿250% and 66% respectively as compared to 150% and 40% in Table II) was due to the fact that NaCl was omitted from the assay mixture which increases basal activity and thereby decreases the percent stimulation.

DISCUSSION

The data presented demonstrates the presence of angiotensin receptors coupled to adenylate cyclase in rat aorta. The inhibition of adenylate cyclase by angioten-

sin II suggests that the angiotensin receptors are negatively coupled to adenylate cyclase in rat aorta. These results are in agreement with the previous reports obtained with liver membranes (7), kidney cortex (8) and adrenal cortex (8), where angiotensin also inhibited adenylate cyclase activity. Furthermore the inhibition by angiotensin II was specific, since it was antagonized by saralasin, which is a well known antagonist of angiotensin. The inhibition of adenylate cyclase by angiotensin II was dependent on the presence of monovalent cations such as Na^+ or Li^+, however Na^+ was more potent than Li^+ in inhibiting adenylate cyclase activity. The requirement of monovalent cations in the inhibition of adenylate cyclase by angiotensin and α-adrenergic agonists in other systems have been reported previously (7-9). However Li^+ was shown to be more potent than Na^+ or K^+ in angiotensin-induced inhibition of liver adenylate cyclase (7).

Similar to the stimulation of adenylate cyclase by hormones, the inhibition of the cyclase by hormones and neurotransmitters has been shown to be dependent on the presence of GTP (19). In the present studies, we have demonstrated that angiotensin-induced inhibition of aorta adenylate cyclase was not only dependent on GTP, but a stable GTP analog, GMP-P(NH)P was as potent as that of GTP in exhibiting the inhibition of adenylate cyclase by angiotensin. The small inhibition of adenylate cyclase by angiotensin observed in the absence of guanine nucleotides might be due to the presence of some endogenous GTP in aorta washed particles. The observed inhibition of adenylate cyclase by angiotensin in the presence of GMP-P(NH)P suggests that in aorta the mechanism by which angiotensin II inhibits adenylate cyclase may be different than that observed in other systems and may not be mediated by the increased hydrolysis of GTP as suggested previously (20). However our data are in agreement with the reports of other investigators which demonstrate the the stable GTP analogs were also able to inhibit adenylate cyclase in other systems (21,22).

Although the presence of adenylate cyclase in a variety of smooth muscles has been demonstrated, the enzyme has been found to have little or no sensitivity to hormones. Recently we have demonstrated the presence of adenosine-sensitive adenylate cyclase in cultured vascular smooth muscle cells from rat aorta (23) and in the present studies we further show that the enzyme prepared by this method is greatly

responsive to epinephrine, norepinephrine, isoproterenol and dopamine and suggests the presence of adrenergic and dopaminergic receptors in aorta.

The inhibition of epinephrine and glucagon-stimulated cAMP levels and adenylate cyclase by angiotensin II has been reported by several investigators (8,24,25). In our studies also, we demonstrate that angiotensin II inhibited catecholamine-sensitive adenylate cyclase activities. The physiological significance of this effect remains to be established.

It is concluded from these studies that angiotensin receptors are present in rat aorta which are negatively coupled to adenylate cyclase and it can be postulated that vasoconstriction elicited by angiotensin may be mediated through adenylate cyclase/cAMP system.

ACKNOWLEDGEMENTS

I wish to thank Dr. Marc Cantin for constant encouragement and Marie-France Nolin, Georges Lepage and Pierre Dupuis for technical assistance. The secretarial help of Vivianne Jodoin is greatly appreciated.

REFERENCES

1. Devynck, M.-A., and Meyer, P. (1978) Biochem. Pharmacol. 27, 1-5.
2. Kobayashi, M., Furukawa, Y., and Chiba, S. (1978) Eur. J. Pharmacol. 50, 17-25.
3. Douglas, J.G., and Catt, K.J. (1978) J. Clin. Invest. 58, 834-843.
4. Moore, T.J., and Williams, G.H. (1982) Circ. Res. 51, 314-320.
5. Baudouin, M., Meyer, P., and Worcel, M. (1971) Biochem. Biophys. Res. Commun. 42, 434-444.
6. Mukherjee, A., Kulkarni, P.V., Haghani, Z., and Sutko, J.L. (1982) Biochem. Biophys. Res. Commun. 105, 575-581.
7. Jard, S., Cantan, B., and Jakobs, K.H. (1981) J. Biol. Chem. 256, 2603-2606.
8. Woodcock, E.A., and Johnston, C.I. (1982) Endocrinology 111, 1687-1691.
9. Marie, J., and Jard, S. (1983) Febs Letters 159, 97-101.
10. Companile, C.P., Crane, J.K., Peach, M.J., and Garrison, J.C. (1982) J. Biol. Chem. 257, 4951-4958.
11. Robison, G.A., Butcher, R.W. and Sutherland, E.W. (1971) cAMP, pp. 146-228. Academic Press, New York.
12. Baudouin, M., Meyer, P., Fermandjian, S., and Morgat, J.L. (1972) Nature, Lond. 235, 336-338.
13. Morvan, P.L., and Palaic, D. (1975) J. Pharmac. Exp. Ther. 195, 167-175.
14. Anand-Srivastava, M.B., and Cantin, M. (1983) Arch. Biochem. Biophys. 223, 468-476.
15. Salomon, Y., Londos, C., and Rodbell, M. (1974) Anal. Biochem. 58, 541-548.
16. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
17. Woodcock, E.A., Johnston, C.I., and Olsson, C.A. (1980) J. Cyclic Nucleotide Res. 6, 261-269.
18. Leung, E., Johnston, C.I., and Woodcock, E.A. (1983) Biochem. Biophys. Res. Commun. 110, 208-215.
19. Jakobs, K.H., Aktories, K., and Schultz, G. (1981) in Dumont, J.E., Greengard, P. and Robison, G.A. (eds) Advances in Cyclic Nucleotide Res. vol. 14. Raven Press, New York, pp. 173-187.

20. Jakobs, K.H., Aktories, K., Lasch, P., Bitsch, R., Gabel, G., Steinmeyer, C., and Schultz, G. (1982) in Dumont, J., Nunez, J., and Schultz, G. (eds) Hormones and Cell Regulation, vol. 6, Elsevier Biomedical Pres, pp. 131-142.
21. Aktories, K., Schultz, G., and Jakobs, K.H. (1983) Febs Letter 156, 88-92.
22. Hildebrandt, J.D., Hanoune, J., and Birnbaumer, K. (1982) J. Biol. Chem. 257, 14723-14725.
23. Anand-Srivastava, M.B., Franks, D.J., Cantin, M., and Genest, J. (1982) Biochem. Biophys. Res. Commun. 108, 213-219.
24. Morgon, N.G., Exton, J.H., and Blackmore, P.F. (1983) Febs Letter 153, 77-80.
25. Crane, J.K., Campanile, C.P., and Garrison, J.C. (1982) J. Biol. Chem. 257, 4959-4965.