Inhibitory effect of atrial natriuretic factor on adenylate cyclase activity in adrenal cortical membranes

Madhu B. Anand-Srivastava*, Jacques Genest and Marc Cantin

Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec H2W 1R7, Canada

Received 24 December 1984

The effect of rat synthetic atrial natriuretic factor (ANF) on adenylate cyclase activity was studied in adrenal cortical membranes. Synthetic ANF (Arg 101–Tyr 126) inhibited adenylate cyclase activity in a concentration-dependent manner. The maximum inhibition observed was about 25% with an apparent k_i of 5×10^{-11} to 10^{-10} M. Various hormones such as isoproterenol, dopamine, prostaglandin (PGE_1) and ACTH-stimulated adenylate cyclase to various degrees and ANF inhibited the stimulatory effect of all these hormones to some degree but never abolished it. In addition, ANF was also able to inhibit the stimulation exerted by forskolin which activates adenylate cyclase by receptor-independent mechanism. This is the first study demonstrating the inhibitory effect of ANF on adrenal cortical adenylate cyclase. From these results it can be suggested that the inhibition of adenylate cyclase may be one of the mechanisms through which ANF exerts the inhibitory effect on steroidogenesis stimulated by various hormones and agents.

Atrial natriuretic factor *Adenylate cyclase* *Adrenal cortical membrane* *ACTH* *Prostaglandin* *Forskolin*

1. INTRODUCTION

Mammalian atria have been shown to contain a factor that exhibits diuretic and natriuretic activity. This factor has been called atrial natriuretic factor [1]. The atrial natriuretic factor was also shown to exert vasodilatory and vasorelaxant effects [2–4]. The purification and amino acid sequence of atrial natriuretic factor has been reported by several groups of investigators [5–8]. Very recently we reported that synthetic atrial natriuretic factor inhibits adenylate cyclase activities in heart, vascular smooth muscle from aorta, mesenteric artery and renal artery [9], anterior and posterior pituitary [10] but not in non-target tissues such as spleen, skeletal muscle, testis and adrenal medulla [9]. ANF has also been shown to inhibit steroidogenesis stimulated by angiotensin, prostaglandin, ACTH and forskolin [11] and angiotensin II and ACTH [12] in zona glomerulosa cells. Since the steroidogenesis stimulated by prostaglandins, ACTH and forskolin has been shown to be a cyclic AMP-dependent phenomenon, it is possible that the inhibitory effect of ANF on steroidogenesis stimulated by these agents [11] may be mediated through the inhibition of adenylate cyclase. The present studies were therefore undertaken to investigate the effects of ANF on adenylate cyclase activity in the presence of various stimulators which have been shown to influence steroidogenesis.

* Canadian Heart Foundation scholar

Abbreviations: ANF, rat synthetic atrial natriuretic factor (Arg 101-Tyr 126); PGE_1, prostaglandin; ACTH, adrenocorticotropic hormone; FSK, forskolin

2. MATERIALS AND METHODS

2.1. *Materials*

GTP, ATP, cyclic AMP, isoproterenol and dopamine were purchased from Sigma (St. Louis, MO). Creatine kinase (EC 2.7.3.2) and myokinase

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

(EC 2.7.4.3) were purchased from Boehringer Mannheim, Canada. [α-^{32}P]ATP was purchased from Amersham. ACTH was from Penninsula and forskolin was obtained from Calbiochem-Behring Corp. (San Diego, CA). Synthetic rat atrial natriuretic factor (Arg 101–Tyr 126) was a gift from Dr R. Nutt of Merck, Sharp & Dohme Research Laboratories.

2.2. *Preparation of adrenal cortical membranes*

Adrenal glands were dissected out from Sprague Dawley rats (200–300 g). The capsular zone (zona glomerulosa) was separated from the subcapsular area (zona fasciculata) and was homogenized using a teflon-glass homogenizer in a buffer containing 10 mM Tris and 1 mM EDTA (pH 7.5). The homogenate was centrifuged at 20000 × *g* for 10 min. The supernatant was discarded and the pellet was homogenized in the above buffer with a glass-teflon homogenizer. This preparation was used for adenylate cyclase determination.

2.3. *Adenylate cyclase activity determination*

Adenylate cyclase activity was determined by measuring [^{32}P]cAMP formation from [^{32}P]ATP as in [9,13]. Typical assay medium contained 50 mM glycylglycine (pH 7.5), 0.5 mM MgATP, [α-^{32}P]ATP (1–1.5 × 10^6 cpm), 5 mM $MgCl_2$ (in excess of the ATP concentration, 0.5 mM cAMP, 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 membranes (50–100 μg) to the reaction mixture which had been thermally equilibrated for 2 min at 37°C. Incubations 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. [14]. 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. [15] with crystalline bovine serum albumin as standard.

3. RESULTS

Fig.1 shows the effect of various concentrations of synthetic rat ANF on adenylate cyclase activity in rat adrenal cortical membranes. ANF inhibited adenylate cyclase activity in a concentration-dependent manner with an apparent K_i between 0.05 to 0.1 nM. The maximal inhibition observed was about 25%. A similar inhibitory effect of ANF was also observed on adenylate cyclase in bovine adrenal cortical membranes from zona glomerulosa cells and bovine cultured zona glomerulosa cells (not shown). These data suggest the presence of ANF receptors in adrenal cortex which are negatively coupled to adenylate cyclase.

Various hormones have been shown to stimulate adenylate cyclase and steroidogenesis in adrenal cortex [16]. Recently it was shown that ACTH-, prostaglandin- and forskolin-stimulated steroidogenesis in cultured bovine zona glomerulosa cells was significantly inhibited by ANF [11]. Since all these agents also stimulate adenylate cyclase, it is possible that the inhibitory effect of ANF on steroidogenesis may be mediated through the adenylate cyclase/cAMP system, we were tempted to in-

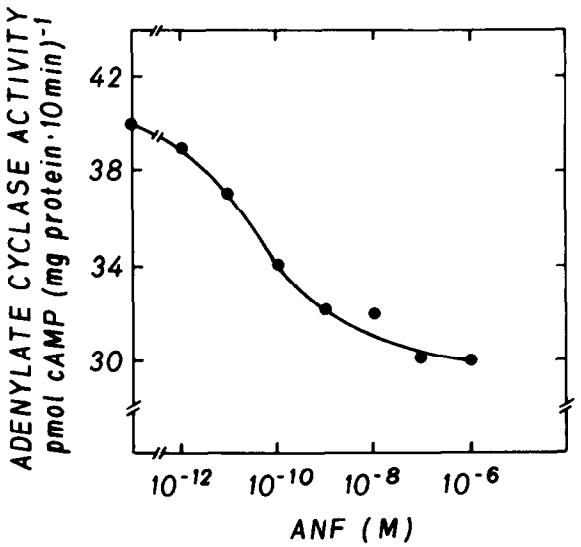

Fig.1. Effect of various concentrations of ANF on adenylate cyclase activity in adrenal cortical membranes. Adenylate cyclase activity was determined as in section 2. Values are means of triplicate determinations from one of three different experiments.

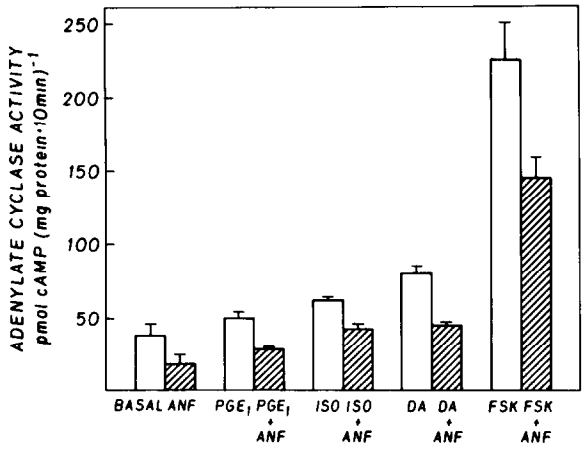

Fig.2. Effect of ANF on basal or stimulated adenylate cyclase activity by various agonists in adrenal cortical membranes. Adenylate cyclase activity was determined in the absence or presence of 1 μM prostaglandin (PGE_1), 50 μM isoproterenol (ISO), 100 μM dopamine (DA), 50 μM forskolin (FSK) alone (□) or in combination with 10 nM ANF (▨) as in section 2. Values are means ± SE of triplicate determinations from one of three different experiments.

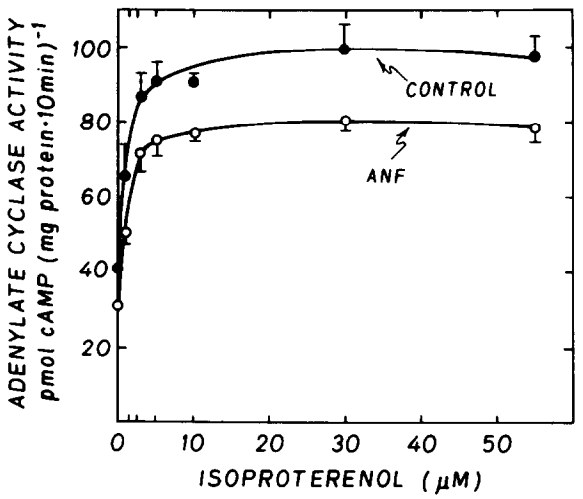

Fig.4. Effect of various concentrations of isoproterenol on adenylate cyclase activity in adrenal cortical membranes in the absence (●—●), or presence of 10 nM ANF (○—○). Adenylate cyclase activity was determined as in section 2. Values are means ± SE of triplicate determinations from one of two different experiments.

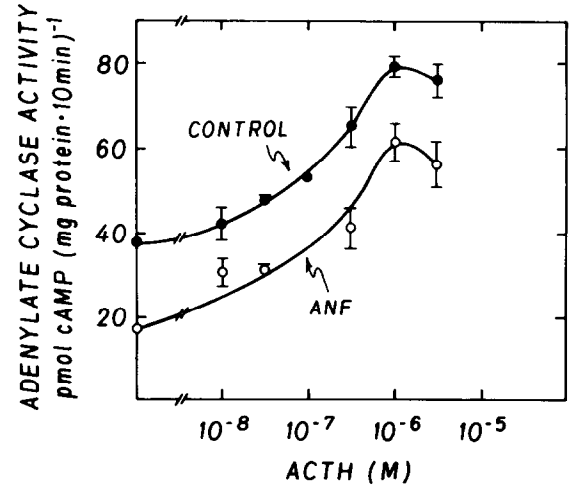

Fig.3. Effect of various concentrations of adrenocorticotropic hormone (ACTH) on adenylate cyclase activity in adrenal cortical membranes in the absence (●—●), or presence of 10 nM ANF (○—○). Adenylate cyclase activity was determined as in section 2. Values are means ± SE of triplicate determinations from one of two different experiments.

vestigate the effect of ANF on hormone-responsive adenylate cyclase activities. The results are shown in fig.2. Prostaglandin (PGE_1), isoproterenol, dopamine and forskolin all stimulated adenylate cyclase to various degrees and ANF inhibited the stimulatory responses of all these agonists. In no case, however, was a complete inhibition observed.

The effect of ANF was also studied on the entire dose–response curve of ACTH-sensitive adenylate cyclase activity. As shown in fig.3, ACTH stimulated adenylate cyclase activity in a concentration-dependent manner, the maximal stimulation being observed at 1 μM, and ANF inhibited the stimulatory effect of ACTH on adenylate cyclase at all the concentrations used in the present studies.

Similar results were also obtained when the effect of ANF was studied on the entire concentration curve of isoproterenol (fig.4). The inhibitory effects of ANF on ACTH-, and isoproterenol-stimulated adenylate cyclase activities appear to be associated with a decrease in V_{max} and not an increase in K_a.

4. DISCUSSION

The data presented demonstrate the presence of ANF receptors coupled to adenylate cyclase in adrenal cortex. The inhibition of adenylate cyclase by ANF suggests that ANF receptors are negatively coupled to adenylate cyclase in adrenal cortex. The similar inhibition of adenylate cyclase by ANF has also been reported previously in target tissues such as heart, vascular smooth muscle [9], and anterior and posterior pituitary [10]. The apparent K_i observed in the present studies (5×10^{-11}–10^{-10} M) is comparable with the values observed with the other tissues [9,10]. The inhibitory effect of ANF on basal and PGE_1-, ACTH- and FSK-stimulated adenylate cyclase is interesting and could explain the ability of ANF to inhibit steroid secretion stimulated by these agents. However, the inhibitory effect of ANF on angiotensin II-stimulated steroidogenesis [11,12] which does not appear to involve cAMP, cannot be explained by the above-mentioned mechanism, because angiotensin II has been shown to inhibit adenylate cyclase activity [17,18]. The possible mechanism(s) by which ANF exerts its inhibitory effect on angiotensin II-stimulated steroidogenesis may involve calcium movements, phosphatidyl inositol turnover or cGMP. Nevertheless, the present studies indicate that ANF inhibits basal and hormone-stimulated adenylate cyclase activities in adrenal cortical membranes, and suggest that inhibition of adenylate cyclase by ANF may be one of the mechanisms involved in the inhibition of steroid production.

ACKNOWLEDGEMENTS

We wish to thank Sylvie Picard and Gizala Ali for the excellent technical assistance and Lyne Guindon for secretarial help. This work was supported by grants from the Quebec Heart Foundation, the Medical Research Council of Canada and from a group grant from the Medical Research Council of Canada to the Multidisciplinary Research Group on Hypertension.

REFERENCES

[1] De Bold, A.J., Borenstein, H.B., Veress, A.T. and Sonnenberg, H. (1981) Life Sci. 28, 89–94.
[2] Misono, K.S., Fukumi, H., Grammer, R.T. and Inagami, T. (1984) Biochem. Biophys. Res. Commun. 119, 524–529.
[3] Garcia, R., Thibault, G., Cantin, M. and Genest, J. (1984) Am. J. Physiol. 247, R-34–R-39.
[4] Currie, M.G., Geller, D.M., Cole, B.R., Baylar, W., Yu Sheng, S.W. and Holmberg, P. (1983) Life Sci. 221, 71–73.
[5] Seidah, N.G., Lazure, C., Chretien, M., Thibault, G., Garcia, R., Cantin, M., Genest, J., Nutt, R.F., Brady, S.F., Lyle, T.A., Paleveda, W.J., Colton, C.D., Ciccarone, T.M. and Veber, D.F. (1984) Proc. Natl. Acad. Sci. USA 81, 2640–2644.
[6] Thibault, G., Garcia, R., Cantin, M., Genest, J., Lazure, C., Seidah, N.G. and Chretien, M. (1984) FEBS Lett. 167, 352–356.
[7] Flynn, T.C., De Bold, M.L. and De Bold, A.J. (1983) Biochem. Biophys. Res. Commun. 117, 859–865.
[8] Currie, M.G., Geller, D.M., Cole, B.R., Siegel, N.R., Fok, K.F., Adams, S.P., Eubanks, S.R., Gallupi, G.R. and Needelman, P. (1984) Science 223, 67–69.
[9] Anand-Srivastava, M.B., Cantin, M. and Genest, J. (1985) Life Sci., in press.
[10] Anand-Srivastava, M.B., Cantin, M. and Genest, J. (1985) Life Sci., in press.
[11] De Lean, A., Racz, K., Gutkowska, J., Nguyen, T.T., Cantin, M. and Genest, J. (1984) Endocrinology 115, 1636–1638.
[12] Chartier, L., Schiffrin, E., Thibault, G. and Garcia, R. (1984) Endocrinology 115, 2026–2028.
[13] Anand-Srivastava, M.B. and Cantin, M. (1983) Arch. Biochem. Biophys. 223, 468–476.
[14] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[15] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[16] Halkerston, I.D.K. (1975) Adv. Cyclic Nucleotide Res. 6, 99–136.
[17] Woodcock, E.A. and Johnston, C.I. (1982) Endocrinology 111, 1687–1691.
[18] Anand-Srivastava, M.B. (1983) Biochem. Biophys. Res. Commun. 117, 420–428.