Volume 281, number 1,2, 73–76 FEBS 09553 April 1991

ADONIS 001457939100263Z

Dual regulation of atrial natriuretic factor-dependent guanylate cyclase activity by ATP

Ravi B. Marala[1], Ari Sitaramayya[2] and Rameshwar K. Sharma[1]

[1]*Section of Regulatory Biology, Department of Brain and Vascular Research, The Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195-5068, USA* and [2]*Pennsylvania College of Optometry, 1200 West Godfrey Avenue, Philadelphia, PA 19141, USA*

Received 20 December 1990; revised version received 28 January 1991

The 'second messenger' of certain atrial natriuretic factor (ANF) signals is cyclic GMP. One type of ANF receptor linked to the synthesis of cyclic GMP is a transmembrane protein which contains both the ANF-binding and guanylate cyclase activities. The consensus is that the maximal activity of this guanylate cyclase is observed in the presence of ATP. We now show that depending upon the cofactors Mg^{2+} or Mn^{2+}, ATP stimulates or inhibits the ANF-dependent guanylate cyclase activity in the testicular plasma membranes: stimulation in the presence of Mg^{2+} and inhibition in the presence of Mn^{2+}. With Mg^{2+} as cofactor neither ATP nor ANF stimulate the cyclase activity – it is only when the two are together that the enzyme is activated. Furthermore, this investigation for the first time demonstrates binding of ATP to the ANF receptor guanylate cyclase, suggesting that ATP-mediated responses could occur by direct ATP binding to the cyclase.

Atrial natriuretic factor; Guanylate cyclase; Cyclic GMP; ATP-binding protein; Atrial natriuretic factor receptor guanylate cyclase

1. INTRODUCTION

Atrial natriuretic factor (ANF) is one of the members of structurally-related peptide hormones that is released from atria in response to volume expansion and regulates sodium excretion, water balance, blood pressure [1–4] and steroidogenesis [5–7]. The mechanism, or mechanisms, by which this hormone exhibits pleotropic activities is not known. But one of the second messengers of certain ANF signal transductions is cyclic GMP [8,9]. In direct support of this concept, the original biochemical studies demonstrated that one type of ANF receptor linked to the synthesis of cyclic GMP is a transmembrane protein, containing both the ANF-binding and guanylate cyclase activities [10–12]. Recent genetic evidence reveals such a receptor family; two family members – GC-A and GC-B – have been cloned from rat brain [13,14] and a partial-length cDNA from rat adrenal, representing a potential third family member (provisionally termed GC-C) has recently been identified [15].

There is, however, an enigmatic observation; despite the fact that the pure enzyme binds ANF stoichiometrically, its activity is not hormonally stimulated [10,11].

Correspondence address: R.K. Sharma, Section of Regulatory Biology, Department of Brain and Vascular Research, The Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA.

To account for this anomalous observation, currently two hypotheses are under active consideration. Both are based on the original observation that ATP enhances the ANF-dependent guanylate cyclase activity in the particulate fractions of rat liver [16]. In one hypothesis, ATP binds directly to a site located in the putative protein kinase domain [17]. Such an allosteric site has not yet been identified – although it was shown that deletion of the presumed protein kinase domain from GC-A results in the loss of activation by ANF [17]. On the other hand, the second hypothesis suggested a separate ATP-binding protein mediates the ATP stimulatory effect [18]. Formulation of both of these concepts is based on the studies which used Mg^{2+} as a cofactor in the cyclase assays.

ATP also inhibits basal particulate guanylate cyclase activity in a dose-dependent fashion with Mn^{2+} as the cofactor [19]. This raised the curious possibility that in this case ATP might in fact be reversing the normal stimulatory operation of ANF-dependent guanylate cyclase, causing inhibition instead of stimulation of the hormone-dependent guanylate cyclase activity. This would then indicate that ATP regulates ANF-dependent guanylate cyclase activity in two opposing manners – stimulation with Mg^{2+} and inhibition with Mn^{2+}.

Results of our present study with the model system of rat testes indicate that this indeed is the case. In addition, this investigation for the first time demonstrates specific binding of ATP to a homogeneous preparation

of guanylate cyclase, providing one possibility that ATP mediates these responses by direct binding to the cyclase.

2. MATERIALS AND METHODS

The operational steps to prepare rat particulate fractions were at 4°C [19], unless stated otherwise: testes were homogenized in 5 mM Tris-HCl buffer, pH 7.5, containing 1 mM $MgCl_2$, 250 mM sucrose and a combination of protease inhibitors (50 μg/ml lima bean trypsin inhibitor, 8 mM benzamidine-HCl, and 70 μg/ml phenylmethylsulfonyl fluoride); post-mitochondrial supernatant was centrifuged for 60 min at 35 000 × g, the pellet was resuspended in 50 mM Tris-HCl, pH 7.5, containing 4 mM $MgCl_2$ with the above described protease inhibitors. Guanylate cyclase activity was measured at 37°C in a 50 mM Tris-HCl buffer, pH 7.6, containing 10 mM theophylline, 15 mM creatine phosphate, 20 U/ml creatine phosphokinase (250 U/mg protein) and 10–20 μg membrane protein in a total incubation volume of 100 μl. The reactions were started with the addition of substrate (1 mM GTP and 4 mM $MgCl_2$ or $MnCl_2$), continued for 10 min and terminated by the addition of 0.9 ml ice-cold 50 mM sodium acetate, pH 6.2, followed by heating for 3 min in a boiling water bath. To determine the effect of the ANF, the membrane fraction was preincubated with or without ANF (10^{-7} M) for 10 min on ice prior to addition of substrate. The cyclic GMP formed was measured by radioimmunoassay as described earlier [20] and expressed as pmol cGMP formed per mg protein per min.

To determine whether ATP binds to the ANF receptor guanylate cyclase, the homogeneous immunopurified 180-kDa ANF receptor guanylate cyclase [21] was applied as a dot blot on a nitrocellulose membrane. The protein-binding sites were blocked by incubating the nitrocellulose membrane with 200 μl 0.3% bovine serum albumin for 10 min. This was followed by incubation for 60 min with [α-^{32}P]ATP (1 μCi/ml) in the absence or presence of the indicated non-radioactive ATP in the binding buffer containing 50 mM Tris-HCl, 0.3% Tween 20, 5 mM $MgCl_2$ and 1 mM EDTA, pH 7.5. The membrane was washed four times (15 min each wash) with the binding buffer, air-dried and autoradiographed using Kodak X-OMAT AR film.

ANF (rat, 8-33) used in these studies was a 26-amino acid peptide H-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-OH purchased from Peninsula Laboratories.

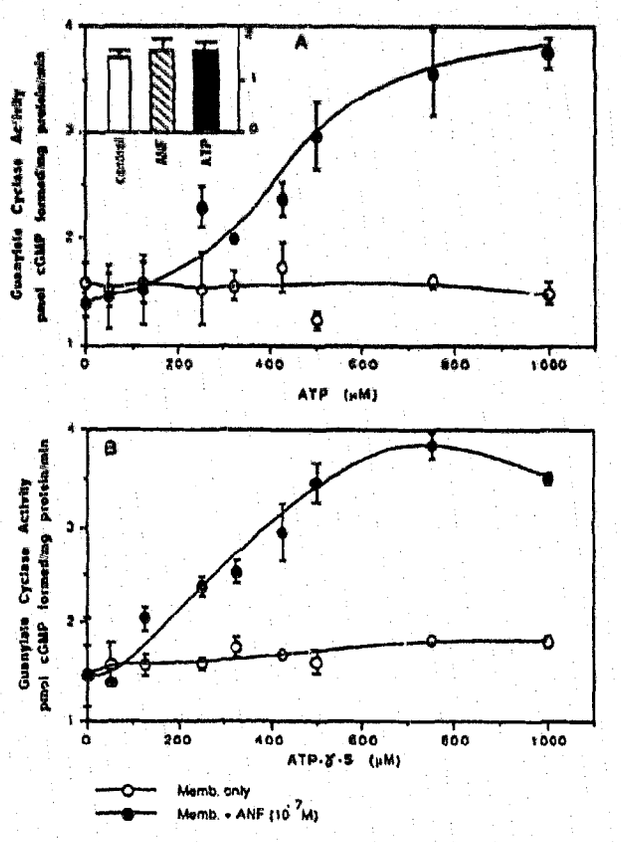

Fig. 1. Effect of ATP and ATPγS on basal and ANF-dependent guanylate cyclase activities in the presence of Mg^{2+} as cofactor. Rat testis membranes were assayed for guanylate cyclase activity in the presence or absence of ANF (10^{-7} M) with increasing concentrations of (A) ATP and, (B) ATPγS. The experiments were done in triplicate and repeated three times, although the data depicted are from one typical experiment. (Inset) Effect of ANF (10^{-7} M) or ATP (0.5 mM) alone on rat testis membrane guanylate cyclase activity. Each bar represents 7 separate experiments each done in triplicate. The means ± SE are shown. Abbreviations: Memb. = rat testis membranes.

3. RESULTS AND DISCUSSION

To determine the effect of ATP on basal and ANF-dependent guanylate cyclase activities in the presence of Mg^{2+} or Mn^{2+} as cofactors, rat testis membranes were incubated with a series of increasing concentrations of ATP with or without ANF. With Mg^{2+} as cofactor, neither ATP nor ANF altered the cyclase activity (Fig. 1A, inset); but, ATP in the presence of ANF (10^{-7} M) stimulated guanylate cyclase activity in a dose-dependent fashion (Fig. 1A). The ATP concentration causing half-maximal response (EC_{50}) was 400 μM. Similar response-patterns were obtained with the ATP analogues, ATPγS and AMP-PNP: ATPγS by itself stimulated guanylate cyclase activity only marginally, but when present with ANF caused a dose-dependent stimulation with an EC_{50} of 400 μM (Fig. 1B).

There are several intriguing aspects of these results obtained with rat testis membranes. Like those obtained with rat lung membranes [16,18] and other rat tissues [16] – kidney, heart, brain and adipocytes – ATP potentiates ANF-dependent guanylate cyclase activity. But, in contrast to all those tissues [16,18], ATP alone does not stimulate the testis membrane guanylate cyclase activity. Again, in contrast to all other above-mentioned tissues [16,18] where ANF independently (without the presence of ATP) stimulated guanylate cyclase activity, it did not do so in the testis membranes; the presence of both ANF and ATP was obligatory in this case.

In contrast to the above results, when the membranes were incubated with Mn^{2+} instead of Mg^{2+} as a cofactor, ANF (10^{-7} M) by itself stimulated the guanylate cyclase activity (Fig. 2A). Furthermore, ATP inhibited both the basal and ANF-dependent cyclase activities in a dose-dependent manner with a half-maximal concentration of ~400 μM in each case (Fig. 2B). Similar patterns of inhibition of both basal and hormone-dependent cyclase activities were observed upon

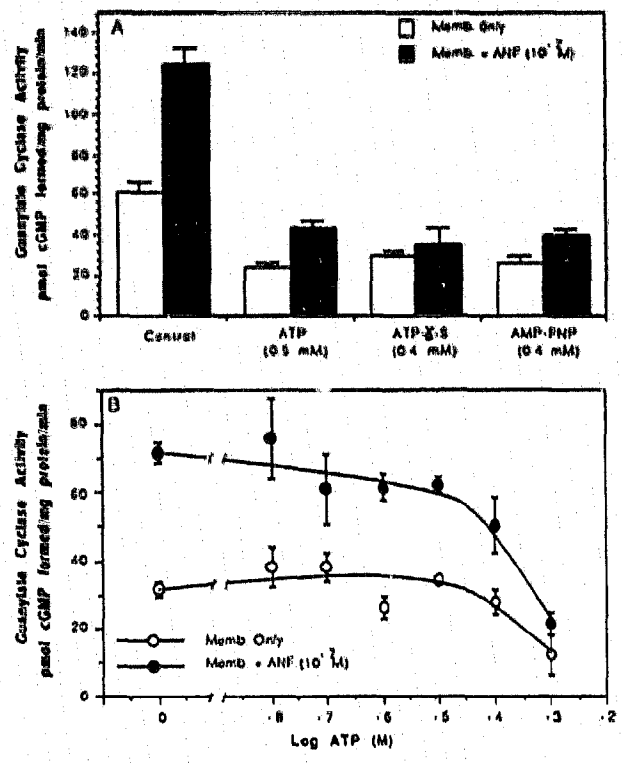

Fig. 2. Effect of ATP and its analogs on basal and ANF-dependent guanylate cyclase activities when Mn^{2+} was used as cofactor. (A) Basal and ANF (10^{-7} M)-dependent guanylate cyclase activities from rat testis membranes in the absence or presence of ATP (0.5 mM), ATPγS (0.4 mM) or AMP-PNP (0.4 mM). (B) Dose-dependent inhibition of basal and ANF (10^{-7} M)-dependent guanylate cyclase activities by ATP. The experiments were done in triplicate and repeated three times, although the data depicted are from one typical experiment. The means ± SE are shown. Abbreviations: Memb. = rat testis membranes.

substitution of ATP with its analogues, ATPγS or AMP-PNP (Fig. 2A). We do not yet know the physiological significance of the inhibitory effect of ATP in the presence of Mn^{2+}, because the concentrations of this divalent cation are very low in the tissues.

These contrasting stimulatory and inhibitory ATP-effects suggest a dual regulation of ANF-dependent guanylate cyclase activity by this nucleotide. To account for the stimulatory response, an original study had suggested an allosteric ATP-binding site on the ANF-receptor guanylate cyclase [16], and a recent study with transfected ANF receptor guanylate cyclase supported the ATP-dependent potentiation of ANF-responsive guanylate cyclase [17]; but so far an ATP-binding site on the ANF-receptor guanylate cyclase has not been demonstrated. We now show that ATP binds to the homogeneous ANF receptor guanylate cyclase (Fig. 3), providing direct support to a possible mechanism in which ATP-enzyme interaction at an allosteric site results in the ANF-dependent regulation of guanylate cyclase activity.

Direct ATP-binding to the enzyme may not be the sole regulatory mechanism of ANF-dependent guanylate cyclase activity. An additional mechanism might involve the participation of an accessory protein that binds ATP [18]. To support such a mechanism, it is important to demonstrate the existence of a protein in membranes containing ANF-responsive guanylate cyclase; in preliminary studies [22], we have identified a 72-kDa protein in rat testis membranes which binds ATP. The bound ATP could be displaced by ATP, ATPγS or AMP-PNP, but not by GTP. Studies are in progress to isolate this protein, reconstitute with

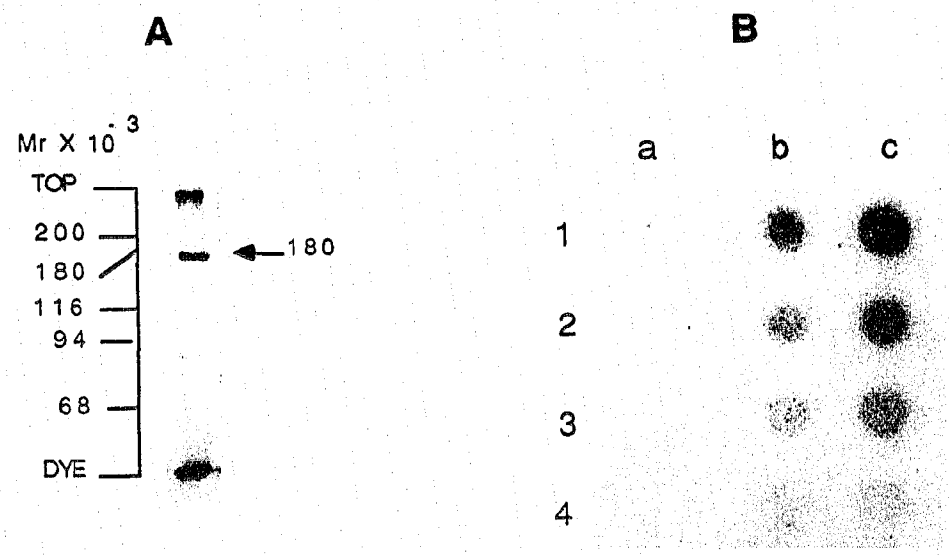

Fig. 3. ATP binding to the purified 180-kDa ANF-receptor guanylate cyclase. The guanylate cyclase from rat testis was purified by immunoaffinity chromatography utilizing antibodies raised against the 180-kDa membrane guanylate cyclase [22]. The purified 180-kDa guanylate cyclase was (A) radioiodinated and analyzed on SDS-7.5% polyacrylamide gel electrophoresis followed by autoradiography, and (B) applied to nitrocellulose membrane at indicated protein concentrations: (lane a) 0; (lane b) 0.5 μg; and (lane c) 1 μg. The blots were incubated with [α-^{32}P]ATP (1 μCi/ml) in the presence of: (1) no competitor; (2) 10 μM ATP; (3) 50 μM ATP; (4) 200 μM ATP as described in Materials and Methods, followed by autoradiography.

purified guanylate cyclase and test if it confers ATP-dependent ANF sensitivity to the cyclase.

Acknowledgements: We appreciate the support by the National Institutes of Health (NS-23744, EY07158) and the National Science Foundation (DCB 83-00500).

REFERENCES

[1] deBold, A.J. (1985) Science 230, 767-770.
[2] Schwartz, D. (1985) Science 229, 397-400.
[3] Atlas, S.A. and Laragh, J.H. (1986) Annu. Rev. Med. 37, 397-414.
[4] Cantin, M. and Genest, J. (1985) Endocr. Rev. 6, 107-127.
[5] Bex, F. and Corbin, A. (1985) Eur. J. Pharmacol. 115, 125-126.
[6] Mukhopadhyay, A.K., Bohnet, H.G. and Leidenberger, F.A. (1986) FEBS Lett. 202, 111-116.
[7] Pandey, K.N. and Singh, S. (1990) J. Biol. Chem. 265, 12342-12348.
[8] Waldman, S.A., Rapport, R.M. and Murad, F. (1984) J. Biol. Chem. 259, 14332-14334.
[9] Hamet, P., Tremblay, J.P., Pang, S., Garcia, R., Thibault, G., Gurtkowska, J., Cantin, M. and Genest, J. (1984) Biochem. Biophys. Res. Commun. 123, 515-527.
[10] Kuno, T., Andresson, W., Kamisaki, Y., Waldman, S.A., Chang, L.Y., Saheki, S., Leitman, D.C., Nakane, M. and Murad, F. (1986) J. Biol. Chem. 261, 5817-5823.
[11] Paul, A.K., Marala, R.B., Jaiswal, R.K. and Sharma, R.K. (1987) Science 235, 1224-1226.
[12] Takayanagi, R., Inagami, T., Snajdar, R.M., Inada, T., Tamura, M. and Misono, K.S. (1987) J. Biol. Chem. 262, 12104-12113.
[13] Chinkers, M., Garbers, D.L., Chang, M-S., Lowe, D.G., Chin, H., Goeddel, D.V. and Schulz, S. (1989) Nature 338, 78-83.
[14] Schultz, S., Singh, S., Bellet, R.A., Singh, G., Tubb, D.J., Chin, H. and Garbers, D.L. (1989) Cell 58, 1155-1162.
[15] Sharma, R.K., Chalberg, S.C. and Marala, R.B. (1990) in: A Decade of ANF Research, Ottawa Symposium on Atrial Natriuretic Factor, Ottawa, Ontario, Canada, June 21-23, 1990 (Abstr. no. 19).
[16] Kurose, H., Inagami, T. and Ui, M. (1987) FEBS Lett. 219, 375-379.
[17] Chinkers, M. and Garbers, D.L. (1989) Science 245, 1392-1394.
[18] Chang, C.-H., Kohse, K.P., Chang, B., Hirata, M., Jiang, B., Douglas, J.E. and Murad, F. (1990) Biochim. Biophys. Acta 1052, 159-165.
[19] Nambi, P. and Sharma, R.K. (1981) Biochem. Biophys. Res. Commun. 100, 508-514.
[20] Nambi, P., Aiyar, N.V., Roberts, A.N. and Sharma, R.K. (1982) Endocrinology 111, 196-200.
[21] Sharma, R.K., Marala, R.B. and Duda, T.M. (1989) Steroids 53, 437-460.
[22] Marala, R.B., Sitaramayya, A. and Sharma, R.K. (1991) Annual FASEB Meeting, April 21-25, 1991 (Abstr.)