Volume 199, number 1 FEBS 3572 April 1986

Atrial natriuretic factor regulation of cyclic GMP levels and steroidogenesis in isolated fasciculata cells of rat adrenal cortex

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Received 22 January 1986

Isolated fasciculata cells of rat adrenal cortex, when incubated with atrial natriuretic factor (ANF), stimulated the levels of cyclic GMP and corticosterone production in a concentration-dependent manner without a rise in the levels of cyclic AMP. The ANF-dependent elevation of cyclic GMP was rapid, with a detectable increment in 30 s. ANF also stimulated the particulate guanylate cyclase. These results not only indicate the coupling of cyclic GMP and corticosterone production with ANF signal, but also demonstrate that, like the ACTH signal, cyclic AMP is not the mediator of ANF-induced adrenocortical steroidogenesis.

Atrial natriuretic factor *cyclic GMP* *Membrane enzyme* *Guanylate cyclase* *Steroidogenesis* *(Rat adrenocortical cell)*

1. INTRODUCTION

Atrial natriuretic factor (ANF) is a peptide hormone that is released from atria and regulates sodium, water balance and blood pressure (review [1]). Although the presence of ANF receptors in tissues like kidney and blood vessels has been demonstrated [2–4], the biochemical mechanism of its action is not known. Since ANF selectively activates particulate guanylate cyclase, the possibility exists that cyclic GMP is the second messenger of ANF signal transduction [5]. We have provided extensive evidence for the mediatory role of cyclic GMP in ACTH-induced steroidogenesis and for the presence of ACTH-dependent particulate guanylate cyclase, in rat adrenocortical cells ([6] and references cited therein). Recently, atriopeptin has been shown to activate selectively particulate guanylate cyclase in rat adrenal cortex [7]. This common property of the two hormones of activating adrenocortical membrane guanylate cyclase provided us with a rationale to scrutinize whether the ANF receptor signal, like that of ACTH, is coupled to the process of steroidogenesis in isolated fasciculata cells of rat adrenal cortex [8]. The results indicate that this indeed was the case. We have discussed the implications of these findings in relationship to the mediatory role of cyclic GMP in steroidogenic signal transduction.

2. MATERIALS AND METHODS

Isolated fasciculata and adrenocortical carcinoma cell preparations were prepared by the trypsin digestion method [8,9]. The cells have been thoroughly characterized, morphologically and biochemically [9,10]. In general, for one experiment, adrenal glands from 16 rats or 1.2 g of the adrenocortical carcinoma tissue were used, and the cells from each adrenal gland (~2 × 10^6 cells) or the corresponding tumor cells were incubated with ANF as described [8]. Assays for cyclic nucleotides and steroids were conducted at 10 min and 2 h,

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

respectively [11]. Steroids were measured fluorometrically and expressed as corticosterone. Cyclic AMP and cyclic GMP were extracted and measured as in [11]. Results are expressed as the mean values (± SD) of 6 separate determinations from 3 different experiments.

3. RESULTS AND DISCUSSION

To determine the correlation between the synthesis of corticosterone and endogenous levels of cyclic GMP and cyclic AMP formed in response to the varying concentrations of ANF, isolated fasciculata cells of rat adrenal cortex were incubated with a series of concentrations of ANF and the levels of corticosterone, cyclic GMP and cyclic AMP measured (fig.1). The results indicate that ANF raises the levels of cyclic GMP, but not of cyclic AMP, in a concentration-dependent manner with a parallel increase in the production of corticosterone. The ED_{50} values of ANF for cyclic GMP and corticosterone production were 0.25×10^{-8} and 10^{-8} M, respectively. Similar results with respect to the elevations of cyclic GMP were

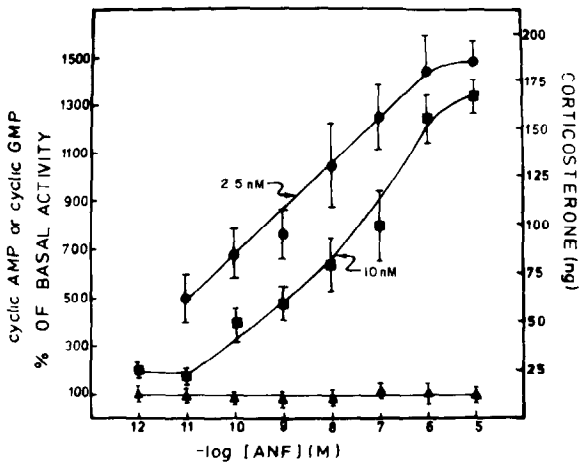

Fig.1. Dose-response curve for the production of cAMP (▲), cGMP (●) and corticosterone (■) in response to increasing concentrations of ANF in normal isolated adrenal cells. Incubation system: 0.8 ml adrenal cell suspension; reagens dissolved in 0.2 ml Krebs-Ringer bicarbonate buffer with glucose and albumin; total volume of incubation mixture 1.0 ml. The data summarize the results of 3 separate experiments, each of which contain duplicate cell incubation mixtures. Basal values (34 ± 2 ng corticosterone, 1.3 pmol cyclic GMP and 11.0 pmol cyclic AMP) have been subtracted.

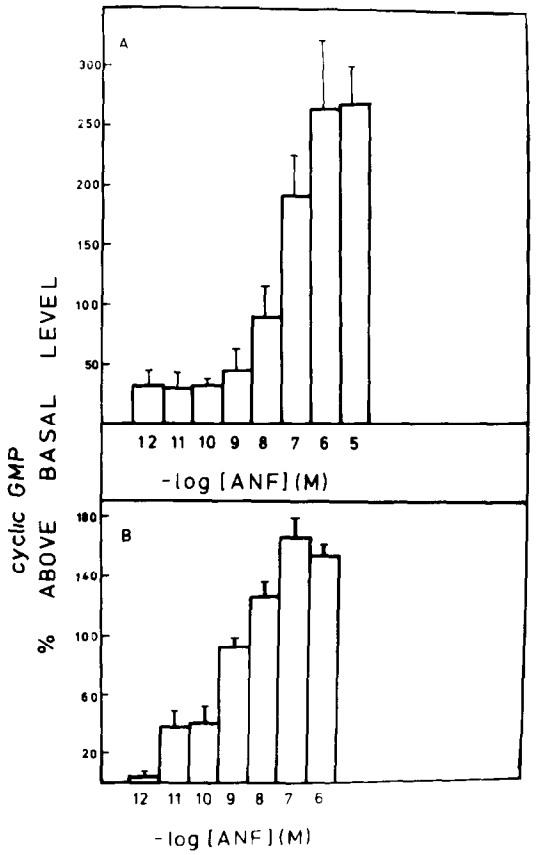

Fig.2. (A) Dose response of cGMP formation by ANF in isolated adrenocortical carcinoma cells. Conditions similar to those in fig.1. Basal value of cyclic GMP (0.84 ± 0.04 pmol) has been subtracted. (B) Dose response of guanylate cyclase activation in adrenocortical carcinoma membranes by ANF. Guanylate cyclase was assayed for 10 min as described [11]. Results are shown as the mean ± SE (n = 3). Basal guanylate cyclase activity, 68 ± 3 pmol/mg protein per 10 min.

observed in adrenocortical carcinoma cells (fig.2A). In these cells, like normal adrenal cells, hormonally dependent particulate guanylate cyclase is intact [6], but the steroidogenic cyclic GMP signal pathway is interrupted beyond cyclic GMP-dependent protein kinase [12]. The ANF-dependent rise of cyclic GMP is rapid, with a detectable rise in less than 30 s (fig.3). Since the isolated fasciculata cells [8] and carcinoma cells [9] used here do not contain cyclic GMP (or cyclic AMP) phosphodiesterase activity [13,14], the elevations in cyclic GMP reflect ANF-dependent

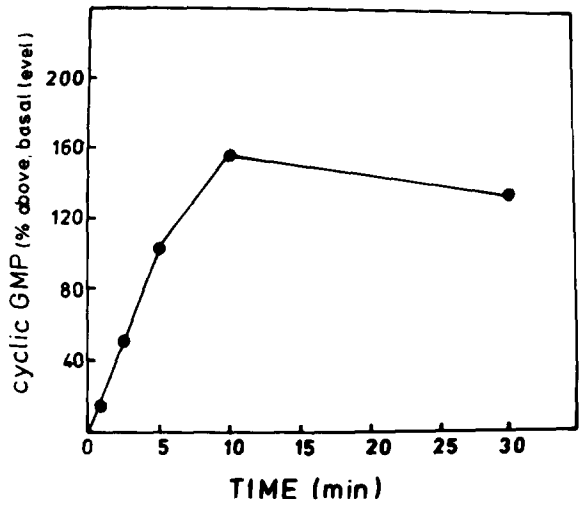

Fig.3. Time-response curve for the formation of cGMP in isolated adrenocortical carcinoma cells in response to 1 μM ANF. Conditions similar to those in fig.1.

guanylate cyclase activities in these cells. This interpretation is supported by the observation that ANF directly stimulates the membrane guanylate cyclase (fig.2B).

Previously, strong evidence for the mediatory role of cyclic GMP in ACTH signal transduction in corticosterone synthesis has been provided ([15] and references cited therein) and it has been shown that in intact isolated fasciculata cells: (i) cyclic GMP stimulates cyclic GMP-dependent protein kinase and corticosterone production; (ii) cyclic GMP directly stimulates the transformation of cholesterol to corticosterone; and (iii) the steroidogenic potential of cyclic GMP and its analogues correlates closely with their ability to stimulate cyclic GMP-dependent protein kinase. In addition, cyclic GMP-dependent protein kinase has been purified to homogeneity, and biochemically characterized in bovine adrenal cortex and isolated fascicula cells of rat adrenal cortex [16].

Firm evidence in support of the 'cyclic GMP model' [15] in adrenocortical steroidogenesis came when the ACTH-dependent particulate guanylate cyclase was demonstrated in rat adrenal cortex and rat adrenocortical carcinoma [6,17,18]. Following this first demonstration of hormonally dependent membrane guanylate cyclase, ACTH-dependent membrane guanylate cyclase in neuronal cells of chick cerebral hemisphere [19] and ANF-dependent particulate guanylate cyclase in various tissues have been demonstrated [5]. We anticipate that our present documentation of the coupling of ANF-receptor mediated steroidogenic signal with cyclic GMP in isolated fasciculata cells of rat adrenal cortex would pave the way for a serious consideration of this cyclic nucleotide as a bona fide second messenger in certain other hormonal transmembrane signal transduction pathways as well.

ACKNOWLEDGEMENTS

We thank Merck Sharp and Dohme Research Laboratories for the sample of synthetic ANF. This research was supported by the National Science Foundation (grant PCM 80-0873).

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