Vol. 145, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1987 Pages 336-343

EFFECTS OF STEROID AND THYROID HORMONES ON SYNTHESIS OF ATRIAL NATRIURETIC PEPTIDE BY CULTURED ATRIAL MYOCYTES OF RAT

H. Matsubara[1,2], Y. Hirata[1], H. Yoshimi[1], S. Takata[1], Y. Takagi[1], T. Iida[1], Y. Yamane[2], Y. Umeda[2], M. Nishikawa[2], and M. Inada[2]

[1]Hypertension-Endocrine Division, National Cardiovascular Center Research Institute, Suita, Osaka, 565, Japan

[2]Second Department of Internal Medicine, Kansai Medical University, Moriguchi, Osaka, 570, Japan

Received January 14, 1987

Summary: The in vitro effects of various steroid and thyroid hormones on synthesis of rat atrial natriuretic peptide (rANP) were studied using new-born rat atrial myocytes in culture. Dexamethasone, testosterone and triiodothyronine markedly stimulated both synthesis and secretion of immunoreactive (IR)-rANP with the same peak after 4-day-culture. Dexamethasone and testosterone dose-dependently (10^{-7}-10^{-6}M) stimulated synthesis of IR-rANP and were the most potent among various steroids tested. Triiodothyronine (T_3) also stimulated synthesis of IR-rANP in a dose-dependent manner (10^{-8}-10^{-7}M), of which effect was more potent than that of tetraiodothyronine, whereas reverse T_3 was ineffective. The present study clearly shows that glucocorticoids, androgens and thyroid hormones directly stimulate synthesis of ANP by atrial myocytes and suggests that ANP may play a potential role in mediating and/or modulating the biological effects by these hormones in the cardiovascular system.

Alpha-atrial natriuretic peptide (α-ANP) is a 28-amino-acid polypeptide hormone with a potent natriuretic and vasoactive effect (1-4). Although the complete amino-acid sequence of γ-ANP, a precursor molecule of α-ANP. has been characterized (5,6), the exact mechanism(s) of its synthesis and secretion has not been elucidated. We have recently established primary rat atrial myocytes in culture and studied the mechanism(s) by which rat (r) ANP is secreted in vitro (7).

It has very recently been reported that glucocorticoids stimulate synthesis of rANP accompanied by an increased accumu-

0006-291X/87 $1.50

lation of messenger (m) RNA for rANP in rat atria (8). The genomic structure for both rANP (9) and human ANP (10) contains DNA sequence homologous to the putative regulatory elements for glucocorticoids to which glucocorticoid-receptor complexes are considered to bind to induce gene transcription (11-13).

Using primary rat atrial myocytes in culture, we have attempted to study the effects of a variety of steroid hormones and thyroid hormones on synthesis of rANP in vitro.

MATERIALS AND METHODS

Cultured rat atrial myocytes were prepared from new-born (3 to 5-day-old) rats with 0.1% collagenase as described elsewhere (7). After atrial myocytes were cultured in Dulbecco's modified Eagle's medium (DMEM; Flow Laboratories, McLean, VA) containing 20 % fetal calf serum (FCS; Flow Laboratories) for 24 hrs, they were replaced with fresh DMEM without FCS. To study the effects of various drugs on synthesis of rANP, cells were usually incubated for 4 days with following drugs: dexamethasone, testosterone, corticosterone, aldosterone, deoxycorticosterone 17α-hydroxyprogesterone, 3,3',5-triiod-L-thyronine (T_3), 3,3',5,5' tetraiodo-L-thyronine (T_4) 3',5',3-triiodothyronine (reverse T_3) (all purchased from Sigma Chemical, St. Louis, MO).

The amounts of immunoreactive (IR)-rANP in cultured atrial myocytes and culture medium were determined by specific RIA as described elsewhere (7). IR-rANP in cultured cells was extracted by homogenization in 1M acetic acid containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 1 mM N-ethylmaleimide). Cellular protein was measured by Lowry's method using bovine serum albumin as standard (25).

RESULTS

As shown in Fig. 1, rat atrial myocytes cultured in serum-free condition synthesized and secreted IR-rANP as a function of time with the same peak on the 4th day, then decreased on the 6th day. Dexamethasone (10^{-6}M) and testosterone (10^{-6}M) similarly and significantly ($p < 0.01$) stimulated secretion of IR-rANP into medium as well as its synthesis, reaching a peak on the 2-4th day and then declined, while T_3 (10^{-7}M) also stimulated both synthesis and secretion of IR-rANP with a peak on the 4th day (Fig. 1). The effects of various steroid hormones in the same concentration (10^{-6}M) on synthesis of IR-rANP by

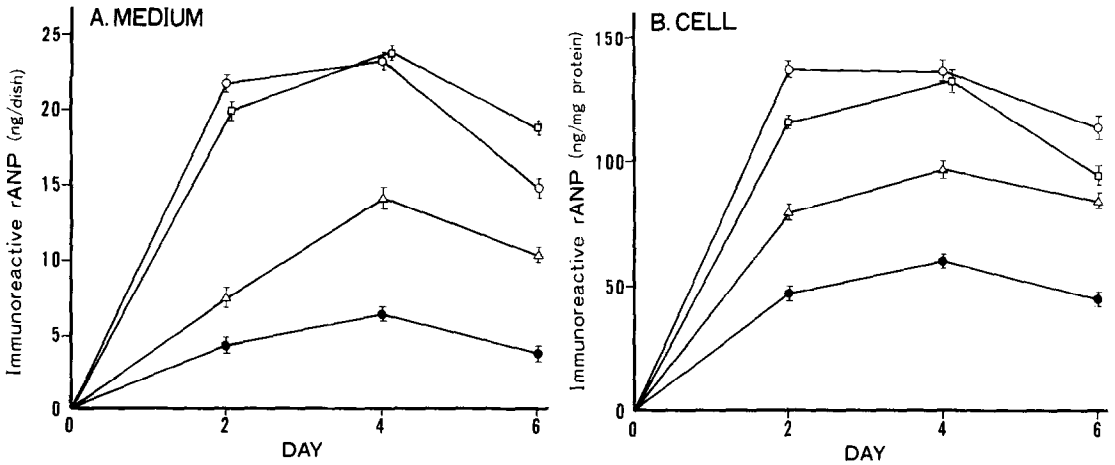


Fig. 1. Effects of dexamethasone, testosterone and triiodothyronine on secretion and synthesis of rANP by cultured rat atrial myocytes. Atrial myocytes were incubated in the absence (●) or presence of 10^{-6}M dexamethasone (DEX : o), 10^{-6}M testosterone (TEST : □) and 10^{-6}M triiodothyronine (T_3 : △). Concentrations of IR-rANP in media and atrial myocytes from different culture days were determined. Average cell numbers were 2 x 10^5 cells/dish (2-day), 5 x 10^5 cells/dish (4-day), and 6 x 10^5 cells/dish (6-day). Each point is the mean of 3 dishes; bar indicates SEM.

cultured rat atrial myocytes were compared as shown in Fig. 2. Dexamethasone and testosterone were almost equipotent (about 2-fold increase over control) and more potent in stimulating

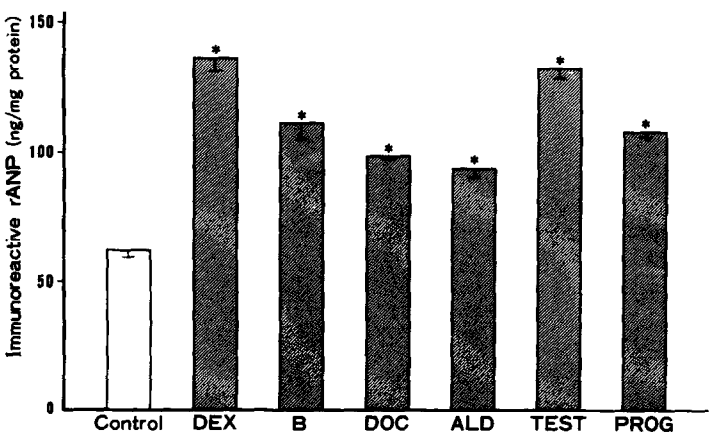


Fig. 2. Effects of various steroid hormones on synthesis of rANP by cultured rat atrial myocytes. Atrial myocytes (5 x 10^5 cells/dish) were incubated for 4 days in the absence (□) or presence (▨) of various steroids (10^{-6}M): dexamethasone (DEX), corticosterone (B), deoxycorticosterone (DOC), aldosterone (ALD), testosterone (TEST) and progesterone (PROG). Each column represents the mean of three dishes; bar indicates SEM.
* : $p < 0.01$ vs control.

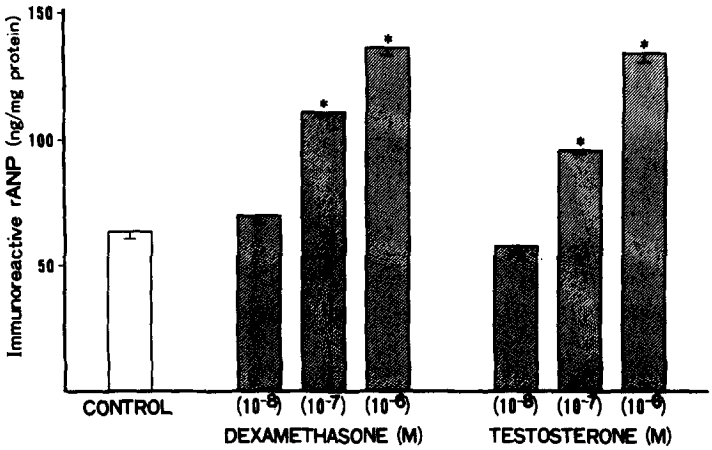


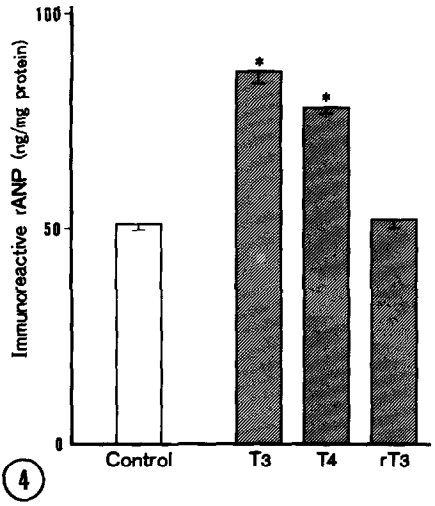
Fig. 3. Dose-responsive effects of dexamethasone and testosterone on synthesis of rANP by cultured rat atrial myocytes. Atrial myocytes (5 x 10^5cells/dish) were incubated for 4 days in the absence (□) or presence (▨) of dexamethasone and testosterone in various doses (10^{-8} - 10^{-6}M). Each column represents the mean of three dishes; bar indicates SEM.
* : $p < 0.01$ vs control.

synthesis of IR-rANP than corticosterone, progesterone (about 1.7-fold), deoxycorticosterone and aldosterone (about 1.5-fold). Both dexamethasone and testosterone stimulated synthesis of IR-rANP in a dose-responsive manner (10^{-7}-10^{-6}M) by cultured rat atrial myocytes (Fig. 3).

The effects of various thyroid hormones in the same concentration (10^{-7}M) on synthesis of IR-rANP were compared (Fig. 4). T_3 had a more potent effect (about 1.8-fold increase over control) in stimulating synthesis of IR-rANP than did T_4 (about 1.6-fold), whereas reverse T_3 was ineffective. Furthermore, T_3 dose-dependently (10^{-8}-10^{-7}M) stimulated synthesis of IR-rANP (Fig. 5).

DISCUSSION

It has recently been reported that administration of dexamethasone together with deoxycorticosterone, but not dexamethasone alone, to the adrenalectomized rats increases plasma rANP levels and atrial rANP contents (14). On the other hand,



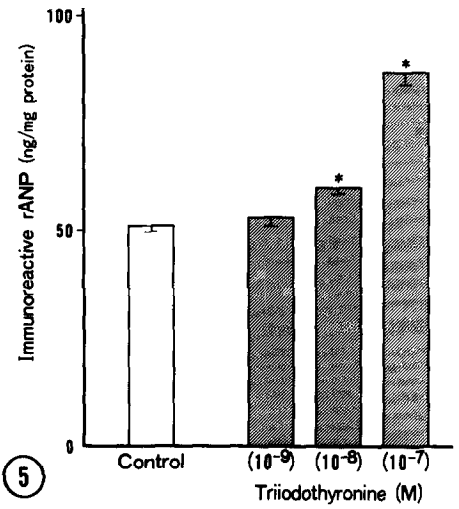


Fig. 4. Effects of various thyroid hormones on synthesis of rANP by cultured rat atrial myocytes. Atrial myocytes (5 x 10^5 cells/dish) were incubated for 4 days in the absence (□) or presence (▨) of 10^{-7}M triiodothyronine (T_3), 10^{-7}M tetraiodothyronine (T_4) and 10^{-7}M reverse T_3 (rT_3). Each column represents the mean of three dishes; bar indicates SEM. * : $p < 0.01$ vs control.

Fig.5 . Dose-responsive effects of triiodothyronine on synthesis of rANP by cultured rat atrial myocytes. Atrial myocytes (5 x 10^5 cells/dish) were incubated for 4 days in the absence (□) or presence (▨) of triiodothyronine (10^{-9} - 10^{-7}M). Each column represents the mean of three dishes; bar indicates SEM. * : $p < 0.01$ vs control.

Gardner et al. have very recently reported that administration of dexamethasone alone increases plasma rANP levels in both intact and adrenalectomized rats, accompanied by an increased accumulation of mRNA for rANP, whereas deoxycorticosterone has no effect (8). The present study using cultured atrial myocytes of new-born rats complements their studies and further demonstrates that not only dexamethasone but testosterone also directly stimulates synthesis of rANP by atrial myocytes. Since specific receptors for glucocorticoids as well as androgens have been demonstrated in the cytosol of the mammalian heart (15-19), it is suggested that glucocorticoids and androgens, after binding to their specific cytosolic receptors to form hormone-receptor complex in atrial myocytes, may activate transcription of the rANP gene, thereby leading to stimulation of synthesis of

rANP. In the present study, aldosterone and deoxycorticosterone, both mineralocorticoids, also had the stimulatory effects on synthesis of rANP, although far less than those of dexamethasone or testosterone in the same concentration. Since it has been already demonstrated that no specific receptors for mineralocorticoids exist in cardiac cytosol (17), it is possible to speculate that mineralocorticoids nonspecifically bind to glucocorticoids receptors to stimulate transcription of the rANP gene. Whether progesterone binds to its specific receptor and/or nonspecifically to other steroid receptors to exert a stimulatory effect on gene expression for rANP remains to be determined.

The present study further shows that T_3 and T_4 stimulate both synthesis and secretion of rANP by cultured rat atrial myocytes, whereas reverse T_3, an inactive derivative of T_3, has no stimulatory effect. It has been well documented that thyroid hormones bind to the nuclear receptors in cardiac cells to regulate gene expression for specific cardiac proteins, such as α-cardiac actin and α-myosin heavy chain (20-22). Although it has been reported that T_4-induced hyperthyroidism in rats causes a decrease of atrial contents of IR-rANP and a reciprocal increase of plasma IR-rANP levels (23), altered hemodynamic effects by T_4, such as increased heart rate, blood pressure and cardiac contractility, may have stimulated secretion of rANP with a concomitant decrease of atrial storage. The lack of direct effect of T_3 on rANP release from rat atrial slice in previous study (24) may be accounted by too short incubation time (4 hrs) employed to express its action on cardiac cells.

In summary, we have clearly shown for the first time that dexamethasone, testosterone and T_3 directly stimulate synthesis and secretion of IR-rANP by cultured rat atrial myocytes. These

findings indicate that synthesis and secretion of ANP is under multihormonal control including glucocorticoids, androgens and thyroid hormones, and suggest that ANP may play a potential role as a mediator and/or modulator of a variety of biological effects of these hormones in the cardiovascular system.

ACKNOWLEDGEMENTS

This study was supported in part by Research Grants for Cardiovascular Diseases (60A-3, 61C-2) from the Ministry of Health and Welfare, Japan.

REFERENCES

1. de Bold, A.J., Borenstein, H.B., Veress A.T., and Sonnenberg, H. (1981) Life Sci. 28: 2311-2318.
2. Currie, M.G., Geller, D.M., Cole, B.R., Boylan, J.C., Yusheng, W, Holmberg, S.W., Needleman, P. (1983) Science 221: 71-73.
3. Kangawa, K., Matsuo, H. (1984) Biochem. Biophys. Res. Commun. 118: 131-139.
4. Misono, K.S., Fukumi, H., Grammer, R.T., Inagami, T. (1984) Biochem. Biophys. Res. Commun. 119: 524-529.
5. Kangawa, K., Tawaragi, T., Oikawa, S., Mizuno, A., Sakuragawa, Y., Nakazato, H., Fukuda, A., Minamino, N., Matsuo, H. (1984) Nature 312: 152-155.
6. Nakayama, N., Ohkubo, H., Hirose, T., Inayama, S., Nakanishi, S. (1984) Nature 310: 699-701.
7. Matsubara, H., Hirata, Y., Takata, S., Takagi, Y., Yoshimi, H., Umeda, Y., Yamane, Y., Nishikawa, M. and Inada, M. (1987) Eur. J. Pharm. in press.
8. Gardner, D.G., Hane, S., Trachewsky, D., Schenk, D. and Baxter, J.D. (1986) Biochem. Biophys. Res. Commun. 139: 1047-1054.
9. Argentin, S., Nemer, M., Drouin, J., Scott, G.K., Kennedy, B.P. and Davies, P.L. (1985) J. Biol. Chem. 260: 4568-4571.
10. Greenberg, B.D., Bencen, G.H., Seilhamer, J.J., Lewicki, J.A. and Fiddes, J.C. (1984) Nature 312: 656-658.
11. Payvar, F., DeFranco, D., Firestone, G.L., Edgar, B., Wrange, O., Okret, S., Gustafsson, J.A. and Yamamoto, K.R. (1983) Cell 35: 381-392.
12. Galas, D. and Schmitz, A. (1978) Nucleic. Acids. Res. 5: 3157-3170.
13. Karin, M., Haslinger, A., Holtgreve, H., Richards, R.I., Krauter, P., Westphal, H.M. and Beato, M. (1984) Nature 308: 513-519.
14. Garcia, R., Debinski, W., Gutkowska, J., Kuchel, O., Thibault, G., Genest, J., and Cantin, M. (1985) Biochem. Biophys. Res. Commun. 131: 806-814.
15. Ballard, P.L., Baxter, J.D., Higgins, S.J., Rousseau, G.G. and Tomkins, G.M. (1974) Endocrinology 94: 998-1002.
16. Hickson, R.C., Galassi, T.M., Kurowski, T.T., Daniels, D.G. and Chatterton, R.T. (1984) Am. J. Physiol. 246: H 761-H767.

17. Funder, J.W., Duval, D. and Meyer P. (1973) Endocrinology 93: 1300 - 1308
18. McGill, H.C., Anselmo, V.C., Buchanan, J.M., Sheridan, P.J. (1980) Science 207: 775-777
19. Krieg, M., Smith, K., Bartsh, W. (1978) Endocrinology 103: 1686-1694.
20. Gustafson, T.A., Markham, B.E., Morkin, E. (1986) Cir. Res. 59: 194-201.
21. Nag. A.C., and Cheng, M. (1984) Biochem. J. 221: 21-26
22. Everett, A.W., Sinha, A.M., Umeda, P.K., Jakovcic, S., Rabinowitz, M., and Zak, R. (1984) Biochemistry 23: 1596-1599
23. Kohno, M., Takaori, K., Matsuura, T., Murakawa, K., Kanayama, Y., and Takeda, T. (1986) Biochem. Biophys. Res. Commun. 134: 178-183
24. Lachance, D., Garcia, R., Gutkowska, J., Cantin, M. and Thibault, G. (1986) Biochem. Biophys. Res. Commun. 135: 1090-1098
25. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193: 265-275