A C T A B I O C H I M I C A P O L O N I C A
Vol. 38 1991 No. 1

We very much regret that Fig. 1 was lost during printing of vol. 38 no 1 therefore the paper in full length is reprinted in this issue

ELŻBIETA BOGUSZ and WIESŁAW H. TRZECIAK

CORTICOTROPIN INDUCED SYNTHESIS OF THE CHOLESTEROL DESMOLASE ENZYMATIC COMPLEX IN CULTURED ADRENAL CORTEX CELLS

Department of Physiological Chemistry, K. Marcinkowski Medical Academy Święcickiego 6; 60-781 Poznań, Poland

Presented at 25th Meeting of the Polish Biochemical Society; September, 1989

Basing on the use of highly specific antibodies it has been proved that ACTH induces synthesis of cytochrome P-450 and adrenodoxin in cultured adrenal cortex cells.

In adrenal cortex cells synthesis of steroid cholesterol is limited by hydroxylation of the latter at positions 22 and 20R [1, 2]. This limiting step is catalysed by the cholesterol desmolase enzymatic complex composed of adrenodoxin reductase (Red), adrenodoxin (Adr) and cytochrome P-450scc (P-450), located in mitochondria [3, 4].

Although many interesting results have unequivocally evidenced that corticotropin (ACTH) stimulates synthesis of steroid hormones by stimulating expression of the genes encoding cytochrome P-450 and Adr, mediated by cyclic AMP (cAMP) and cAMP-dependent protein kinase [5, 6, 7], so far there are no reports on the effect of ACTH on the synthesis of cholesterol desmolase complex in rat adrenal cortex. Purification of the components of cholesterol desmolase to homogenity and preparation of specific antibodies [8] made these studies possible.

MATERIALS AND METHODS

Antigens and antibodies. Polyclonal rabbit antisera against P-450 and Adr, and appropriate antigens were a kind gift from Dr. M. R. Waterman (University of Texas, Dallas, USA).

Cell culture, labelling and immunoisolation of P-450 and Adr. Rat adrenal cortex cells were obtained by digestion with collagenase [9] and cultured on 60 mm Petri dishes (Falcon, U. S. A.) in the medium composed of equal volumes of F12 Hama medium and Eagle's medium (Gibco, U. S. A.) as modified by

Dulbecco [7] by adding 2% foetal calf serum (Serva, F. R. G.), 10% horse serum (Serva) and antibiotics: penicillin (10 U/ml) and streptomycin (0.1 mg/ml).

Protein synthesis was evaluated after 48 h of growth by measuring incorporation of [^{35}S]methionine (spec. act. 1120 Ci/mol) into P-450, Adr and F_1-ATPase [10, 11]. These protein were precipitated from the cell extracts with specific antibodies containing 2×10^6 counts per minute [12, 13], fractionated by polyacrylamide gel electrophoresis under denaturing conditions [14] and detected by autoradiography. In samples of the culture medium, corticosterone concentration was determined by the radioimmunological method [3].

RESULTS AND DISCUSSION

In the preliminary experiments it was found that corticosterone concentration was exponentially dependent on ACTH concentration in the medium; the maximum effect was observed at ACTH concentration of about 1 μm, whereas in the presence of 0.01 μM ACTH the effect was lower by half. With ACTH at the maximum effective dose, corticosterone concentration in the culture medium increased linearly between the 12th and 28th h of incubation.

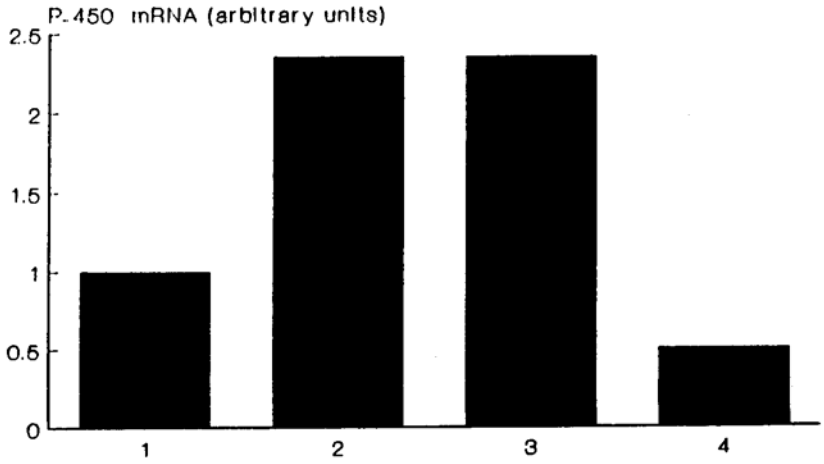
These optimal conditions (1 μM ACTH and 48 h incubation) were applied in the experiments on P-450 and Adr synthesis. In addition, db cAMP, a synthetic derivative of cAMP, was used since it penetrates readily the cellular membrane and acts as an intracellular mediator of ACTH. The db cAMP concentration applied was 1 mM, *i.e.* that which proved to be the most effective for stimulation of corticosterone excretion in bovine adrenal cortex cells [10, 11]. Synthesis of P-450 and Adr was evaluated from incorporation of labelled methionine into these proteins, after their precipitation with specific antibodies.

For comparison purpose, synthesis of F_1-ATPase non-inducible by ACTH and attached to the inner mitochondrial membrane was evaluated in the same way. The antigen-antibody complexes were disrupted by heating, fractionated by polyacrylamide gel electrophoresis, and the labelled antigens were detected by autoradiography. Basing on autoradiograms, densitometric measurements were made (Fig. 1, A, B, C).

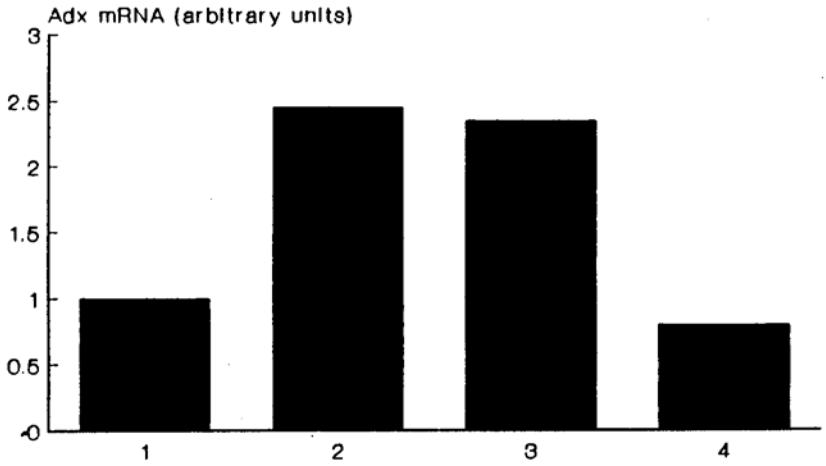
It was found that ACTH and db cAMP stimulate to a high extent synthesis of proteins of molecular mass of about 52 000 and 12 000. These proteins are unequivocally P-450 and Adr, respectively, since they were precipitated with specific antibodies and were removed from the complexes with the purified anti-P-450 and anti-Adr antibodies.

Fig. 1. Densitometric analysis of autoradiograms of A, P-450; B, adrenodoxin; C, F_1-ATPase (two subunits), 1, Without any additions; 2, with 1 μM ACTH; 3, with 1 mM db cAMP; 4, with db cAMP (immunoisolation was performed in the presence of 5 μg of the purified antigen)

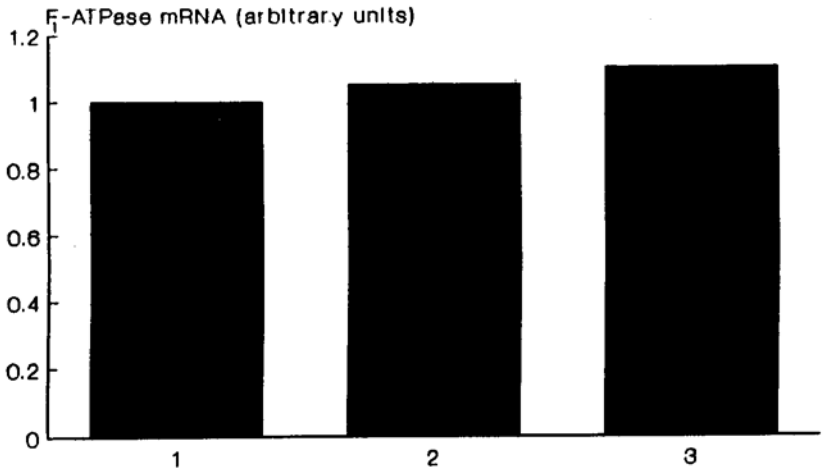
A.


P-450 mRNA (arbitrary units)
2.5
2
1.5
1
0.5
0
1
2
3
4

B.


Adx mRNA (arbitrary units)
3
2.5
2
1.5
1
0.5
0
1
2
3
4

C.


F1-ATPase mRNA (arbitrary units)
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3

Under the same conditions no stimulation of mitochondrial F_1-ATPase was observed (Fig. 1C) which supports specificity of ACTH effect on expression of P-450 and Adr genes.

REFERENCES

1. Beckett, G. S. (1977) *Eur. J. Biochem.*, **72,** 223-233.
2. Ivarie, R. D. & Jones, P. P. (1979) *Anal. Biochem.*, **57,** 24-35.
3. Kramer, R. E., DuBois, R. N., Simpson, E. R., Anderson, C. M., Kashiwagi, K., Lambeth, J. D., Jefcoate, C. R. & Waterman, M. R. (1982) *Arch. Biochem. Biophys.*, **215,** 478-485.
4. Laemmli, U. K. & Favre, M. (1973) *J. Mol. Biol.*, **80,** 575-599.
5. Matocha, M. F. & Waterman, M. R. (1984) *J. Biol. Chem.*, **259,** 8672-8678.
6. Nagshineh, S., Treadwell, C. R., Gallo, L. & Vahouny, G. V. (1974) *Biochem. Biophys. Res. Commun.*, **61,** 1076-1082.
7. Rainey, W. E., Shay, J. W. & Mason, J. I. (1986) *J. Biol. Chem.*, **261,** 7322-7326.
8. Rodgers, R. S., Rodgers, H. F., Waterman, M. R. & Simpson, E. R. (1986) *Reprod. Fert,* **78,** 639-652.
9. Simpson, E. R. (1979) *Mol. Cell Endocrinol.*, **13,** 213-227.
10. Stone, D. & Hechter, O. (1954) *Arch. Biochem. Biophys.*, **51,** 457-469.
11. Trzeciak, W. H., Ahmed, C. E., Simpson, E. R. & Ojeda, S. R. (1986) *Proc. Natl. Sci. U. S. A.*, **83,** 7490-7494.
12. Trzeciak, W. H. & Boyd, G. S. (1973) *Eur. J. Biochem.*, **37,** 327-333.
13. Trzeciak, W. H. & Mathe, D. (1981) *FEBS Lett.*, **130,** 113-118.
14. Trzeciak, W. H., Simpson, E. R., Scallen, T. J., Vahouny, G. V. & Waterman, M. R. (1987) *J. Biol. Chem.*, **262,** 3713-3717.
15. Trzeciak, W. H., Waterman, M. R. & Simpson, E. R. (1986) *Endocrinology,* **119,** 323-330.