EFFECT OF GONADOTROPHINS ON ADENYLATE CYCLASE OF THE OUTER AND INNER MEMBRANE SUBFRACTIONS OF RAT TESTIS MITOCHONDRIA

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Received 12 March 1974

1. Introduction

It has been suggested that 3':5'-cyclic monophosphate (cAMP) functions as an intracellular mediator of gonadotrophin-induced steroidogenesis in the testis [1]. This observation was strengthened by the findings that gonadotrophins stimulate testicular adenylate cyclase [2, 3] and that cAMP and its dibutyryl derivative increased testosterone synthesis in the testis [4, 5]. Furthermore, evidence has been presented that cAMP stimulates steroidogenesis by increasing the conversion of cholesterol to pregnenolone [6]. This reaction is catalyzed in rat testis by the mitochondrial cholesterol side-chain cleavage enzymes [7] and it is assumed to be controlled by gonadotrophic hormones [8].

Recent studies have shown the presence of a gonadotrophin-sensitive adenylate cyclase in rat testis mitochondria [9]. This might suggest that the trophic hormone acts at a mitochondrial locus, and that cAMP locally formed might be utilized in the initiation of the steroidogenic pathway. The present report is concerned with the submitochondrial localization of adenylate cyclase, with particular reference to the effect of gonadotrophins on this enzyme.

The present results indicate that both outer and inner mitochondrial membranes contain adenylate cyclase activity. Gonadotrophic hormones added in vitro to such preparations caused an increase in enzymic activity in the outer membrane subfraction and have no effect on the enzyme attached to the inner mitochondrial membrane.

2. Materials and methods

Human urinary luteinizing hormone (LH) 100 I.U./mg, containing 0.41 I.U. FSH/mg and human urinary follicle stimulating hormone (FSH), 1055 I.U./mg, containing 2.3 I.U. LH/mg were generously supplied by the Instituto Serono (Italy). Human chorionic gonadotrophin (HCG) 3800 I.U./mg was purchased from Ikapharm (Israel). [^{3}H] adenosine 3':5'-cyclic monophosphate (specific activity 27.5 Ci/mmole) was obtained from the Radiochemical Centre (Amersham, England). Testicular tissue was obtained from mature rats (Charles River Colony), 60–80 days old weighing 200–250 g. The rats were killed by a sharp blow on the head and the testes were removed, decapsulated and homogenized in ice-cold 0.25 M Sucrose. Preparation of mitochondria and of submitochondrial fractions was performed as previously described [10]. Treatment of the sonicated mitochondria with Lubrol WX was omitted, since the non-ionic detergent was shown to solubilize the enzyme [9]. Briefly, outer and inner mitochondrial membranes were obtained by subjecting the washed mitochondrial pellets to controlled osmotic lysis, ultrasonic vibration, followed by differential centrifugation [10]. Submitochondrial fractions were suspended in 0.05 M Tris–HCl buffer (pH 7.8). Aliquots (0.1–0.2 ml) of mitochondrial membranes were added to an equal volume of reaction mixture containing 3 mM $M_g SO_4$, 5 mM theophylline, 1 mM ATP, 5 mM phosphoenolpyruvate and pyruvate kinase (60 mg/ml). Incubations were carried out on a shaking incubator at 37°C for

10 min. The reaction was terminated by addition of trichloroacetic acid to give a final concentration of 5%. The cyclic nucleotide was isolated as previously reported [9] and measured by the technique described by Gilman [11], modified by using charcoal plus 2% BSA [12] to separate the bound from the free nucleotide. In order to evaluate the possible interference by excess ATP or other nucleotides present in the submitochondrial fractions with the binding assay, cAMP was isolated from the reaction mixture by both chromatography on aluminium oxide [13] and Dowex-50 resin [14]. The results were found to be identical with those obtained using mitochondrial membranes not chromatographed by these methods. Protein concentration was determined by the procedure of Lowry et al. [15].

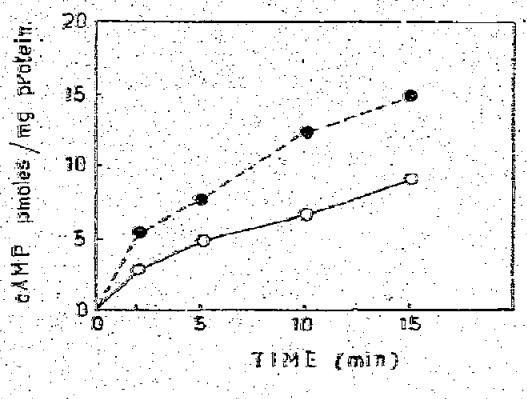

Fig. 1. Time course of cAMP accumulation by an outer mitochondrial membrane in the absence (—) and presence (---) of LH (20 I.U./incubation). The data represent the means of 4–6 determinations.

3. Results and discussion

Adenylate cyclase activity was found to be associated with both outer and inner mitochondrial membranes (table 1). The enzymic activity was higher in the outer membrane subfraction. Each of the subfractions had higher specific activity than intact mitochondria. The 155 000 g supernatant which contained the matrix and the cell sap (see ref. [10]) was not capable of converting ATP to cAMP. The results indicate that during the procedure used to subfractionate the mitochondria the adenylate cyclase enzyme remained attached to the membrane system of this organelle. Table 2 summarized the effect of gonadotrophic hormones and sodium fluoride on cAMP accumulation by mitochondrial membranes isolated from rat testicular tissue. HCG, LH and FSH, 20 I.U. per incubation, caused an increase in cAMP accumulation (40–70%) in the outer mitochondrial membrane, whereas the same amounts of these hormones did not influence the conversion of ATP to cAMP in the inner mitochondrial membrane. Sodium fluoride was found to stimulate the enzyme activity in both submitochondrial fractions, 6–9-fold. The stimulatory effect of LH on the adenylate cyclase of the outer membrane subfraction was higher than that of HCG or FSH. The first detectable increase in cAMP levels occurred two minutes after addition of LH, and the percentage increase over the control levels induced by this hormone (60–75%) was nearly the same after 2, 5, 10 and 15 min (fig. 1). The concentrations of gonadotrophins required to produce an increase in the adenylate cyclase activity by the outer mitochondrial membrane were higher than those required to stimulate this enzyme in an intact mitochondrial preparation [9].

The adenylate cyclase activity of inner mitochondria membrane was activated by sodium fluoride but not by gonadotrophins (table 2). Addition of GTP or ITP which were shown to enhance the adenylate cyclase activity of liver membranes [16, 17] neither increased the basal cyclase activity in the inner sub-

Table 1
Distribution of adenylate cyclase in intact mitochondria and submitochondrial fractions of rat testis

Subcellular fractions	cAMP pmoles/mg protein/ 10 min
Intact mitochondria	3.40 ± 0.04 (6)
Outer membrane	7.10 ± 0.09 (6)
Inner membrane	5.20 ± 0.07 (6)
Matrix + cell sap (155 000 *g* sup.)	0.00

The incubation procedure and the cAMP assay were carried out as described in the section on Methods. The adenylate cyclase activity is expressed as pmoles cAMP formed/mg protein/10 min. incubation. Results are given as mean ± SE of the mean followed by the number of observations in parentheses.

Table 2
Effect of gonadotrophins and sodium fluoride on the adenylate cyclase activity of outer and inner membrane subfractions of rat testis mitochondria

Addition	cAMP pmoles/mg protein/10 min incubation Outer membrane	Inner membrane
No addition	7.10 ± 0.09 (6)	5.20 ± 0.07 (6)
LH 10 IU	9.50 ± 0.10 (5)	—
LH 20 IU	12.40 ± 0.14 (5)	5.80 ± 0.07 (5)
HCG 10 IU	8.75 ± 0.09 (5)	4.90 ± 0.05 (5)
HCG 20 IU	10.40 ± 0.10 (5)	6.00 ± 0.07 (5)
FSH 10 IU	8.30 ± 0.10 (4)	4.50 ± 0.05 (4)
FSH 20 IU	10.70 ± 0.10 (4)	5.40 ± 0.07 (4)
NaF 10 mM	48.50 ± 0.22 (6)	45.60 ± 0.25 (6)

The hormones and sodium fluoride were dissolved in 0.05 M Tris–HCl buffer (pH 7.8) and added to the incubation mixture. Results are given as mean ± SE of the mean followed by the number of observations in parentheses.

mitochondrial membrane nor affected its sensitivity to gonadotrophic hormones. The inner membrane subfraction was suggested to be the locus of mitochondrial conversion of cholesterol to pregnenolone in the adrenal cortex [18, 19]. It is of considerable interest that the adenylate cyclase of the inner mitochondrial membrane was activated by sodium fluoride but not by gonadotrophic hormones. The data indicate that the procedure used to fractionate the mitochondria may have abolished the capacity of gonadotrophins to bind to its receptors, without affecting the adenylate cyclase system.

The results reported here demonstrate the presence of adenylate cyclase on both outer and inner mitochondrial membranes and the capability of the gonadotrophic hormones to stimulate the enzyme attached to the outer membrane subfraction. These results further support our previous findings of the presence of a gonadotrophin-sensitive adenylate cyclase in rat testis mitochondria [9] free of plasma membrane and endoplasmic reticulum [10].

Acknowledgements

This study was supported by Ford Foundation Grant No. 67–470. The skillful assistance of Mrs. M. Kaufman is greatly appreciated.

References

[1] Eik-Nes, K. B. (1971) Recent Progr. Horm. Res. 27, 517–535.
[2] Murad, F., Strauch, B. S. and Vaughan, M. (1969) Biochim. Biophys. Acta 177, 591–598.
[3] Kuehl, Jr., F.A., Patanelli, D.J., Tarnoff, J. and Humes, J. L. (1970) Biol. Reprod. 2, 154–165.
[4] Connell, G. M. and Eik-Nes, K. B. (1968) Steroids 12, 507–516.
[5] Dufau, M. L., Catt, K. J. and Tsuruhara, T. (1971) Biochim. Biophys. Acta 252, 574–579.
[6] Karaboyas, G. S. and Koritz, S. B. (1965) Biochemistry 4, 462–468.
[7] Toren, D., Menon, K. M. J., Forchielli, E. and Dorfman, R. I. (1964) Steroids 3, 381–390.
[8] Menon, K. M. J., Forchielli, E. and Dorfman, R. I. (1967) Biochim. Biophys. Acta 148, 486–494.
[9] Sulimovici, S. and Lunenfeld, B. (1973) Biochem. Biophys. Res. Commun. 55, 673–679.
[10] Sulimovici, S., Bartoov, B. and Lunenfeld, B. (1973) Biochim. Biophys. Acta 321, 27–40.
[11] Gilman, A. G. (1970) Proc. Natl. Acad. Sci. U.S. 67, 305–312.
[12] Brown, B. L., Albano, J. D. M., Ekins, R. P. and Sgherzi, A. M. (1971) Biochem. J. 121, 561–562.
[13] Ramachandron, J. (1971) Anal. Biochem. 43, 227–239.
[14] Chlapowski, F. J. and Butcher, R. W. (1973) Biochim. Biophys. Acta 309, 138–148.
[15] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[16] Leray, F., Chambaut, A. M. and Hanoune, J. (1972) Biochem. Biophys. Res. Commun. 48, 1385–1391.
[17] Swislocki, N. I., Tierney, J. and Sonenberg, M. (1973) Biochem. Biophys. Res. Commun. 53, 1109–1114.
[18] Yago, N. and Ichii, S. (1969) J. Biochem. Tokyo 65, 215–224.
[19] Yago, N., Kobayashi, S., Sekiyama, S., Kurokawa, H., Iwai, Y., Suzuki, I. and Ichii, S. (1970) J. Biochem. Tokyo 68, 775–783.