ADENYLATE CYCLASE IN FOETAL RAT TESTES AND ITS STIMULATION BY LH AND NaF

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Received 19 July 1976

1. Introduction

Rat testes have been demonstrated to synthesize testosterone during foetal life [1,2]. Testosterone secretion could be observed in vitro and enhanced with dibutyryl cyclic adenosine-3′-5′-monophosphate (dibutyryl cyclic AMP) [3] and with LH [4]. It has been accepted that in adult testes, LH-induced testosterone biosynthesis was mediated by cyclic AMP [5]. However, low levels of LH have been found to stimulate steroidogenesis without detectable changes in cyclic AMP levels [6]. Several hypotheses have been advanced: the analytical method for cyclic AMP could be insufficiently sensitive to detect small differences or inadequate to measure eventual variations in a particular intracellular compartment [7]. These disadvantages could be avoided by testing adenylate cyclase activity.

The present communication describes cyclic AMP generation measured by the conversion of radioactive ATP in homogenates of foetal testes. The results obtained suggest that adenylate cyclase activity is present in foetal rat testes and that it can be increased by NaF and by LH. Higher LH concentrations are required to stimulate adenylate cyclase activity than testosterone secretion.

2. Materials and methods

Rat testis tissue was obtained from 15–20-day-old foetuses (Sherman strain). Gestational age was based upon the timing of coitus [8]. Ovine LH (NIH-LH-S19, 1.01 unit/mg) was a gift from the National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Maryland. ATP (disodium salt), cyclic AMP, creatine phosphokinase (214 i.u./mg) and creatine phosphate were obtained from Calbiochem, San Diego, California., Dowex AG50W-X8 cation exchange resin (200–400 mesh, H^+) from Bio-Rad, Richmond, California and neutral aluminium oxide 90 aktiv neutral from Merck, Darmstadt, Germany. Cyclic [^{3}H]AMP (ammonium salt, 27 Ci/mmol) stored at −20°C in water/ethanol (50:50) was purchased from the Radiochemical Centre, Amersham and [α-^{32}P]ATP (ammonium salt, 9.3 to 28.6 Ci/mM) stored at −20°C in aqueous solution from New England Nuclear Corp. Boston.

Testes were homogenized in a Kontes glass homogenizer in Tris–HCl 10 mM, pH 7.4, 0.25 M sucrose and 3.2 mM $MgCl_2$ at 2–4°C; homogenates were used as the source of adenylate cyclase immediately after preparation. In some experiments, homogenates were centrifuged at 800 × g for 10 min and the pellet was washed twice and resuspended in the same volume without sucrose. Reactions were carried out in a final volume of 100 μl which contained 100 mM Tris–HCl, pH 7.4, 0.25 mM ATP, 0.6 μCi [α-^{32}P]ATP, 1 mM cyclic AMP, 20 mM phosphocreatine, 0.8 to 10 mM $MgCl_2$ and 50 to 200 μg of enzyme at 36°C. The reaction was initiated by the addition of enzyme and terminated by adding 150 μl of a diluting solution composed of 50 mM Tris–HCl pH 7.4, 5 mM cyclic AMP, 3 mM ATP and 4.5×10^{-3} μCi of cyclic [^{3}H] AMP and incubating at 60°C for 3 min. Cyclic AMP was separated by sequential chromatography on columns of Dowex cation exchange resin and aluminium oxide [9]; the eluate was collected into plastic counting vials with 5 ml scintillation liquid (Unisolve). The ^{3}H and ^{32}P radioactivities were

determined in an Intertechnique liquid scintillation counter. The ^{32}P content of the samples was corrected for cyclic AMP recovery determined from the ^{3}H content and for the blank value corresponding to a sample diluted immediately after the addition of enzyme. The blank value (with or without enzyme) was 3 to 5 cpm ^{32}P (0.0004%). Recovery of cyclic [^{3}H]AMP was approximately 80%.

Protein was determined according to Lowry et al. [10] with the use of bovine serum albumin standard.

3. Results and discussion

The amounts of cyclic AMP formed were found to increase linearly with incubation time (0 to 30 min) both with total homogenates and pellets. In both cases fluoride stimulated adenylate cyclase activity; a time course relationship was also observed with fluoride (fig.1). A relationship between enzyme concentration (55 to 223 μg) and cyclic AMP production was established (fig.2).

Stimulation of the adenylate cyclase activity was observed when LH was added to the incubation medium (figs.2-3). Basal and LH-stimulated adenylate cyclase activities were measured in the presence of increasing magnesium concentrations ranging from

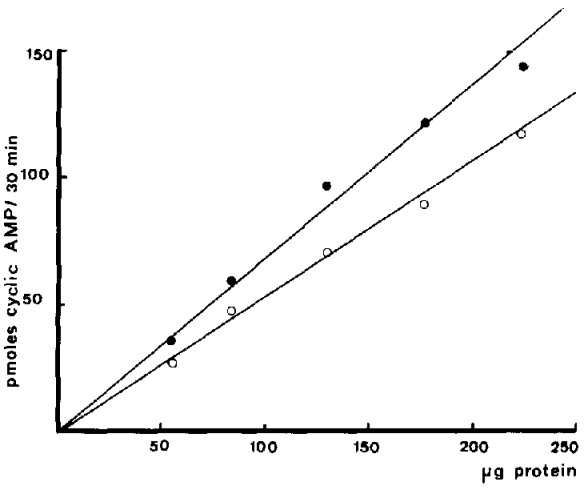

Fig.2. Relationship between enzyme concentration and cyclic AMP production in the absence (○) and presence (●) of LH (50 μg/ml). Total homogenate from 20-day-old testes. Incubation time = 30 min. Incubation medium with 5 mM $MgCl_2$.

0.8 to 10 mM (table 1). Both activities increased until 7.5 mM Mg, but the stimulatory effect of LH was weaker above 2.6 mM Mg. The effect of calcium on the enzymatic response to LH was also examined. When the Ca concentration increased above 10^{-5} M, basal and LH-stimulated cyclase activities were progressively inhibited (table 2). Similar observations

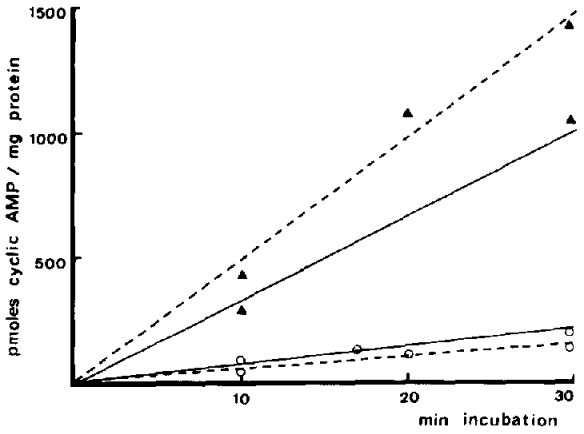

Fig.1. Accumulation of cyclic AMP as a function of time under basal conditions (○) or after stimulation by 10^{-2} M fluoride (▲). Total homogenate = 1058 μg/ml (——) and pellet: 1770 μg/ml (- - - -) from 20-day-old testes. Incubation medium with 0.8 mM $MgCl_2$.

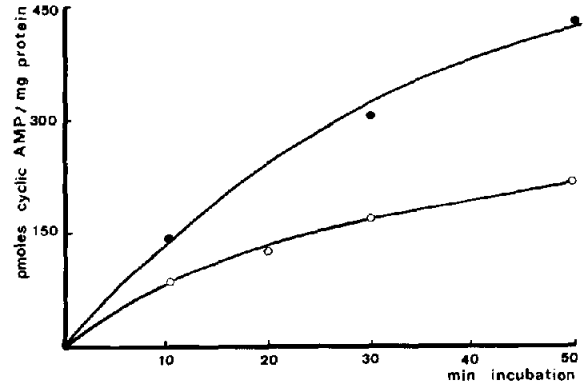

Fig.3. Accumulation of cyclic AMP as a function of time under basal conditions (○) or after stimulation by 500 μg/ml LH (●). Total homogenate from 20-day-old testes (854 μg/ml). Incubation medium with 0.8 mM $MgCl_2$.

Table 1
Effect of magnesium on basal and LH-stimulated adenylate cyclase activities

Mg^{2+} final concentration (mM)	Picomoles cyclic AMP/30 min/mg protein		Ratio
	Control	LH (50 μg/ml)	LH/control
0.8	225	330	1.46
2.6	327	508	1.55
5.0	489	574	1.14
7.5	587	708	1.21
10.0	558	649	1.16

20-day-old testes. Total homogenate (1095 μg/ml). No calcium added. Means of duplicate assays.

Table 2
Effect of calcium on basal and LH-stimulated adenylate cyclase activities

Ca^{2+} final concentration (M)	Picomoles cyclic AMP/30 min/mg protein		Ratio
	Control	LH (50 μg/ml)	LH/control
10^{-6}	511	641	1.26
5×10^{-6}	565	703	1.24
10^{-5}	555	679	1.22
10^{-4}	501	507	1.01
10^{-3}	131	162	1.24

20-day-old testes. Total homogenate (890 μg/ml). Incubation medium with 5 mM $MgCl_2$ – Means of duplicate assays.

have been made in other cyclase systems, especially in ACTH-stimulated adrenal tissue [11].

According to the preceeding results, the dose response relationship between LH and cyclic AMP synthesis was tested in an incubation medium containing 0.8 mM Mg and no Ca (fig.4). It is noteworthy that the stimulation of adenylate cyclase activity induced with LH occurs earlier than the stimulation of steroidogenesis described previously [4], so cyclic AMP could be the second messenger in the foetal testis. LH concentrations from 0.05 μg/ml induced increasing adenylate cyclase activities. A previous study demonstrated a weak effect of 100 μg/ml LH (ovine NIH-LH) on the enzymatic activity of homogenates from adult rat testes incubated with non radioactive ATP [12]. Recent results indicate a more important effect with about 1 μg/ml LH (same origin) on prepubertal rat testes [13]. However concentrations of LH below 0.05 μg/ml were not effective in stimulating adenylate cyclase activity while stimulation of testosterone production could be elicited by 1 ng/ml LH [4]. So the discrepancies previously observed between the concentrations required for the stimulation of testosterone production and enhancement of cyclic AMP concentration [6] were confirmed when adenylate cyclase activity was tested. However

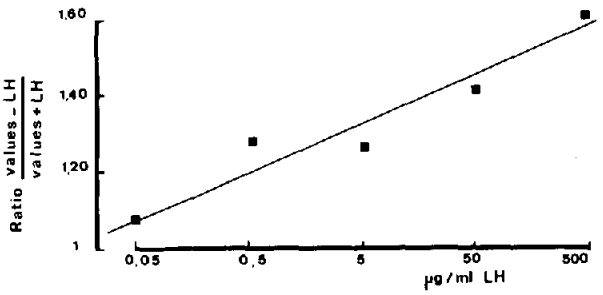

Fig.4. Effect of LH concentration on adenylate cyclase activity. Total homogenate from 20-day-old testes (1060 μg/ml). Incubation time = 10 min. Incubation medium with 0.8 mM $MgCl_2$. Correlation coefficient $r = 0.953$.

Table 3
Adenylate cyclase activities of rat testes during the foetal period.

Age (days)	μg protein/testis	Picomoles cyclic AMP/30 min/testis		
		Control	LH (50 μg/ml)	NaF (10^{-2} M)
15	14	0.44 (2)	0.48 (2)	3.30 (1)
17–18	40	2.39 (2)	2.65 (2)	22.34 (1)
20	98	12.11 (2)	18.16 (2)	91.85 (1)

Total homogenate (420 to 1040 μg/ml). Incubation medium with 0.8 mM $MgCl_2$. No calcium added. In parentheses, number of duplicate assays.

the experimental conditions were not the same; the stimulation of testosterone production was obtained using intact testes in a medium of different composition.

An approximately 1.5-fold increase in adenylate cyclase activity was the maximum that could be obtained in 20-day-old foetal testes following a 10 min incubation with LH. The stimulatory effect was weaker in younger testes (table 3). The effect of LH on testosterone secretion was also more pronounced with 20-day-old testes than with younger ones [4]. To obtain a higher stimulation of adenylate cyclase activity and to demonstrate the specificity of the response to LH, it would be necessary to test the hormone on isolated interstitial cells. Unfortunately such a preparation would be difficult to obtain with foetal testes at a stage when it is not yet possible to separate interstitial cells from seminiferous cords.

Acknowledgements

We would like to thank Dr S. Jard and Mr Ch. Roy (Collège de France, Paris) for their useful advice, the NIH Endocrinology Study Section for gifts of ovine LH and Mrs M. N. Gangnerau for her excellent technical assistance. This work was supported in part by a grant from the Délégation Générale à la Recherche Scientifique et Technique (Grant 75-7-1312).

References

[1] Warren, D. W., Haltmeyer, G. C. and Eik-Nes, K. B. (1973) Biol. Reprod. 8, 560–565.
[2] Bloch, E., Gupta, C., Feldman, S. and Van Damme, O. (1974) Intern. Symp. INSERM 32, 177–190.
[3] Picon, R. (1976) J. Endocrinol. 71, in press.
[4] Picon, R. and Ktorza, A. (1976) FEBS Lett. 68, 19–22.
[5] Sutherland, E. W., Rall, T. W. and Menon, T. (1962) J. Biol. Chem. 237, 1220–1227.
[6] Moyle, W. R. and Ramachandran, J. (1973) Endocrinology 93, 127–134.
[7] Rommerts, F. F. G., Cooke, B. A. and Van Der Molen, H. J. (1974) J. Steroid Biochem. 5, 279–285.
[8] Picon, R. (1967) Arch. Anat. Microsc. Morphol. Exp. 56, 281–290.
[9] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[10] Lowry, O. H., Rosenbrough, N. Y., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[11] Lefkowitz, R. J., Roth, J. and Pastan, I. (1970) Nature 228, 864–866.
[12] Murad, F., Strauch, B. S. and Vaughan, M. (1969) Biochim. Biophys. Acta 177, 591–598.
[13] Braun, T. and Sepsenwol, S. (1976) Mol. Cell. Endocrinol. 4, 183–194.