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PROPERTIES OF PARTICULATE AND DETERGENT-SOLUBILIZED ADENYLATE CYCLASE OF RAT TESTIS

EFFECTS OF FOLLITROPIN STIMULATION

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Summary

Basal, fluoride and follitropin-stimulated activities of adenylate cyclase have been studied in the testes of immature rats. The enzyme was maximally activated (about twice the basal activity) by low concentrations of follitropin, with an apparent K_m of about $9 \cdot 10^{-10}$ M. Both Mg^{2+} and Mn^{2+} activate the enzyme but the apparent K_a for Mg^{2+} is about 10 times that for Mn^{2+}. However, the apparent K_m values for $MgATP^{2-}$ and $MnATP^{2-}$ are nearly the same ($1.4 \cdot 10^{-4}$ M) and the cation activation of the enzyme is mainly through an increase in V. Ca^{2+} inhibited all expressions of testicular adenylate cyclase activity.

Follitropin and fluoride stimulated adenylate cyclase activity at all Mg^{2+} concentrations but did not significantly affect the apparent K_a for Mg^{2+} or the K_m for the substrate ($MgATP^{2-}$). The stimulatory effect of the hormone or fluoride is mainly through an increase in V.

Testicular adenylate cyclase could be solubilized with Triton X-100 or Lubrol-PX. The detergent-solubilized enzyme exhibited K_m for substrate and K_a values for divalent cations similar to those of the membrane-bound enzyme and remained responsive to stimulation by fluoride. The stimulatory effect of follitropin, however, was lost. Responsiveness to follitropin was also lost by membrane-bound adenylate cyclase after treatment with phospholipase, although the fluoride effect was unchanged. These results reflect the essential role of lipids in the regulation of the follitropin-specific response.

Abbreviations: methylisobutyl xanthine, 1-methyl 3′-isobutylxanthine, App(NH)p, 5′-adenylyl imidodiphosphate.

Introduction

Follitropin is known to initiate and control a number of biochemical events in rat testis. One of the early steps in follitropin action is stimulation of adenylate cyclase activity with concomitant increases in cyclic AMP production. Membrane-bound adenylate cyclase is a useful model system for studying molecular events associated with expression of receptor function. With the exception of a recent report describing unmasking of both follitropin and lutropin receptors in homogenized preparations of seminiferous tubules [1] it is generally felt that follitropin-specific adenylate cyclase activity is localized in seminiferous tubules [1—6], while lutropin adenylate cyclase activity is localized in Leydig cells [7—9]. Although detailed studies have appeared on the fundamental properties of lutropin- and choriogonadotropin-sensitive adenylate cyclase from Leydig cells [7—9] and ovaries [7,9—14] very little has been reported on the properties of the follitropin-sensitive testicular enzyme.

An important goal of our current efforts is reconstitution of an follitropin-responsive system composed of solubilized testicular receptor and adenylate cyclase. We have already reported on solubilization of follitropin receptor from testis [15]. In this report we describe basic properties of the follitropin-sensitive membrane bound adenylate cyclase as well as solubilization of the testicular enzyme by detergents.

Materials and Methods

Testes were obtained from 12-day old immature Sprague-Dawley rats. [α-^{32}P] ATP (200 Ci/mmol) and [^{3}H]cyclic AMP (30—50 Ci/mmol) were obtained from New England Nuclear. Unlabeled nucleotides, creatine phosphate, creatine phosphokinase, bovine serum albumin, neutral alumina and Lubrol-PX from Sigma Chemical Co. 5′-guanylyl-imidodiphosphate and 5′-adenylyl-imidodiphosphate from ICN Pharmaceuticals, 1-methyl-3-isobutyl xanthine from Aldrich Chemical Co., Triton X-100 from Rohm and Haas, Dowex AG-50 WX4 (200—400 mesh, H^+ form) from BioRad Laboratories.

Highly purified human follitropin (LER 1801-3, 4019 I.U. follitropin/mg, 130 I.U. lutropin/mg) and (LER-1577, 895 I.U. follitropin/mg, 5.7 I.U. lutropin/mg) as well as highly purified human lutropin (LER-960, 4620 I.U. lutropin/mg, 2 I.U. follitropin/mg) were used in this study. Iodination of human follitropin was carried out as described previously [15]. Calculations for molar concentrations were based on a mol. wt. of 33 000 for follitropin and lutropin [1].

Preparation of testis homogenate. 12 days old immature rats were killed by CO_2 asphyxiation. Testes from 20 rats were rapidly excised, the tunica albuginea removed and the testicular tissue was homogenized in 0.25 M sucrose/0.02 M Tris/HCl buffer (pH 7.5). The homogenate was filtered through 4 layers of cheesecloth and then centrifuged. The supernatants were then centrifuged at 32 000 $\times g$ for 20 min at 4°C. The pellets were resuspended in 0.02 M Tris/HCl buffer (pH 7.5)/0.1% bovine serum albumin and used immediately after preparation for the assay of adenylate cyclase activity and binding of human [^{125}I]-follitropin.

Isolation of seminiferous tubules. Testes from 40 immature rats (12 days old) were rapidly excised and the tunica albuginea removed. The testicular tissue was minced at 4°C in Krebs-Ringer-bicarbonate buffer (pH 7.4) using 5 strokes of a loose-fitting hand homogenizer pestle. The resulting suspension was filtered through a single layer of cheesecloth. The tubules retained by the cheesecloth were placed in a petri dish and separated from the interstitial tissue with fine forceps. The microdissected tubles were then transferred to another petri dish and washed once for 2 min with hypotonic buffer solution [16], and then immediately washed 3 times with the Krebs-Ringer buffer. The tubules were centrifuged at 3000 × *g* for 10 min at 4°C and resuspended in Krebs-Ringer buffer to give a concentration of 150 mg tubules/ml.

Solubilization of adenylate cyclase from testes. For solubilization of testicular adenylate cyclase the 32 000 × *g* particles (prepared from the testicular homogenate) were suspended in 1% Lubrol PX 0.02 M Tris/HCl buffer (pH 7.5) and stirred at 4°C for 60 min followed by centrifugation for 90 min at 300 000 × *g*. The high-speed supernatant was diluted with 0.02 M Tris/HCl buffer (pH 7.5) to 0.2% Lubrol PX and then assayed for adenylate cyclase activity and human [^{125}I]follitropin binding.

Assay of adenylate cyclase. The assay of adenylate cyclase was performed as described by Salomon et al. [17]. Unless stated otherwise, the assay of homogenate was performed in a final volume of 200 µl in a medium containing 40 mM Tris/HCl buffer (pH 7.5), 5 mM $MgCl_2$, 0.5 mM cyclic AMP, 0.1% bovine serum albumin, 10 mM creatine phosphate, 0.1 mg/ml creatine phosphokinase, 1 mM [α-^{32}P]ATP (approx. 2 µCi) and 0.25 mM methylisobutylxanthine. Incubation was carried out at 32°C for 15 min and the reaction was stopped by immersing the incubation tubes in a boiling water bath for 5 min. Approx. 30 000 cpm cyclic [^{3}H] AMP was added for recovery and water was added to a final volume of 1.0 ml. The mixture was then clarified by centrifugation for 10 min at 7000 × *g* in a Sorvall Centrifuge at 4°C. The supernatants were subjected to chromatography on Dowex-AG50-WX4 columns followed by chromatography on neutral alumina. The radioactivity in the eluates was then determined in a Beckman LS-100C Liquid Scintillation Spectrometer.

The composition of the medium used for the incubation of seminiferous tubules was the same as that described for the homogenate. After incubation had been stopped by boiling, the contents of the tubes were clarified by centrifugation for 10 min at 7000 × *g* in the Sorvall centrifuge. The supernatants were then subjected to chromatography on the Dowex-AG50-WX4 followed by adsorption on the neutral alumina and the radioactivity in the eluates was counted as described above.

Other assays. Protein was assayed according to the method of Lowry et al [18] using bovine serum albumin as standard. Binding of human [^{125}I]-follitropin to testis particles and solubilized testis preparations was performed as previously described [15,16].

Results

Hormonal activation of testicular adenylate cyclase. We have previously reported specific binding of human [^{125}I]follitropin to microdissected rat

tubules cleansed of residual contamination with Leydig cells by exposure to hypotonic buffers [16]. Human [^{125}I]lutropin did not bind to such tubule preparations to any significant degree. We examined the effect of gonadotropin binding on adenylate cyclase activity in homogenates of testis as well as of highly purified testes tubules prepared as described ealier [16]. Highlu purified human follitropin (LER-1801-3, 4019 I.U./mg) stimulated adenylate cyclase activity in a dose-related manner, with maximum response at nearly 200 ng human follitropin/ml. A double reciprocal plot of adenylate cyclase activity vs. the concentration of human follitropin (Fig. 1) gave half-maximal stimulation at a hormone concentration of about 30 ng/ml ($9 \cdot 10^{-10}$ M). The effect of various follitropin, lutropin and non-gonadal hormone preparations on stimulation of adenylate cyclase activity is summarized in Table I. 8 I.U. (200 ng) human follitropin fraction LER-1801-3 stimulated adenylate cyclase activity in homogenates of whole testis from immature rats 2.4-fold (Table IA) and in homogenates of purified seminiferous tubules 2.7-fold. LER-1801-3 has an lutropin contamination of 130 I.U./mg. This much lutropin added as the highly purified fraction LER-960 (4620 I.U./mg) stimulated adenylate cyclase activity in testes homogenates 1.6-fold, but had no effect on adenylate cyclase activity in homogenates of seminiferous tubules (compare Table I, D and A). Also, the human follitropin preparation LER-1577 (895 I.U./mg), having an follitropin/lutropin ratio 10 times that of LER-1801-3, with lutropin contamination in 200 ng estimated at only about $1 \cdot 10^{-3}$ I.U., stimulated adenylate cyclase activity in whole testis and seminiferous tubule homogenates to approximately the same extent as LER-1801-3 (compare Table I, C with B). When highly purified human follitropin and lutropin were added together, adenylate cyclase activity was essentially additive in the whole testis homogenate (for example, compare Table IE with B and D) indicating that separate adenylate cyclase systems were responding to hormonal stimulation. In homogenates of testis tubules, however, no additive effect was seen and the response was to follitropin stimulation only. A variety of other hormones such as glucagon, pro-

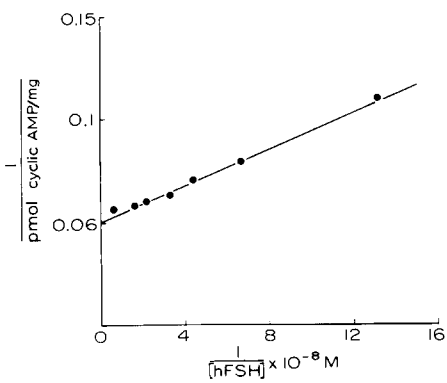

Fig. 1. Effect of varying follitropin concentration on stimulation of testicular adenylate cyclase. Adenylate cyclase was ayssayed in the presence of 250 μg enzyme protein. Concentrations of human follitropin (hFSH, LER 1801-3) were varied as described. Incubations were carried out for 15 min at 32°C. Values represent the mean of duplicate experiments and are expressed as activity in the presence of human follitropin minus activity in the absence of the hormone.

TABLE I

STIMULATION OF TESTICULAR ADENYLATE CYCLASE BY VARIOUS HORMONES

Effect of various hormones on the activity of testicular adenylate cyclase. Assay of adenylate cyclase was carried out in the presence of 250 μg testis or tubule homogenate. Seminiferous tubules from 12-day old rats were freed from Leydig cells by washing in hypotonic buffer solution. Concentrations of various hormones used were as listed. Incubation was carried at 32°C for 15 min. (h) FSH and LH, (human) follitropin and lutropin, respectively.

Addition preparation		Dose/ml	Adenylate cyclase activity (pmol cyclic AMP/min per mg)	
			Homogenate of testes	Homogenate of seminiferous tubules
A	None	—	7.1	6.7
B	hFSH LER-1801-3	200 ng	16.9	18.4
C	hFSH LER-1577	600 ng	21.7	19.2
D	hLH LER-960	200 ng	11.4	6.9
E	LER-1801-3 + LER-960	200 ng + 200 ng	27.6	18.9
F	LER-1577 + LER-960	600 ng + 200 ng	34.7	20,1
G	Glucagon	5 μg	7.2	6.2
H	Prolactin	5 μg	7.2	6.4
I	Thyrotropin	10 μg	7.0	6.5
J	Growth hormone	10 μg	6.8	6.4
K	NaF	10 mM	74.5	48.2

lactin, thyrotropin and growth hormone had no effect on adenylate cyclase activity in either testicular preparation. The reason for the discrepancies of our observation with those of Braun and Sepsenwol [1] are not understood at present.

Properties of follitropin-sensitive adenylate cyclase in testis. The amount of cyclic [α-^{32}P]AMP formed increases linearly with time of incubation up to about 15 min, at 32°C in the presence or absence of follitropin or fluoride, and was also linear with enzyme protein concentrations of up to 500 μg.

Effect of divalent cations. It has been proposed that divalent metal cations activate adenylase cyclase from various sources, possibly by lowering the concentration of the uncoupled ATP [20,21]. In this study we compared the effects of increasing concentrations of Mg^{2+} and Mn^{2+} on the activity of testicular adenylase cyclase. Fig. 2 shows that both Mg^{2+} and Mn^{2+} were capable of stimulating the testicular enzyme. However, maximal velocities at optimal Mn^{2+} concentration (2 mM) were significantly greater than those obtained with saturating Mg^{2+} concentration (15 mM). Mn^{2+} also saturated the enzyme at a lower concentration than Mg^{2+}. The apparent K_a for Mg^{2+} was determined to be 4.3 mM while that of Mn^{2+} was approx. 0.6 mM. Concentrations of Mn^{2+} above 3 mM were found to be inhibitory.

Fluoride stimulated adenylate cyclase activity when Mn^{2+} was substituted for Mg^{2+} and the extent of stimulation was greater with Mn^{2+} than with Mg^{2+}. However, in the presence of human follitropin the extent of stimulation by Mg^{2+} or Mn^{2+} was nearly the same.

We also compared the effect of Mn^{2+} or Mg^{2+} on the activation of the testicular adenylate cyclase at various concentrations of ATP and in the presence of

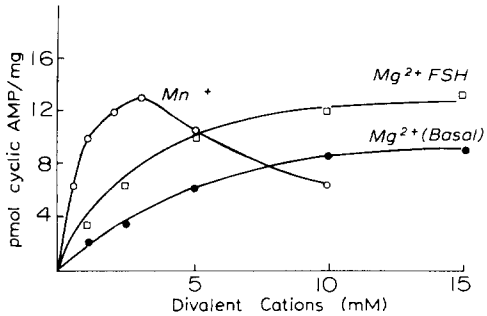

Fig. 2. Effect of Mg^{2+} and Mn^{2+} concentration of testicular adenylate cyclase. Mg^{2+} or Mn^{2+} concentration was varied as indicated. Human follitropin (LER 1801-3), when present, was at 200 ng/ml. Incubation was carried at 32°C for 15 min. Each point represents the mean of duplicate determinations FSH, follitropin.

maximal effective concentration of either divalent cation. The results in Fig. 3 show that the apparent K_m values for ATP in the presence of Mn^{2+} or Mg^{2+} were nearly the same, being 0.14 mM for $MgATP^{2-}$ and 0.12 mM for $MnATP^{2-}$, and that the cation activation of the enzyme is mainly through an increase in the V.

On the other hand, Ca^{2+} inhibits basal, fluoride or follitropin-stimulated adenylate cyclase in the testis (Table II). Also under similar assay conditions, the particulate enzyme exhibited no measurable activity when Ca^{2+} was the only divalent cation used. This result is in contrast to the requirement of Ca^{2+} for the activity of the enzyme from bovine cerebral cortex [24].

Substrate requirements. It has been established that the true substrate for the adenylate cyclase system from various sources in $MgATP^{2-}$ or $MnATP^{2-}$ and that free ATP is inhibitory at higher concentrations. Thus the effect of vary-

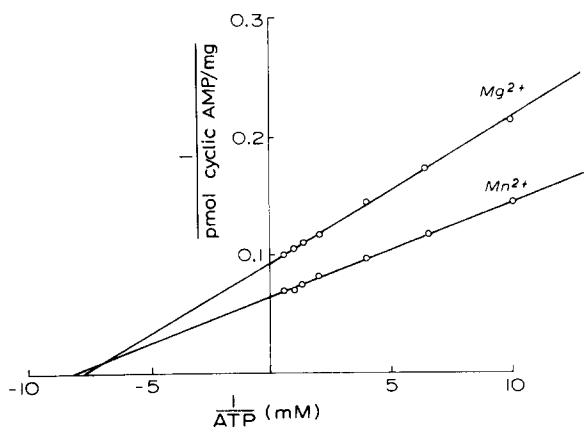

Fig. 3. Comparison of $MgATP^{2-}$ and $MnATP^{2-}$ on the activity of testicular adenylate cyclase. [α-^{32}P]ATP concentration was varied as described in the presence of 10 mM $MgCl_2$ or 2 mM $MnCl_2$. Incubation was carried at 32°C for 15 min and in the presence of 250 μg testicular homogenate and 5 units/ml adenosine deaminase. Each point represents the mean of duplicate determinations.

TABLE II

EFFECT OF Ca^{2+} ON TESTICULAR ADENYLATE CYCLASE ACTIVITY

Effect of calcium on testicular adenylate cyclase activity. Adenylate cyclase was assayed in the absence and presence of 200 ng/ml human follitropin (hFSH) (LER 1801-3) or 10 mM NaF. 250 μg testis homogenate was used. Concentration of Ca^{2+} was varied as described. Incubation was carried at 32°C for 15 min.

Addition	Basal	Adenylate cyclase activity (pmol cyclic AMP/min per mg)	
		+ hFSH 200 ng/ml	+ Fluoride 10 mM
None	8.5	17.4	72.8
Ca^{2+} 0.2 mM	6.9	9.8	48.3
0.5 mM	4.6	6.9	36.6
1.0 mM	2.4	2.5	—

ing ATP concentration on the testicular adenylate cyclase was also studied in the absence and presence of fluoride or human follitropin. The results in Fig. 4 show that basal, fluoride and follitropin-stimulated activities of the testicular enzyme were enhanced by increasing ATP concentration from 0.1 to 2 mM. Double reciprocal plots also show that fluoride or follitropin did not change the affinity of the enzyme for the substrate $MgATP^{2-}$ (Fig. 4). The apparent K_m of the enzyme for ATP in the absence and presence of fluoride or follitropin is nearly the same ($1.5 \cdot 10^{-4}$ M). In this respect fluoride or follitropin

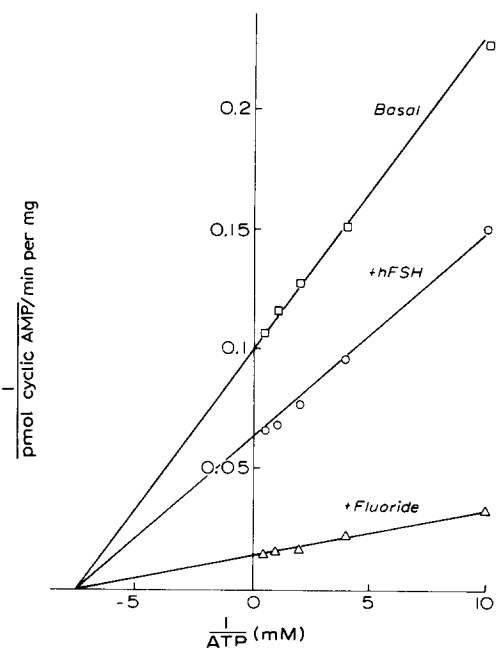

Fig. 4. Effect of ATP concentration on the activity of testicular adenylate cyclase. Assay mixtures in the absence (basal) and in the presence of 200 ng human follitropin (hFSH) or 10 mM NaF as well as testis homogenate were preincubated separately at 32°C for 5 min. The amount of [α-^{32}P]ATP was varied to give the indicated concentrations. Mg^{2+} concentration was fixed at 10 mM. The reaction was started by the addition of 250 μg of testicular homogenate to the assay mixtures. Incubation was carried at 32°C for 15 min. Each point is the mean of results from duplicate experiments.

stimulation of the enzyme activity is mainly through an increase in V. Free ATP, in excess of Mg^{2+} was found to inhibit all expressions of the testicular enzyme (unpublished data).

The effect of FSH or fluoride on the affinity for the metal cation (Mg^{2+}) was studied by varying the concentrations of Mg^{2+} (1—20 mM) in presence of a fixed concentration of ATP. The results shown in Fig. 5 indicate that follitropin or fluoride did not significantly affect the affinty of the enzyme for Mg^{2+}, but mainly stimulated the enzyme through an increase in V in both cases. The apparent K_a for the cation in the absence and presence of fluoride or human follitropin is nearly the same (4 mM).

The results suggest that the stimulatory action of the hormone or fluoride on the enzyme activity is primarily from an increase in reaction velocity without affecting the apparent K_m for the substrate ($MgATP^{2-}$) or the K_a for the cation (Mg^{2+}).

Addition of varying concentrations of ATP or 5'-App(NH)p to the incubation medium containing [α-^{32}P]ATP caused a proportional reduction in the amount of cyclic [^{32}P]AMP formed. (Table III). App(NH)p, therefore, can be also used by the testicular adenylate cyclase as a substrate instead of ATP.

Solubilization of testicular adenylate cyclase. There have been numerous reports on the solubilization of adenylate cyclase from various endocrine tissues either in a hormone-sensitive or hormone-insensitive form. The enzyme is associated with the plasma membranes of animal cells and can usually be dislodged from the membranes only through use of detergents [25]. This strongly suggests that some part of the enzyme surface must be in contact with lipids or hydrophobic regions of membrane proteins.

The results in Table IV show the effect of Lubrol PX and Triton X-100 on the solubilization of the adenylate cyclase. Particulate testicular fractions were treated with various concentrations of Lubrol PX or Triton X-100 in 0.05 Tris/HCl buffer pH 7.5 for 60 min, followed by centrifugation at 300 000 × g for 90 min at 4°C and then assayed for adenylate cyclase activity in the high-speed

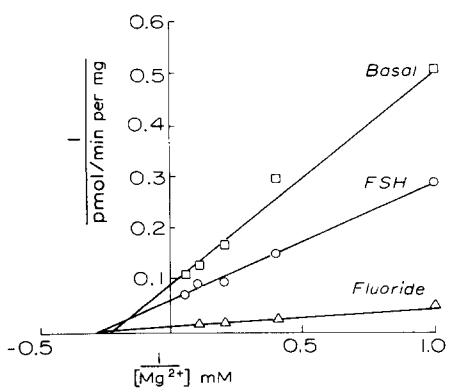

Fig. 5. Effect of Mg^{2+} concentration on testicular adenylate cyclase activity. Mg^{2+} was added to the assay mixtures at the indicated concentrations and in the presence of 0.4 mM ATP and 250 μg enzyme protein. Incubation was carried at 32°C for 15 min. Each point represents the mean of results from duplicate experiments. FSH, follitropin.

TABLE III

EFFECT OF UNLABELED ATP OR App(NH)p ON FORMATION OF CYCLIC [^{32}P]AMP FROM [α-^{32}P]ATP

Effect of unlabeled ATP or App(NH)p on formation of [^{32}P]cyclic AMP from [α-^{32}P]ATP. Adenylate cyclase was assayed in the presence of 250 μg testis homogenate, 1 mM [α-^{32}P]ATP and 200 ng/ml human follitropin (LER 1801-3). Various concentrations of unlabeled ATP or App(NH)p were added to separate incubation mixtures and the amount of cyclic [^{32}P]AMP formed was determined. Incubation was carried at 32°C for 15 min. The results represent the mean of duplicate experiments.

Addition	Adenylate cyclase activity (pmol cyclic [^{32}P]AMP formed)	
	+App(NH)p	+ATP
None	17.4	16.9
0.25 mM	10.6	10.2
0.5 mM	8.7	7.8
0.75 mM	6.0	5.7
1.0 mM	4.8	4.6

supernatant as well as in the washed sedimented pellets. About 70% of the fluoride-stimulated adenylate cyclase present in the control homogenate was solubilized by 1% Lubrol PX and 40% was solubilized by 1% Triton X-100. As shown in Table IV, solubilized, fluoride-stimulated activity was somewhat enhanced in the presence of 0.25 or 0.5% detergent. This effect was also noticed when the particulate fractions of testis were dispersed in these same concentrations of detergent. On the other hand, the solubilized enzyme was no longer responsive to follitropin. However, as can be seen in Table IV, loss of adenylate cyclase stimulation by the hormone was not associated with absence of follitropin receptor, since appreciable amounts of human [^{125}I]FSH bound specifically to detergent-solubilized receptors present in the testicular extract.

TABLE IV

EFFECT OF DETERGENTS ON THE ACTIVITY OF TESTICULAR ADENYLATE CYCLASE

Effect of detergents on the activity of testicular adenylate cyclase. Aliquots of particulate fractions (32 000 × *g*) were centrifuged and the pellets utilized with or without the indicated concentrations of Lubrol PX or Triton X-100 for 60 min at 4°C and then centrifuged at 300 000 × *g* for 90 min. Adenylate cyclase activity was assayed in the high-speed supernatants in the absence and presence of 200 ng/ml of human follitropin (hFSH, LER 1801-3) or 10 mM NaF. Incubation was carried out at 32°C for 15 min. Specific binding of human [^{125}I] follitropin to soluble receptors was carried out in the presence of 10 ng of human [^{125}I] follitropin and after diluting the detergent concentration to 0.2%.

Treatment		Adenylate cyclase activity (pmol cyclic AMP/mg protein)			Binding of ^{125}I-labeled hFSH (Mol × 10^{-15} protein)
		Basal	+NaF	+FSH	
Particulate		9.2	48.9	25.4	$22 \cdot 10^{-15}$
Lubrol PX	0.25%	7.8	59.2	8.3	
	0.5%	7.2	57.6	8.6	
	1%	5.0	48.2	3.3	$13 \cdot 10^{-15}$
Triton X-100	0.25%	6.6	51.6	7.8	
	0.5%	5.3	49.8	7.0	$11 \cdot 10^{-15}$

The hormone responsiveness in such a detergent solubilized enzyme could not be restored by diluting the detergent or removing most of the detergent by Bio-Beads SM-2. This seems different from the ovarian enzyme which maintains a slight degree of hormone responsiveness to gonadotropin after detergent-solubilization [7,26,33].

Detergents have been found to enhance the fluoride-stimulated adenylate cyclase in interstitial tissue of testis [7] and the particulate enzyme from cerebral cortex [27,28], however, the activity of the detergent solubilized cerebral cortex enzyme was no longer stimulated by fluoride [28].

The properties of the detergent-solubilized enzyme are nearly the same as those of the membrane-bound enzyme. Both enzyme activities are stimulated by Mg^{2+} and Mn^{2+}. The extent of stimulation by Mn^{2+} is higher and nearly double that obtained with Mg^{2+} and reaches maximum at level 2 mM above which Mn^{2+} became inhibitory. Mg^{2+} concentration, on the other hand, continued to increase the activity of the enzyme up to 15 mM. The apparent K_a of the solubilized enzyme for Mg^{2+} is 4.7 mM and for Mn^{2+} 0.4 mM.

The apparent K_m values of the basal and fluoride-stimulated activity for the substrates $MgATP^{2-}$ in the solubilized preparation were 0.16 and 0.18 mM respectively.

Table V shows the effect of phospholipase *C* on the responsiveness of the testicular enzyme to follitropin or fluoride. Particulate fractions of the testis were treated with phospholipase *C* for 10 min at 25°C followed by centrifugation and washing the particles with 0.05 M Tris/HCl buffer (pH 7.5) before assaying for the enzyme activity. The results show that treatment with phospholipase significantly abolished the follitropin response without marked effect on the fluoride-stimulated activity.

The results show that the use of lipid modifying agents as detergents or phospholipase caused the loss of membrane components responsible for the hormone response without significantly affecting the active catalytic moiety of the enzyme as reflected by its fluoride stimulation.

TABLE V

EFFECT OF PHOSPHOLIPASE *C* ON TESTICULAR ADENYLATE CYCLASE

Effect of phospholipase *C* on testicular adenylate cyclase. Aliquots of particulate fractions of testis homogenate (32 000 × *g*) were incubated with and without phospholipase *C* for 10 min at 24°C in 0.02 M Tris/HCl buffer (pH 7.5)/0.2 mM $CaCl_2$. At the end of incubation, the tubes were immediately centrifuged and the pellets washed twice. The pellets were then suspended in 1 ml 0.02 M Tris/HCl buffer (pH 7.5) and assayed for adenylate cyclase activity in the presence of 200 ng/ml human follitropin (hFSH, LER 1801-3) or 10 mM NaF. Incubation was carried at 32°C for 15 min. The results represent the mean of duplicate experiments.

Phospholipase *C* units/ml	Adenylate cyclase activity (pmol cyclic AMP/mg protein)	
	+hFSH (200 ng/ml)	+Fluoride (10 mM)
None	18.6	46.5
20	10.2	49.7
30	5.8	45.2
50	2.1	42.6

Discussion

The addition of a highly purified preparation of human follitropin resulted in the stimulation of adenylate cyclase activity in homogenates of whole testis and isolated, Leydig cell-free, seminiferous tubules of immature rat testis. Human lutropin stimulated adenylate cyclase activity in testis homogenate but not in the purified tubules. When concentrations of the two hormones giving maximum stimulation of adenylate cyclase were used in combination, the effect was additive in testis homogenate but not in tubules. Follitropin and lutropin are, therefore, stimulating separate adenylate cyclase systems in the testis. The results confirm that an lutropin-sensitive adenylate cyclase is not present in the seminiferous tubules. This is in agreement with previous reports which show that follitropin stimulates adenylate cyclase system in seminiferous tubules and lutropin stimulates an adenylate cyclase enzyme in interstitial cells [2,4,19,29]. It differs however from the observations of Braun and Sepsenwol [1] who reported stimulation of lutropin and follitropin-responsive adenylate cyclase in seminiferous tubules from testis of young rats, explained by unmasking of functional lutropin receptors by homogenization.

Follitropin stimulated adenylate cyclase in immature rat testis in a dose-related fashion (see Results).

The difference between Mg^{2+} and Mn^{2+} activation of testicular adenylate cyclase was apparently not due to lowering of the concentration of ATP uncomplexed with the divalent cation.

The results in Fig. 3 (see also Results) suggests that divalent cations activate the testicular enzyme directly by interacting with a cation-binding site independent of the catalytic site, thus increasing the V. Such effects were independent of the ratios of free ATP to complexed ATP. The testicular enzyme in this respect behaves similarly to hepatic glucagon-sensitive adenylate cyclase [22] but differs from the brain enzyme [23] where the K_m for $MnATP^{2-}$ was found to be 1/4—1/7 of that for $MgATP^{2-}$.

The stimulatory effect of follitropin and fluoride on the testicular adenylate cyclase appears to be mainly due to an increase in the V of the enzyme. In each case there was no significant effect on the apparent K_m for the substrate ($MgATP^{2-}$) or the apparent K_a for the cation (Mg^{2+}). This reflects mainly an increased reactivity of the catalytic moiety with the substrate, possibly through dissociation of an inhibitor · catalytic components complex. The results obtained agree with those of Drummond et al. [30] for the epinephrine-stimulated adenylate cyclase activities from rabbit and guinea pig heart and brain. Hepp et al. [31] also showed that the cation of glucagon and fluoride on particulate adenylate cyclase from rat and mouse liver was to increase the V of the enzyme without affecting its affinity for the cation. On the other hand, Birnbaumer et al. [32] reported that the stimulatory effect of corticotropin on the fat cell enzyme, like that of fluoride, was mainly due to increased affinity of the enzyme for Mg^{2+}.

Treatment of testicular particulate fractions with Lubrol PX or Triton X-100 solubilized and seemed to enhance the fluoride-stimulated activity of adenylate cyclase. The solubilized enzyme has nearly the same apparent K_m for the substrate or K_a for the divalent cation. However, the solubilized enzyme was no

longer responsive to follitropin even after the removal of detergent. In this respect, the follitropin-sensitive testicular enzyme is different from the ovarian adenylate cyclase [33] which remains somewhat responsive to the gonadotropin after detergent solubilization.

The loss of hormone responsiveness in the detergent solubilized adenylate cyclase with the enhancement of the fluoride-stimulated activity may reflect the loss of an essential component of the system (mainly lipids) required for the hormone response but not necessary for fluoride stimulation. This was also confirmed by treatment of testicular membranes with phospholipase whereupon the follitropin response was completely lost but the fluoride-stimulated activity was not significantly affected. The enhancement of the fluoride-stimulated adenylase cyclase with detergents has been noticed in other systems [9, 26]. We have described elsewhere the modulation of follitropin-sensitive adenylase cyclase in immature rat testis by guanyl nucleotides [34] as well as solubilization of the follitropin-specific receptor in rat testis [15]. Solubilization of testicular adenylate cyclase as described here, should allows an investigation of factors related to establishment of the hormone-specific activation of adenylate cyclase in a solubilized and purified system.

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