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# $Mn^{2+}$-SENSITIVE, SOLUBLE ADENYLATE CYCLASE IN RAT TESTIS

## DIFFERENTIATION FROM OTHER TESTICULAR NUCLEOTIDE CYCLASES

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## Summary

In subcellular fractions prepared from homogenate of adult rat testis adenylate cyclase (ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1) activity was found in the particulate, primarily 600 × *g* for 10 min, fractions, as well as in the cytosol. The properties of the adenylate cyclase in the cytosol differs substantially from the adenylate cyclase system associated with the 600 × *g* for 10 min particulate fraction. The cytosol enzyme, in contrast to the particulate adenylate cyclase, was found to be fluoride- and gonadotropin hormone-insensitive. The cytosol adenylate cyclase appears to be located in the germ cell while the particulate enzyme system in the non-germ cell component of the seminiferous tubules. The cytosol adenylate cyclase was found to be distinct also from the guanylate cyclase present in the rat testis cytosol. The adenylate cyclase appears to be located in the germ cell component while the guanylate cyclase, in the non-germ cell tubular component. Furthermore, it was found that the cytosol guanylate cyclase develops at an earlier stage of spermatogenesis, and precedes the development of the cytosol adenylate cyclase.

## Introduction

The presence of a $Mn^{2+}$-sensitive adenylate cyclase (ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1) in the cytosol isolated from rat testis homogenate has been described in our previous studies [1]. This communication extends our previous findings and presents data distinguishing the $Mn^{2+}$-sensitive adenylate cyclase from other adenylate cyclases present in rat testis. It is shown that the $Mn^{2+}$-sensitive, soluble adenylate cyclase does not develop in the testis which does not contain germ cells, while the particulate and gonadotropin hormone-stimulated adenylate cyclase clearly does.

The rat testis cytosol contains, in addition to the $Mn^{2+}$-sensitive adenylate cyclase, a $Mn^{2+}$-sensitive guanylate cyclase [2]. Both enzymes were found to exhibit an obligatory requirement for $Mn^{2+}$ for catalytic activity, and were hormone- and fluoride-insensitive. Moreover, the stimulatory effect of $Mn^{2+}$ was augmented by $Ca^{2+}$. The above activities in the cytosol could be attributed to a single enzyme with preferential affinity for ATP or GTP under the in vitro assay conditions, or could reflect the presence of two separate enzymes. The findings presented in this communication indicate that the two cyclases in the cytosol are separate entities. This is concluded, in part, from the fact that these two enzymes develop in different cell types in the seminiferous tubules, i.e. the $Mn^{2+}$-sensitive adenylate cyclase develops in the germ cells, while the guanylate cyclase develops in a non-germ cell tubular component. Furthermore, it was found that the guanylate cyclase develops in the pre-meiotic stage of spermatogenesis, while the adenylate cyclase in the cytosol develops in the post-meiotic stage.

## Materials and Methods

*Experimental animals.* Charles River CD® rats were used. Immature rats younger than 21 days were kept in groups of ten with their mothers. Time-bred pregnant rats were obtained on the 15th day of gestation. Purina Chow diet and water were provided ad libitum. Rats were maintained at a temperature of 22—23° C on a schedule of a 14-h light and 10-h dark period each day.

*X-irradiation of pregnant rats.* On the 19th day of gestation rats (to be irradiated) were placed individually in a wooden box and exposed to a single whole body dose of 300 R using a Picker model X-ray unit. The radiation factors were 270 kV (peak), 20 mA, target distance to top of box 50 cm, output in air 79.2 R/min.

*Tissue and cell preparations.* Seminiferous tubules were isolated from testis tissue by microdissection [3,4] and interstitial cells were isolated by collagenase treatment of testis tissue, using procedures previously described [1]. Homogenates of either total testis tissue, isolated seminiferous tubules or interstitial cells were prepared in tight-fitting Dounce glass homogenizers using 5 mM Tris · HCl buffer (pH 7.2). Subcellular fractions from whole homogenates were isolated by differential centrifugation. The supernatant fractions were filtered through 0.22-$\mu$m millipore filter. The filtered supernatants did not contain membrane fragments or vesicles as determined by electron microscopy of negatively stained preparations.

*Adenylate cyclase activity.* This was determined by measuring the rate of conversion of [$\alpha$-$^{32}$P]ATP to cyclic[$^{32}$P]AMP. The assay procedure and separation of the formed radioactive cyclic AMP from other radioactive substances was previously described [1,5].

*Guanylate cyclase activity.* This was determined by measuring the conversion of [$\alpha$-$^{32}$P]GTP into cyclic[$^{32}$P]GMP. Before assaying for guanylate cyclase activity homogenates and subcellular fraction were treated with 30 mg/ml dry neutral aluminum oxide [6]. The standard assay mixture contained in a total mixture of 50 $\mu$l: 40 mM Tris · HCl buffer (pH 7.6), 3 mM MnCl, 0.5 mM unlabeled cyclic GMP, 0.1% bovine serum albumin, 0.1 mM EDTA, 10 mM

mercaptoethanol, 10 mM creatine phosphate, 0.2 mg/ml creatine phosphokinase (25 units/mg) and 0.2 mM [$\alpha$-$^{32}$P]GTP. The $Mn^{2+}$ was added to the incubation mixture shortly before the start of the assay to prevent Tris-catalyzed oxidation of $Mn^{2+}$. The reaction was started by adding [$\alpha$-$^{32}$P]GTP and incubated for 10—20 min at 37°C. Adding 50 $\mu$l of unlabeled 10 mM GTP, GDP, 5′-GMP and cyclic GMP and 20 mM EDTA, the reaction was stopped by boiling for 3 min. The addition of EDTA before boiling prevents nonenzymatic formation of cyclic GMP in the presence of $Mn^{2+}$ and creatine phosphate [7]. The cyclic[$^{32}$P]GMP formed was separated from other radioactive substances by one-dimensional repetitive adsorption chromatography on alumina thin-layer sheets (Dods, R. and Braun, T., in preparation) developed in a methanol water (1 : 1) solvent system [8].

*Histology.* Samples of intact testis tissue were minced into 1 $mm^3$ blocks, fixed 90 min in 2.5% glutaraldehyde + 1.0 M cacodylate buffer at pH 7.5, post-fixed 60 min in 1% $OsO_4$ + 0.1 M collidine buffer at pH 7.5, dehydrated in graded acetone and embedded in Epon 812. Sections 1—2 $\mu$ thick were cut with a glass knife and stained with 1% Toluidine Blue in 1% borate buffer.

*Radioisotopes and chemicals.* [$\alpha$-$^{32}$P]ATP and [$\alpha$-$^{32}$P]GTP were purchased from International Chemical and Nuclear Corporation. Tris · HCl (Trizma-HCl), unlabeled ATP, GTP, 5′ AMP, 5′ GMP, cyclic AMP and cyclic GMP were purchased from Sigma; chloride salts (analytical grade) of $Mg^{2+}$ and $Ca^{2+}$, from Mallinckrodt; $MnCl_2$ and NaF from Fisher; bovine serum albumin (Fraction V), from Armour; collagenase, from Worthington; creatine kinase and creatine phosphokinase, from Calbiochem; chloroform and methanol (reagent grade) from Fisher; Silica gel thin-layer and alumina chromatography sheets with fluorescent indicator were purchased from Eastman Kodak and millipore filters from Millipore Corporation.

*Hormones.* Follitropin (ovine, NIH-FSH-S9) and Lutropin (ovine, NIH-LH-S-16) were generously supplied by NIAMDD, NIH, Bethesda.

## Results

### *Differentiation of the soluble, $Mn^{2+}$-sensitive adenylate cyclase from other testis adenylate cyclases*

Table I shows that adenylate cyclase activity was present in the whole homogenate and in the 600 × *g* for 10 min particulate fraction prepared from testis of either immature (11-day old) or adult (80-day old) rats. Adenylate cyclase activity was not detectable in the cytosol of 11-day old rats, but was present in the cytosol of 80-day old rats. The properties of the enzyme in the particulate fraction and in the cytosol were found to differ substantially. The activity of the enzyme system in the 600 × *g* for 10 min particulate fraction, like that in the whole homogenate, was stimulated by fluoride (F′), follitropin and lutropin. Stimulation of the particulate enzyme system by F′ or gonadotropin hormones was attained regardless of whether $Mg^{2+}$ (Table I) or $Mn^{2+}$ (Table II) was used for the formation of the metal · ATP substrate complex. The $Mn^{2+}$-sensitive adenylate cyclase in the cytosol of adults rats was not stimulated by F′, follitropin or lutotropin regardless of whether $Mg^{2+}$ (Table I) or $Mn^{2+}$ (Table II) was used as the divalent cation. F′ was found to be ineffective in stimulating

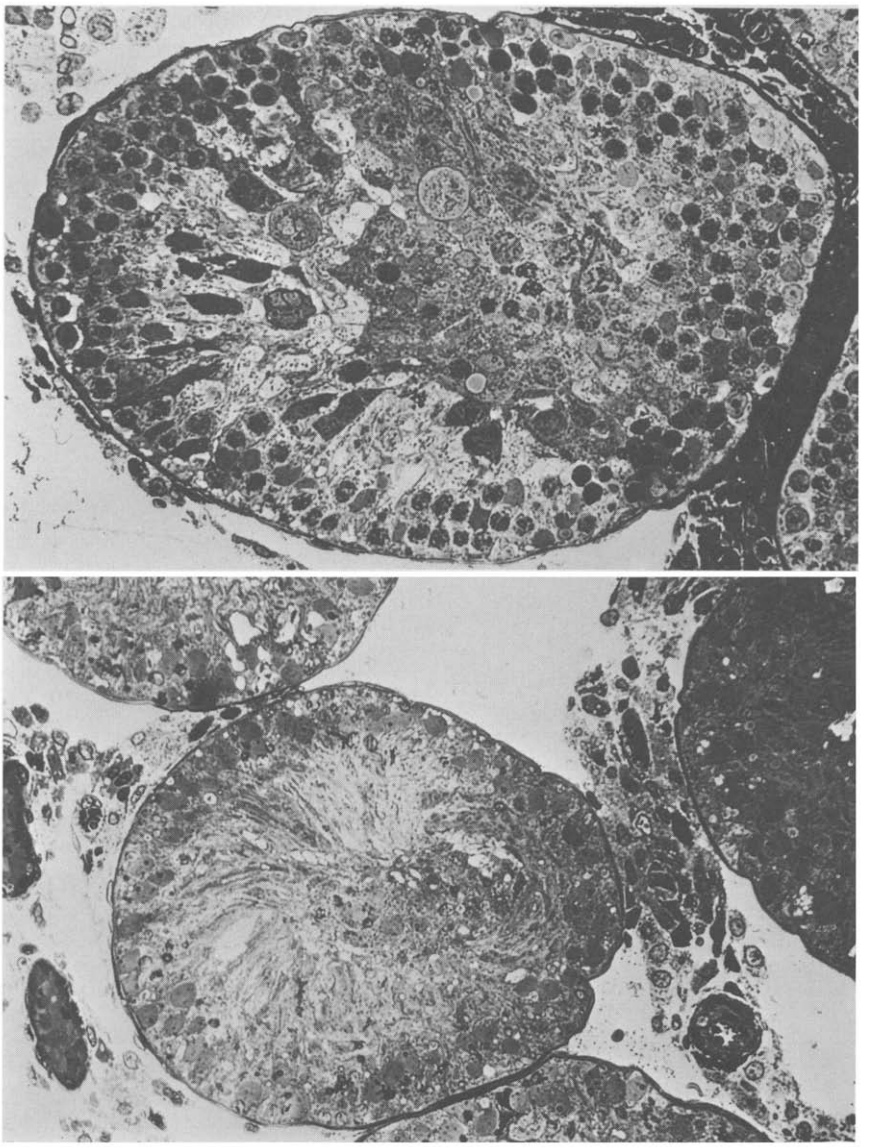

Fig. 1. (Upper) Intact testis, normal rat, 40 days old. Germ cell types include B-type spermatogonia at the perimeter, late pachytene spermatocytes, and early spermatids in the center of the tubule. Pale nuclei adjacent to peripheral spermatogonia belong to Sertoli cells. Note islands of interstitial tissue applied to the tubules. Epon embedment, Toluidine Blue staining. Magnification X350. (Lower) Intact testis, prenatally irradiated rat (300 R), 40 days old. The seminiferous tubule diameter is significantly smaller than normal. Only pale Sertoli cell nuclei are visible in the interior. Long Sertoli cell cytoplasmic processes fill the central portion of the tubule, but enclose no germ cells of any type. The peritubular cell layer delimiting the tubule and interstitial tissue appear normal. Epon embedment, Toluidine Blue staining. Magnification X350.

the enzyme activity in the cytosol isolated from testis of sexually maturing or mature rats. However, $F^-$ considerably increased the activity of the particulate adenylate cyclase (Table III).

The soluble $Mn^{2+}$-sensitive adenylate cyclase was further distinguished from

TABLE I

EFFECT OF $Mn^{2+}$, $Mg^{2+}$, F′ AND GONADOTROPIN HORMONES ON ADENYLATE CYCLASE ACTIVITY IN TESTIS FROM IMMATURE AND MATURE RATS

Enzyme activity measured in the whole homogenate, 600 × *g* for 10 min particulate fraction (pellet) and cytosol (105 000 × *g* for 60 min supernatant) using either 5 mM $Mn^{2+}$ or $Mg^{2+}$ and 0.2 mM ATP. Values are averages ± S.E. of triplicate determinations. ND: not detected; FSH: follitropin; LH: lutropin. The effect of 10 μg/ml of the hormones and 10 mM F′ is shown in the table.

| Additions | pmol of cAMP per mg protein per min | | |
|---|---|---|---|
| | Whole homogenate | Pellet | Supernatant |
| In 11-day old rats: | | | |
| $Mn^{2+}$ | 2.9 ± 0.1 | 14.9 ± 0.2 | ND |
| $Mg^{2+}$ | 4.3 ± 0.4 | 30.6 ± 3.6 | ND |
| $Mg^{2+}$ + F′ | 60.1 ± 5.5 | 405.4 ± 33.3 | ND |
| $Mg^{2+}$ + FSH | 19.1 ± 1.6 | 73.0 ± 11.4 | ND |
| $Mg^{2+}$ + LH | 8.4 ± 0.9 | 42.2 ± 3.3 | ND |
| In 80-day old rats: | | | |
| $Mn^{2+}$ | 6.9 ± 0.2 | 6.9 ± 0.3 | 14.8 ± 0.8 |
| $Mg^{2+}$ | 1.3 ± 0.1 | 1.3 ± 0.6 | 0.8 ± 0.2 |
| $Mg^{2+}$ + F′ | 13.4 ± 0.5 | 43.6 ± 1.2 | 0.4 ± 0.1 |
| $Mg^{2+}$ + FSH | 3.3 ± 0.0 | 5.7 ± 0.5 | 0.4 ± 0.1 |
| $Mg^{2+}$ + LH | 2.2 ± 0.2 | 2.4 ± 0.1 | 0.4 ± 0.2 |

TABLE II

EFFECT OF F′ AND GONADOTROPIN HORMONES ON RAT TESTIS ADENYLATE CYCLASE ACTIVITY

Enzyme activity measured in the whole homogenate and in the cytosol using 5 mM $Mn^{2+}$ as the divalent cation. Values are averages ± S.E. of triplicate determinations. Testes from 77-day old rats were used. FSH: follitropin; LH: lutropin. The effect of 10 μg/ml of the hormones and 10 mM F′ is shown in the table.

| Additions | pmol of cAMP per mg protein per min | |
|---|---|---|
| | Whole homogenate | Cytosol |
| $Mg^{2+}$ | 1.1 ± 0.2 | 1.2 ± 0.2 |
| $Mn^{2+}$ | 7.0 ± 0.4 | 27.1 ± 1.8 |
| $Mn^{2+}$ + F′ | 16.5 ± 0.6 | 27.4 ± 1.2 |
| $Mn^{2+}$ + FSH | 10.6 ± 0.4 | 26.3 ± 0.7 |
| $Mn^{2+}$ + LH | 8.9 ± 0.7 | 25.3 ± 1.0 |

TABLE III

EFFECT OF F′ ON RAT TESTIS ADENYLATE CYCLASE ACTIVITY

Enzyme activity measured in the whole homogenate and in the cytosol using either 5 mM $Mg^{2+}$ or $Mn^{2+}$ and 0.2 mM ATP. Values are averages of triplicate determinations. Testes from 36-day old (Exp. 1), 57-day old (Exp. 2) and 82-day old (Exp. 3) rats were used. The effect of 10 mM F′ is shown in the table.

| Additions | pmol of cAMP per mg protein per min | | | | | |
|---|---|---|---|---|---|---|
| | Whole homogenate | | | Cytosol | | |
| | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 |
| $Mg^{2+}$ | 0.8 | 0.1 | 1.8 | 0.4 | 0.6 | 1.3 |
| $Mg^{2+}$ + F′ | 6.3 | 6.4 | 9.7 | 0.0 | 0.0 | 1.0 |
| $Mn^{2+}$ | 5.1 | 13.2 | 11.9 | 11.1 | 18.7 | 22.5 |
| $Mn^{2+}$ + F′ | 12.9 | 19.9 | 15.3 | 9.5 | 19.5 | 18.9 |

the particulate enzyme systems by their differential response to $Ca^{2+}$ ions. Table IV shows that the effect of 1 mM $Mn^{2+}$ on the formation of cyclic AMP by the cytosol adenylate cyclase was considerably augmented by the simultaneous addition of $Ca^{2+}$ to the assay mixture. On the other hand, the effect of $Mn^{2+}$ on the particulate enzyme system derived from immature (11-day old) or adult (80-day old) rat testes was not substantially affected by $Ca^{2+}$.

When pregnant rats are X-irradiated on the 19th day of gestation, their male offspring have no germ cells in their seminiferous tubules [9—11]. As expected, we found no germ cells in the seminiferous tubules from rats X-irradiated in the uterus (Fig. 1). Irradiated rats, free of germ cells, were then used to determine the cell type(s) in which these adenylate cyclase activities develop. As illustrated in Table V, the particulate, hormone- and F′-responsive adenylate cyclase is present in the whole homogenate from testis of both X-irradiated and non-irradiated (control) rats. The activity of the particulate adenylate cyclase was profoundly greater in X-irradiated than in control rats. The cytosol, $Mn^{2+}$-sensitive adenylate cyclase activity, however, did not develop in testes free of germ cells. A small amount of F′-stimulated adenylate cyclase activity was found in the cytosol of X-irradiated rats, but not in the cytosol from non-irradiated (control) rats.

*Differentiation of $Mn^{2+}$-sensitive adenylate cyclase from guanylate cyclase activity in the cytosol from rat testis homogenates*

Using [$\alpha$-$^{32}$P]ATP or [$\alpha$-$^{32}$P]GTP and $Mn^{2+}$ for the formation of the nucleotide-metal substrate complex, both enzyme activities were detected in the cytosol of the testis from adult, sexually mature rats. Table VI shows that guanylate cyclase activity was detectable in the cytosol derived from suckling rats 13 days old, while cytosol $Mn^{2+}$-sensitive adenylate cyclase activity was undetectable. At 27 days of age the activity of the cytosol guanylate cyclase was considerably greater than that of the adenylate cyclase. In contrast to younger

TABLE IV

AUGMENTATION BY $Ca^{2+}$ OF THE STIMULATORY EFFECT OF $Mn^{2+}$ ON THE CYTOSOL ADENYLATE CYCLASE OF RAT TESTIS

Enzyme activity measured in the whole homogenate, 600 × *g* for 10 min particulate fraction (pellet) and cytosol. Incubations carried out with 1 mM $Mn^{2+}$ and 0.2 mM ATP in the absence or presence of 3 mM $Ca^{2+}$. Values are averages ± S.E. of triplicate determinations. ND: not detected.

| Additions | pmol of cAMP per mg protein per min | | |
|---|---|---|---|
| | Whole homogenate | Pellet | Supernatant |
| In 80-day old rats: | | | |
| $Mn^{2+}$ | 8.9 ± 0.1 | 5.8 ± 0.3 | 10.8 ± 0.6 |
| $Ca^{2+}$ | 0.2 ± 0.0 | ND | 0.5 ± 0.0 |
| $Mn^{2+}$ + $Ca^{2+}$ | 8.9 ± 0.1 | 7.9 ± 0.4 | 24.0 ± 1.1 |
| In 11-day old rats: | | | |
| $Mn^{2+}$ | 2.3 ± 0.3 | 4.5 ± 0.6 | ND |
| $Ca^{2+}$ | ND | 0.5 ± 0.1 | ND |
| $Mn^{2+}$ + $Ca^{2+}$ | 1.3 ± 0.4 | 4.6 ± 0.8 | ND |

TABLE V

EFFECT OF X-IRRADIATION ON ADENYLATE CYCLASE ACTIVITIES IN RAT TESTIS

Enzyme activity measured in the 20 000 × *g* for 10 min supernatant (filtered through 0.22-μm millipore filter). Values are averages ± S.E. of triplicate determinations. Rat testes obtained from control (non-irradiated) and X-irradiated rats at 40 days of age, i.e. about 42 days after irradiation in the uterus. FSH: follitropin; LH: lutropin. The effect of 10 μg/ml of the hormones and 10 mM $F^-$ is shown in the table.

| Additions | pmol of cAMP per mg protein per min | |
|---|---|---|
| | Whole homogenate | Supernatant |
| In control rats: | | |
| $Mn^{2+}$ | 12.1 ± 0.9 | 16.9 ± 0.4 |
| $Mg^{2+}$ | 1.3 ± 0.2 | 0.4 ± 0.1 |
| $Mg^{2+}$ + $F^-$ | 29.3 ± 0.5 | 1.8 ± 0.2 |
| $Mg^{2+}$ + FSH | 4.9 ± 0.3 | 0.9 ± 0.1 |
| $Mg^{2+}$ + LH | 1.6 ± 0.2 | 0.6 ± 0.1 |
| In X-irradiated rats: | | |
| $Mn^{2+}$ | 12.4 ± 1.0 | 1.8 ± 0.1 |
| $Mg^{2+}$ | 5.2 ± 0.2 | 0.8 ± 0.2 |
| $Mg^{2+}$ + $F^-$ | 99.9 ± 4.8 | 8.9 ± 1.1 |
| $Mg^{2+}$ + FSH | 18.1 ± 0.2 | 1.2 ± 0.2 |
| $Mg^{2+}$ + LH | 7.2 ± 0.3 | 1.1 ± 0.1 |

rats, the activity of the cytosol adenylate cyclase from adults was found to be much higher than the activity of the guanylate cyclase.

In our previous studies the cytosol, $Mn^{2+}$-sensitive adenylate cyclase was shown to be localized in the seminiferous tubules of rat testis [1]. In this study we have compared the activity of the soluble guanylate cyclase in whole testis tissue, isolated seminiferous tubules and in an interstitial-cell-enriched fraction. Table VII shows that the level of the soluble guanylate cyclase activity isolated either from whole testis tissue or isolated seminiferous tubules is similar. The guanylate cyclase activity recovered in the supernatant derived from the interstitial-cell-enriched fraction was only 16—18% of the activity found in isolated seminiferous tubules or whole testis tissue. This level of guanylate cyclase activity in the interstitial-cell-enriched fraction can be accounted for by the presence of contaminating tubular cells (usually about 15% of the total cells in this fraction).

TABLE VI

ADENYLATE AND GUANYLATE CYCLASE ACTIVITY IN THE TESTIS CYTOSOL IN RELATION TO THE AGE OF RATS

Enzyme activities measured in the presence of either 5 mM (adenylate cyclase) or 3 mM (guanylate cyclase) of $Mn^{2+}$ and 0.2 mM either ATP or GTP. Values are averages ± S.E. of triplicate determinations.

| Age of rats (days) | pmol of cyclic nucleotide per mg of protein per min | |
|---|---|---|
| | Cyclic AMP | Cyclic GMP |
| 13 | 0.2 ± 0.1 | 10.9 ± 0.7 |
| 27 | 2.6 ± 0.0 | 34.9 ± 1.1 |
| 90 | 29.5 ± 0.5 | 8.7 ± 0.6 |

TABLE VII

DISTRIBUTION OF GUANYLATE CYCLASE ACTIVITY BETWEEN ISOLATED SEMINIFEROUS TUBULES AND INTERSTITIAL CELLS FROM RAT TESTIS

Enzyme activity measured in the 20 000 × *g* for 10 min supernatant (filtered through 0.22-μm millipore filter) and particulate fraction (pellet) resuspended in Tris · HCl buffer. Values are averages ± S.E. of triplicate determinations. Testes from 27-day old rats were used.

| Preparation | pmol of cGMP per mg protein per min | | |
|---|---|---|---|
| | Whole homogenate | Supernatant | Pellet |
| Whole testis tissue | 5.7 ± 0.5 | 24.4 ± 0.7 | 2.9 ± 0.0 |
| Isolated seminiferous tubules | 9.9 ± 0.8 | 27.3 ± 1.0 | 6.3 ± 0.9 |
| Interstitial cell fraction | 0.7 ± 0.1 | 4.4 ± 0.7 | 3.0 ± 0.0 |

TABLE VIII

EFFECT OF X-IRRADIATION ON THE SOLUBLE, $Mn^{2+}$-SENSITIVE ADENYLATE AND GUANYLATE CYCLASE ACTIVITIES IN RAT TESTIS

Enzyme activities were measured in the 20 000 × *g* for 10 min supernatant fraction (filtered through 0.22-μm millipore filter) in the presence of 3 mM $Mn^{2+}$ and 0.2 mM of either ATP or GTP. Values are averages ± S.E. of quadruplicate (adenylate cyclase) or triplicate (guanylate cyclase) determinations.

| Group | pmol of cyclic nucleotide per mg of protein per min | |
|---|---|---|
| | Cyclic AMP | Cyclic GMP |
| Control | 9.2 ± 0.4 | 17.5 ± 6.3 |
| X-irradiated | 2.2 ± 0.1 | 15.0 ± 0.1 |

We have also searched for guanylate cyclase activity in the soluble fraction isolated from testes homogenates of prenatally X-irradiated rats. Table VIII shows that guanylate cyclase activity in the soluble fraction from testis homogenates of X-irradiated rats did not substantially differ from that in the supernatant derived from non-irradiated rats. In contrast, only marginal adenylate cyclase activity was detected in the soluble fraction isolated from testis of X-irradiated rats.

## Discussion

Our findings show that the cytosol adenylate cyclase is separate from the gonadotropin hormone- and F′-stimulated adenylate cyclases. These F′- and hormone-stimulated adenylate cyclases are particulate and originate from non-germ cells in the testis as indicated by their presence in the testis of X-irradiated rats containing no germ cells.

The cytosol adenylate cyclase was found to develop at about the time of the first meiotic divisions. We interpreted this to mean that the cytosol enzyme forms either in the secondary spermatocytes or early spermatids [1]. Our finding that the enzyme activity does not develop in the absence of germ cells in testis concurs with the idea that the site of its biogenesis is in a certain population of germ cells.

The results of the present study also indicate that the rat testis cytosol adenylate and guanylate cyclases, which both exhibit an obligatory $Mn^{2+}$ requirement for activity, are separate entities. It was found that the two enzyme activities appear at different stages of testis development, and in different cell types.

The guanylate cyclase in the cytosol, in contrast to the cytosol adenylate cyclase, was found to develop at an earlier stage, i.e. during the first two weeks of postnatal life, and was present in testis with no germ cells in consequence of X-irradiation. Since the guanylate cyclase activity has been found primarily in the seminiferous tubules, the tubular component possessing the enzyme is thus narrowed to either Sertoli or peritubular cells. On the basis of present findings, we cannot distinguish between these alternatives.

The physical properties of these two soluble testicular enzymes could not be compared since attempts to separate them by physical methods have not thus far given clear results. Recently Neer and Sukiennik [12] have solubilized and separated the adenylate and guanylate cyclase enzymes from rat renal medulla and found their physical properties to be very similar. It is thus conceivable that although the two enzymes might, in general, exhibit similar physical properties, their presence in testis, in different and functionally specialized cell types, suggest that they are separate systems with different functions.

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