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MECHANISMS AND INTERACTIONS IN TESTICULAR STEROIDOGENESIS AND PROSTAGLANDIN SYNTHESIS

LeGrande C. Ellis, Dean K. Sorenson and Louis E. Buhrley

Departments of Biology and Chemistry, Utah State University, Logan, Utah 84322, U.S.A.

SUMMARY

Prostaglandin synthesis by rat testicular tissue was observed to be gonadotrophin and adrenal dependent, and to have the same distribution in the gonad as the steroidogenic enzymes. The synthesis was increased by 5-HT, but decreased by melatonin. Lipid peroxidation increases with age, FSH treatment, linoleic and arachidonic acid and melatonin, but was decreased by serotonin, c-AMP, LH, prolactin and ephinephrine. Testicular lysosomes were labilized by LH, adrenal demedullation, testosterone, Vit. A and acetylcholine, but were stabilized by c-AMP, epinephrine and cortisol. Contractility of rat seminiferous tubules was observed to be prostaglandin dependent, stimulated by 5-HT, and inhibited by melatonin and c-AMP. Evidence was obtained indicating that a phospholipase A_2 is present in rat testicular lysosomes, and that it is liberated by radiation, heat or certain chemicals. This enzyme hydrolyses PUFA from membrane phospholipids, the former of which are converted into prostaglandins. The prostaglandins appear to initially stimulate contractility of seminiferous tubules, but at higher concentrations they stimulate adenyl cyclase, which increases c-AMP levels in the tubules thereby inhibiting contractility and cellular division. The process of prostaglandin synthesis also produced malonaldehyde, and endoperoxides that contribute to alterations in steroidogenesis by Schiff's base formation and to cellular lysis by membrane permeability changes. Prostaglandins appear to be synthesized physiologically from PUFA derived from cholesterol esters, but pathologically in much larger quantities from membrane phospholipids. Prostaglandins apparently inhibit androgen synthesis by their effects on cholesterol esterase and other steroidogenic enzymes rather than by changing blood flow.

INTRODUCTION

Investigations were undertaken in our laboratory in the mid 1960's to ascertain what effects irradiation and certain endogenous and exogenous antioxidants with radioprotective properties had on steroid biotransformations *in vitro*. These studies demonstrated antioxidants have specific effects on both steroid biotransformations and lipid peroxidation [1–3]. Lipid peroxides are now known to be intermediates in the synthesis of prostaglandins (PG's) [4]. Similarities in distribution of the steroid biotransforming and the lipid peroxidative mechanisms between the interstitial cells and the stable elements of the seminiferous tubules, reductions in both entities after hypophysectomy, plus their gonadatrophin dependency [1, 2] led us to conclude that the testis is a dual endocrine organ producing both androgens and prostaglandins [5]. Investigations by other workers support this conclusion.

The presence of prostaglandins in the testis [7, 8] and the rapid rate at which testicular tissue inactivates them [9, 10] further implicates these compounds with respect to testicular function. Additional impetus for us to study testicular-prostaglandin relationships came from recent observations that exogenous PG's reduce testicular weight and androgen synthesis [6, 11–14]. Although we had established a physiological role for PG's in regulating testicular capsular contractions, the synthesis of PG's and their overall effects on testicular tissue remain largely undefined. We therefore undertook the following investigations to elucidate further the mechanisms of synthesis and the activities of these biologically important compounds.

EXPERIMENTAL

Animals and treatments

Care of animals. All rats (males) were purchased from commercial sources (hypophysectomized and adrenalectomized animals from Hormone Assay Laboratories, Inc. and all others from Holtzman Laboratories). The animals were maintained in professional animal care units in our small-animal laboratory, which has a Southern exposure. Temperature was kept at 22°C with a relative humidity of 50%. Feed (laboratory chow) and water were given *ad lib.* All animals were sacrificed by decapitation.

Rabbits of mixed breed were obtained locally and maintained in the small-animal laboratory with feed and water given *ad lib.*

Hypophysectomy. Six control and six surgically treated rats (hypophysectomized at three weeks of age) were sacrificed when six weeks old. Six additional hypophysectomized animals were sacrificed when eight weeks old. All testes were removed and assayed for PG synthetase activity.

Adrenalectomy and adrenal demedullation. Six control and six rats adrenalectomized at 12 weeks of age were sacrificed 10 days after surgery. Their testes were

Table 1. Prostaglandin synthetase activity of rat testicular tissue after hypophysectomy and adrenalectomy expressed on a per animal basis

Treatment	Number of animals	Prostaglandin synthetase activity (O.D. units)	P Value
Control	6	1·166 ± 0·133*	
Hypophysectomy (3 weeks)	6	0·905 ± 0·149	<0·10
Hypophysectomy (5 weeks)	6	0·323 ± 0·029	<0·01
Control	6	1·955 ± 0·158	
Adrenalectomy (10 days)	6	0·503 ± 0·130	<0·001

* Mean values ± S.E.M.

removed and assayed for PG synthetase activity. Seven control and seven rats adrenal demedullated at 12 weeks of age were subjected to feed deprivation (7·5–8·0 g per day) for 23 days and sacrificed. The testes were removed and assayed for lysosomal stability. Both 0·9 NaCl and tap water were given *ad lib.* to the adrenalectomized animals.

Aging study. Five male rats were sacrificed on each of the following days after birth: 4, 8, 12, 20, 24, 26, 28, 30, 34, 36 and 90. Both the testes were removed and assayed for either androgen synthesis or lipid peroxidation.

Testicular implants. Intratesticular implants of either c-AMP or dibutyryl c-AMP were made by filling tubes (Dow medical grade Silastic tubing 0·058″ I.D., 0·077″ o.d., 5 mm long) with either 2 or 5 mg of the compounds. The tubes were sealed with medical grade "382" Silastic elastomer. Empty capsules were implanted in the control animals. The animals were anesthetized with ether. A small incision was made in the scrotum to expose the testes, and the implants were inserted into one of the testes *via* a small incision in the capsule. The scrotal incision was closed with wound clamps.

Intratesticular injections of caffeine and c-AMP. Fifteen adult male rats were irradiated at 12 weeks of age with 450 R of whole-body X-irradiation. Of these animals, five immediately had 0·03 ml of a 10^{-3} M solution of caffeine in 0·1 M phosphate buffer, pH 7·4, injected into one of their testes, five were injected with 0·03 ml of a 10^{-3} M solution of c-AMP, and five were injected with 0·03 ml of a 10^{-3} M solution of both caffeine and c-AMP. Five control rats were injected with buffer only.

Assays. Androgen synthesis was measured by incubating aliquots of testicular tissue in the presence of [7α-^{3}H]-pregnenolone and [4-^{14}C]-progesterone as described elswhere [15, 16]. Lipid peroxidation was measured spectrophotometrically using the thiobarbituric assay for malonaldehyde formation [17]. Lysosomal fragility was measured spectrophotometrically using the acid phosphatase assay [18].

Contractility of testicular capsules and seminiferous tubules. Rabbit testicular contractions were recorded *in vitro* and *in vivo* as described elsewhere [19]. Contractility of teased seminiferous tubules [20] was ascertained using a special water-jacketed chamber fitted to the stage of a microscope containing an ocular micrometer.

RESULTS

Hypophysectomy and adrenalectomy resulted in a rapid progessive decline of PG synthetase activity (Table 1) indicative of gonadotrophin and adrenal dependency. Lipid peroxidation was high postnatally (Fig. 1) on a per mg of tissue basis, but low on a per animal basis. Lipid peroxidation was low on day-26 but increased on day-28. A second reduction in

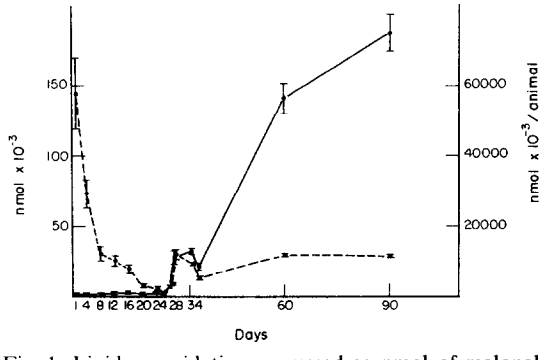

Fig. 1. Lipid peroxidation expressed as nmol of malonaldehyde produced by rat testicular tissue from animals of 1–90 days of age expressed on a per mg of tissue basis (hatched line) and a per animal basis (solid line).

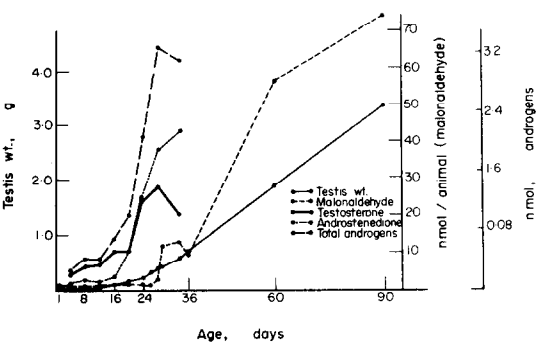

Fig. 2. The effect of age on testicular weight and the production of androstenedione, testosterone, malonaldehyde and total androgens expressed on a per animal basis.

Table 2. Effects of serotonin and melatonin on lipid peroxidation (malenaldehyde production) by testicular minced preparation, contractions of the testicular capsule and prostaglandin synthetase activity

Molar	TBA O.D. units per 0·5 ml inc. mixture	P Value	Smooth muscle contraction	Prostaglandin synthetase assay	P Value
Serotonin					
Control	313 ± 0·6*			373 ± 39	
5×10^{-6}	300 ± 0·3	<0·20	Stimulates		
5×10^{-5}	236 ± 0·5	<0·001	Stimulates		
5×10^{-4}	181 ± 0·3	<0·0001	Stimulates	841 ± 43	<0·001
5×10^{-3}	142 ± 0·4	<0·0001	Stimulates		
Melatonin					
Control	362 ± 0·7			391 ± 35	
5×10^{-6}	423 ± 1·2	<0·001	Inhibits	167 ± 21	<0·001

* Mean values ± S.E.M.

activity was observed on day-36 followed by another increase. Testosterone synthesis was low initially on a per animal basis (Fig. 2), but high on a per mg of tissue basis (data not shown). Testosterone production was markedly reduced compared with that of androstenedione on days 28–34, concomitant with the increased lipid peroxidation. Although not shown in Fig. 1, androstenedione and testosterone production was increased on days 60 and 90. Except for the period of 20–36 days of age, androgen synthesis and lipid peroxidation closely paralleled changes in testicular weight (Fig. 2).

5-HT inhibited malonaldehyde formation in a dose-dependent manner (Table 2). Melatonin, on the other hand, stimulated malonaldehyde production, inhibited capsular motility and decreased PG synthetase activity (Table 2). Similarly, injections of melatonin also reduced contractility of rat seminiferous tubules (Table 3). Testicular implants of Silastic tubing containing either c-AMP or dibutyryl c-AMP effectively

Table 3. Effects of melatonin injections on contractility of rat seminiferous tubules

Treatment	Number of animals	Diameter of tubules (μ)	Frequency of contractions (C/M)	Depth of contractions (μ)
Control	7	346·8 ± 9·8*	7·64 ± 0·54	6·79 ± 0·74
250 μg melatonin	7	339·4 ± 4·6	6·50 ± 1·01	4·72 ± 0·25†
500 μg melatonin	7	349·3 ± 6·3	6·14 ± 0·44§	3·91 ± 0·62†
1000 μg melatonin	7	366·0 ± 16·1	4·52 ± 0·76†	2·48 ± 0·29‡

* Mean value ± S.E.M.
† $P < 0·01$.
‡ $P < 0·001$.
§ $P < 0·20$.

Table 4. Effect of cyclic AMP and dibutyryl cyclic AMP silastic implants on contractility of seminiferous tubules

Treatment	Number of animals	Diameter of tubule (μ)	Frequency of contractions (C/M)	Depth of contractions (μ)
Control	5	354·4 ± 4·2*	7·80 ± 0·26	5·57 ± 0·31
2 mg C-AMP	5	374·8 ± 8·3†	4·40 ± 0·83‡	2·45 ± 0·54‡
Control	4	362·5 ± 12·7	7·91 ± 0·60	6·23 ± 0·96
5 mg C-AMP	4	417·0 ± 28·3†	2·71 ± 0·41§	1·50 ± 0·18‡
Control	8	361·5 ± 6·6	7·25 ± 0·49	5·45 ± 0·24
2 mg dibutyryl C-AMP	15	396·8 ± 7·4‡	3·13 ± 0·81‡	2·19 ± 0·47§
5 mg dibutyryl C-AMP	18	387·1 ± 9·3‖	2·39 ± 0·67§	2·0 ± 0·42§

* Mean values ± S.E.M.
† $0·10 > P < 0·20$.
‡ $P < 0·01$.
§ $P < 0·001$.
‖ $0·05 > P < 0·10$.

Table 5. The effect of changing the bathing media on contractility of rat seminiferous tubules

Treatment	Number of observations	Diameter of tubules (μ)	Frequency of contractions (C/M)	Depth of contractions (μ)
Control	8	363·5 ± 9·3	7·25 ± 0·37	6·49 ± 0·36
After 1st change	8	329·5 ± 8·6†	6·01 ± 0·60‡	4·64 ± 0·69†
After 2nd change	8	365·0 ± 9·1[1]	4·01 ± 0·57§	2·95 ± 0·44§
After addition of original bathing media	8	337·5 ± 7·7†[,2]	7·38 ± 0·57§[,2]	5·71 ± 0·51§[,2]

* Mean values ± S.E.M.
† $P < 0{\cdot}05$.
‡ $0{\cdot}05 > P < 0{\cdot}10$.
§ $P < 0{\cdot}001$.
[1] $P < 0{\cdot}05$ when compared with 1st change of bathing medium.
[2] Comparisons are made with respect to the 2nd change of the bathing medium.

increased the diameter of the tubules, but decreased the rate and intensity of contractions (Table 4). Successive changes of the medium also effectively decreased the frequency and intensity of contractions, along with inducing a biphasic change in diameter of the tubules (Table 5). Placing the seminiferous tubules in the original bathing medium increased both the frequency and the intensity of contractions as well as decreased the diameter of the tubules.

Pre-treatment of rats with indomethacin (10 mg/kg body wt. in oil twice daily for two days) reduced the frequency and intensity of contractions of seminiferous tubules, while the addition of 10^{-6} M $PGF_{1\alpha}$ to the medium increased both phenomena (Table 6). Pre-treatment of rats with indomethacin followed by its addition to the bathing medium more effectively inhibited contractility of the seminiferous tubules than did injections of this compound.

Intratesticular injections of either caffeine or c-AMP failed to significantly affect testicular weight after irradiation, but a combination of the two did decrease it (Table 7). Moreover, there was a radioprotective effect of the combination treatment on the contralateral testis when compared with the caffeine treated group. Intratesticular implants of Silastic tubing containing c-AMP decreased both testicular weight and stabilized the lysosomes (Table 8).

Lysosomal fragility (i.e., acid phosphatase release) was increased by LH and testosterone in a dose-dependent manner (Fig. 3), while FSH had no demonstrable effect. Lipid peroxidation was increased maximally by FSH, but was depressed by hypophysectomy, prolactin, LH and a combination of FSH, LH and prolactin (Fig. 4). C-AMP (Fig. 5) and dibutyryl c-AMP (data not shown) both increased lysosomal fragility (Fig. 5) while c-AMP decreased it. Vitamin A also stimulated lipid peroxidation (data not shown). Arachidonic acid increased lipid peroxidation whereas linoleic acid moderately increased it (Fig. 6). Epinephrine markedly decreased both lysosomal fragility and lipid peroxidation, while adrenal demedullation increased acid phosphatase release (Table 9).

Table 6. The effect of indomethacin *in vivo* and *in vitro* on contractility of rat seminiferous tubules

Treatment	Number of samples	Diameter of tubules (μ)	Frequency of contractions (C/M)	Depth of contractions (μ)
Control	8	347·0 ± 7·8*	7·50 ± 0·46	6·18 ± 0·23
10 μ/ml *in vitro* indomethacin	8	368·0 ± 16·8	5·00 ± 1·11†	3·74 ± 0·79†
10 μ/ml *in vitro* indomethacin + 10^{-6} $PGF_{1\alpha}$	8	362·0 ± 8·6	9·00 ± 1·24†[,2]	5·85 ± 0·82‡[,2]
Control	8	329·5 ± 5·2	7·00 ± 0·38	4·65 ± 0·44
10 mg/kg I.P. 2 × daily + 10μ/ml indomethacin	8	351·5 ± 6·7†	3·50 ± 0·33§	2·95 ± 0·27[1]

* Mean values ± S.E.M.
† $P < 0{\cdot}05$.
‡ $0{\cdot}05 > P < 0{\cdot}10$.
§ $P < 0{\cdot}001$.
[1] $P < 0{\cdot}01$.
[2] Comparisons were made with the 10 μg/ml indomethacin group.

Table 7. Effects of 450 R whole-body X-irradiation and intratesticular injections of caffeine, cyclic-AMP or a combination of both on rat testes *in vivo*

Treatment	Animals	Weight of non-injected testis (g)	Weight of injected testis (g)	P Value	Difference in testicular wt.* (g)	P Value
Control	5	1·630 ± 0·069	1·633 ± 0·069	<0·50	(+) 0·003 ± 0·011†	
Caffeine	5	1·254 ± 0·036	1·288 ± 0·039	<0·50	(−) 0·026 ± 0·035	<0·50
C-AMP	5	1·304 ± 0·030	1·264 ± 0·032	<0·50	(−) 0·040 ± 0·029	<0·40
Combination	5	1·388 ± 0·029	1·312 ± 0·025	>0·40	(−) 0·072 ± 0·027	<0·05

* Difference between treated and untreated testes.
† Mean values ± S.E.M.
Statistical comparisons for non-injected testes only:
Control vs caffeine $P < 0·01$.
Control vs C-AMP $P \ll 0·001$.
Control vs Combination $P < 0·02$.
C-AMP vs Combination $P < 0·05$.
Caffeine vs Combination $P < 0·02$.

Cortisol decreased lysosomal fragility at 10^{-6} to 10^{-7} M, but lost this effect at higher concentrations (Table 9).

DISCUSSION

The rapid decline in PG synthetase activity after hypophysectomy and adrenalectomy (Table 1) indicates a gonadotrophin and adrenal dependency[2]. A positive correlation between the localization of steroidogenic and lipid peroxidizing enzymes in the teased seminiferous tubules and minced tissue, the dependency of both mechanisms on NADPH [2], and the positive correlation between lipid peroxidation and steroidogenesis in the developing rat (Figs. 1, 2) further suggest a gonadotrophin dependency.

The rapid fluctuations in lipid peroxidation in animals between 26 and 36 days of age, concomitant with changes in the androstenedione/testosterone ratio, reflect some biochemical adjustments in the testis at this time. In this respect, unsaturated fatty acids do increase lipid peroxidation (Fig. 6), and preliminary data from our laboratory plus observations on the adrenal gland [21] show that lipid peroxidation does alter specific steroid biotransformations.

It is noteworthy that 5-HT decreased lipid peroxidation, but stimulated PG synthetase activity as well as contractility of the capsule and seminiferous tubules, while melatonin stimulated lipid peroxidation, but decreased PG synthetase activity as well as contractility of the capsule (Table 2) and seminiferous tubules (Table 3). These observations are consistent with those of others [22–25] that the types and amounts of PG produced are determined by the concentration and nature of the cofactors (antioxidants) present in the incubation medium. Thus, malonaldehyde formation apparently can be separated from PG synthesis and it may accumulate under certain conditions at the expense of PG synthesis.

Abolition of seminiferous tubular contractility by successive changes of the bathing medium (Table 5), the restoration of motility by adding the original bathing medium, the abolition of contractility by indomethacin and its reestablishment with exogenous PG (Table 6) suggest that contractility of this tissue is PG-dependent similar to that of the capsule [19]. Thus, PG's serve a function in mammalian testes by modulating the contractility of the capsule and seminiferous tubules. That this action might be important to testicular function is indicated by the observation that $PGF_{2\alpha}$ increased the number of sperm in the deferent duct of rabbits [26], while PGE_2 and $F_{2\alpha}$ decreased the time lag between injection of [^{3}H]-thymidine and appearance of labeled

Table 8. Effects of silastic implants containing either cyclic-AMP or dibutyryl C-AMP testes *in vivo*

Treatment	Number of animals	Difference in testicular wt.* (g)	P Value	Difference in acid phosphate activity (O.D. units)	P Value
C-AMP					
Control	4	(−) 0·040 ± 0·002†	<0·01	0·02	<0·05
2 mg C-AMP	5	(−) 0·118 ± 0·004	<0·02	(−) 0·12	<0·01
5 mg C-AMP	3	(−) 0·223 ± 0·064	<0·001	(−) 0·16	<0·01
Dibutyryl C-AMP					
Control	7	0·046 ± 0·010			
2 mg	7	(−) 0·130 ± 0·016	0·01		
5 mg	6	(−) 0·220 ± 0·052	0·001		

* Difference between treated and untreated testes.
† Mean values S.E.M.

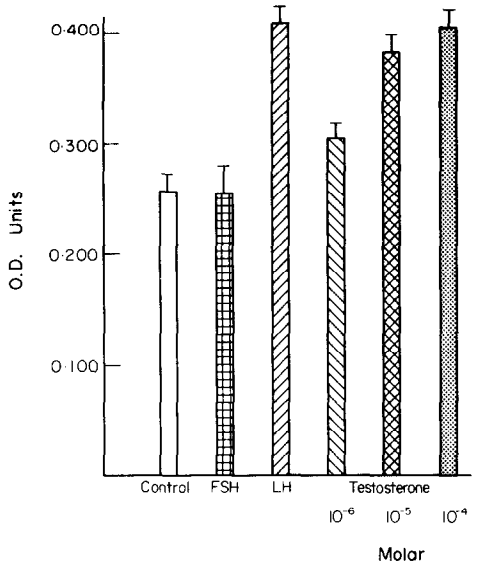

Fig. 3. The effects of FSH, LH and testosterone on rat testicular lysosomal fragility (release of acid phosphatase activity) *in vitro*.

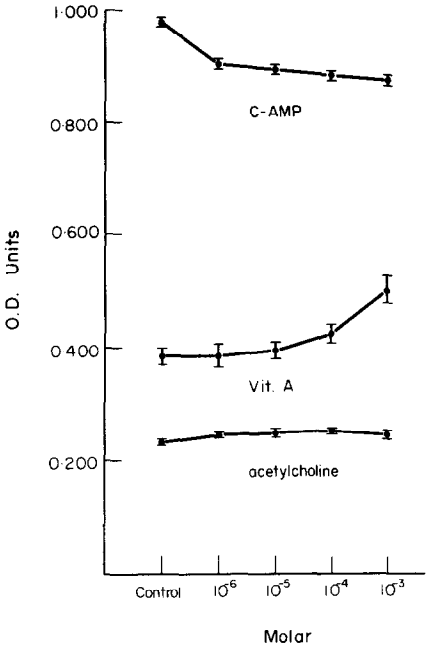

Fig. 5. The effects of c-AMP, Vit. A and acetylcholine on lysosomal fragility (release of acid phosphatase activity) *in vitro*.

spermatozoa in the semen [27]. In addition to facilitating sperm transport, PG's might also propel lymph and blood from the testes [8, 19, 28] and regulate blood flow [29].

Of particular importance is the fact that intratesticular Silastic implants of either c-AMP of dibutyryl c-AMP inhibited contractility of the seminiferous tubules while stabilizing the lysosomes (Table 8) and decreasing testicular size. Thus c-AMP appears to be of importance to the testes not only in stimulating androgen synthesis, but also in modulating contractility of the capsule [30] and seminiferous tubules and in regulating lysosomal activity and cellular divisions. In addition, epinephrine stabilizes lysosomes with a concomitant reduction in lipid peroxidation (Table 9), and may also serve as a cofactor in PG synthesis [22–25]. The observed decrease in acid phosphatase activity after demedullation (Table 9) is consistent with epinephrine's role in regulating testicular lysosomal integrity as has been reported for this amine in the preputial glands [26]. Cortisol appears to stabilize lysosomes (Table 9) while LH and testosterone labilize them (Fig. 3). Moreover, the steroidal effects appear to be exercised directly on the lysosomal membrane [31, 32], while the effects of most other agents (LH, epinephrine, theophylline, caffeine, Vit.

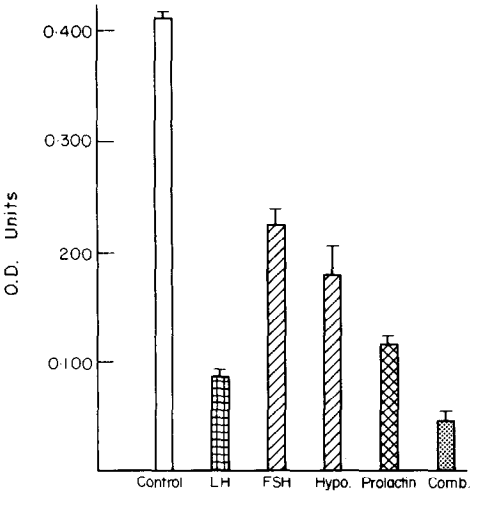

Fig. 4. The effects of hypophysectomy and injections of prolactin and a combination of FSH, LH and prolactin on lipid peroxidation (malonaldehyde production).

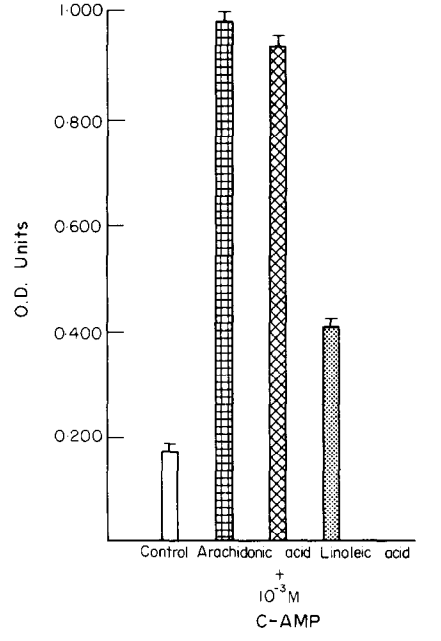

Fig. 6. The effect of arachidonic acid and linoleic acid on lipid peroxidation (malonaldehyde formation) *in vitro* and the inhibiting effect of c-AMP.

Table 9. The effects of epinephrine, adrenal demedullation and cortisol on lipid peroxidation and lysosomal acid phosphatase release

Treatment	Number of animals	Acid phosphatase activity (O.D. units)	P Value	Lipid peroxidation (O.D. units)	P Value
Epinephrine					
Control	5	0·803 ± 0·015*		0·282 ± 0·009	
10^{-6} M	5	0·685 ± 0·008	<0·001	0·272 ± 0·010	<0·50
10^{-5} M	5	0·654 ± 0·008	<0·001	0·240 ± 0·011	<0·02
10^{-4} M	5	0·526 ± 0·005	<0·001	0·160 ± 0·009	<0·001
10^{-3} M	5	0·647 ± 0·012	<0·001	0·068 ± 0·008	<0·001
Cortisol					
Control	5	0·560 ± 0·012			
10^{-6} M	5	0·519 ± 0·011	<0·03		
10^{-5} M	5	0·485 ± 0·014	<0·005		
10^{-4} M	5	0·511 ± 0·013	<0·02		
10^{-3} M	5	0·585 ± 0·011	<0·10		
Control	5	0·298 ± 0·026			
Demedullated	5	0·391 ± 0·006	<0·005		

* Mean values ± S.E.M.

A and acetylcholine), are mediated through either c-AMP or c-GMP [33]. Since LH and prolactin both decrease lipid peroxidation while increasing testosterone synthesis [34] the effect is apparently mediated through c-AMP as c-AMP decreases lipid peroxidation (Fig. 4). The increase in malonaldehyde production induced by FSH can be logically attributed to a decrease in c-AMP through an increase in phosphodiesterase activity.

The hormonal control of lysosomes is important since the lysosomes are necessary for the differentiation of spermatocytes to spermatids [35]. Of particular importance is the fact that cortisol both stabilizes lysosomes (Fig. 5) and inhibits spermatogenesis [36], while c-AMP also stabilizes lysosomes, inhibits testicular DNA synthesis [37], reduces testicular weights (Table 7) and inhibits cellular division of some cells [38, 39]. By contrast Vit. A labilizes lysosomes (Fig. 5, ref. 40) and is necessary for spermatogenesis [41]. In the liver Vit. E apparently stimulates a liver phosphodiesterase that appears to preferentially inactivate c-AMP with little effect of c-GMP [33].

With respect to steroidogenesis, lipid peroxidation and PG synthesis (Fig. 7), three polyunsaturated fatty acids (PUFA) are known to serve as precursors for PG synthesis [4]: dihomo-γ-linolenic acid, arachidonic acid and 5,8,11,14,17-eicosapentaenoic acid. The concentrations of the three PUFA are an important limiting factor in the endogenous biosynthesis of PG's [42, 43]. An important control of endogenous PG biosynthesis is the enzymic release of the PUFA from membrane phospholipids. In this respect rat liver lysosomes contain a soluble acid and a membrane-bound alkaline phospholipase A_2 (pH optima 7·0–8·0) [45]. Our data suggest that the same relationship exists for the testis.

On this basis anything that labilizes lysosomes should increase PG synthesis and could elevate c-AMP levels. Indeed, when heat was applied to the scrotum, acid phosphatase accumulated about the damaged pachytene primary spermatocytes and persisted until the damaged cells had been removed [46]. Moreover, rat testicular lysosomes are more subject to thermal lability than are those from rat liver or chicken liver and testis [47]. Acid phosphatase is also located in mature germ cells and disappears as the cells are lost. Similarly, contractility of the seminiferous tubules increased markedly *in vitro* with temperature as did lysosomal fragility [48], indicating an increase in PG synthesis. Contractility was gradually lost with time after cryptorchidism [48] which indicated an increase in c-AMP synthesis. Lastly, c-AMP increased in the testes after cryptorchidism and hypophysectomy [49]. Cryptorchidism also increases androgen synthesis [50].

HCG increased c-AMP levels in the media of decapsulated testes incubated *in vitro* [51, 52]. LH increased both c-AMP [53–55] and testosterone synthesis [54, 56] by isolated rat testicular interstitial tissue *in vitro*, while FSH increased c-AMP in isolated seminiferous tubules [54, 56]. C-AMP accumulated only in the presence of theophylline [54] or in young or hypophysectomized animals [56], indicating a high phosphodiesterase activity in this tissue. Although the function of c-AMP in the testis remains to be fully elucidated, its concentration in the male gonad decreases with age and maturation of the germinal cells, but increases with the reduction in germ cell numbers associated with cryptorchidism and hypophysectomy [49]. Moreover, a highly specific testicular c-AMP phosphodiesterase is associated with sexual maturation [57].

In view of the above, it is tenable that any noxious environmental change in the testes—e.g., from ionizing irradiation; a temperature increase due to cryptorchidism, externally applied heat, or microwaves; or introduction of some chemicals—could labilize the lysosomes thereby releasing phospholipase A_2. The liberated PUFA, once converted into PG's could stimulate adenyl cyclase and form c-AMP. The nucleotide could then inhibit DNA synthesis [37] and cellular division, giving rise to depressed stem cells that would

result in the maturation depletion of germ cells in the testes that is known to occur after noxious treatments. Some PG's stabilize lysosomes [58] consistent with their role in elevating c-AMP levels in cells and possibly as a feedback control mechanism.

Evidence showing that irradiation does release enzymes from the lysosomes (thereby increasing lipid peroxidation) [59], and that PG's do increase c-AMP in the testes [19, 60–62] especially in the presence of caffeine [61] supports our proposal. It is also relevant that c-AMP, caffeine, theophylline and some prostaglandins inhibit cellular division [38, 39, 63–65] in various tissues and ameliorate some radiation-induced responses in cells [66]. Recent information shows that c-AMP actually affords radioprotection to cells [67] and that the radioprotective effects of cysteamine on mouse liver are mediated by c-AMP [68]. The fact that c-AMP inhibits testicular DNA synthesis [37] and is radioprotective to specific cells [67] fits well into our proposed radioprotective scheme for the testes [69, 70].

C-AMP does provide radioprotection to the testes but not intratesticularly. The time of injection and dosage may be important in determining the response shown.

The products of lipid peroxidation also have deleterious effects on the lysosomes [71], hemoprotein catabolism [72], microsomes [73], sulfhydryl metabolism [1, 74], enzyme protein [75] and DNA [76].

Of concern is the observation that fluoride induces infertility in male mice and can activate adenyl cyclase [78]. Also, microwave radiation depletes the testis of germinal cells and the damage is directly related to the rise in temperature in the gonad [79],

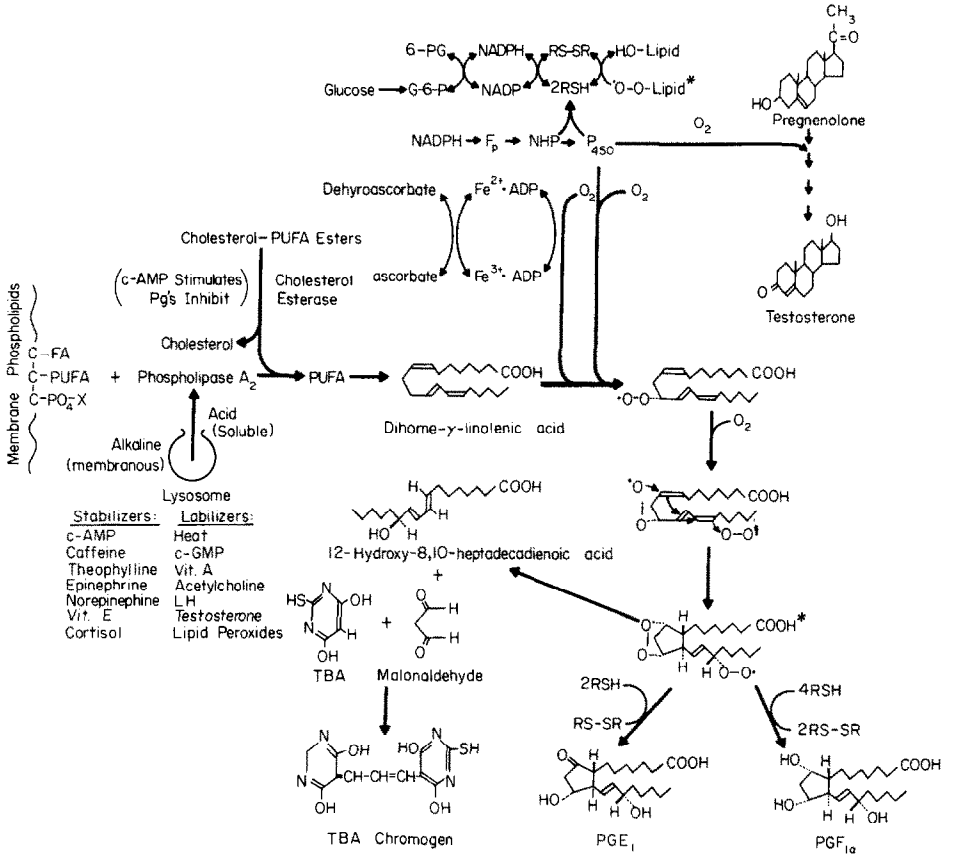

Fig. 7. Proposed scheme showing the apparent routes for the synthesis of malonaldehyde, prostaglandin E_1 and prostaglandin $F_{1\alpha}$ and testosterone synthesis from pregnenolone. The asterisks are placed on the lipid peroxides to indicate their effects on the conversion of sulfhydryl compounds to disulfide bridges. During lipid peroxidation, free radicals are formed. These free radicals are brought to the ground state by accepting protons from other organic compounds. RSH is used in this scheme to represent either enzyme-protein sulfhydryl groups, glutathione sulfhydryl groups, or any organic RH_2 moiety. Once disulfide bridges are formed from sulfhydryl groups, the sulfhydryl groups may be regenerated through glutathione-reductase involving glucose-6-phosphate and NADPH as indicated at the upper-left-hand corner of the figure. NHP represents non-heme iron: P_{450} represents the iron containing pigment that develops an ultraviolet absorption spectrum with maximum absorption at 450 nmeters in the presence of carbon monoxide. The TBA-chromogen is the pigment measured with thiobarbituric acid reagent as an indicator of lipid peroxidation. Two methods are shown for the peroxidation of lipids. The first and predominant method is heat labile and enzymatic. This route involves NADPH, non-heme iron (NHP), and the P_{450} pigment. The second method involves ascorbic acid, ADP, and Fe^{2+}. It is heat stable and has a slower rate of synthesis. It is not known whether ascorbic acid acts as a proton donor in regenerating Fe^{2+} from Fe^{3+} or possibly as a complex with Fe^{2+} (From Ellis and Baptista, [1]).

which further implicates PG's and c-AMP in the etiology of infertility under these conditions.

A number of investigators have now shown that certain PG's reduce testicular weight [11–13] and androgen levels in the blood [6, 11–13, 80, 81]. PG's may reduce testicular blood flow [29, 81], but recent investigations show however, that ischemia is not the causative factor as norepinephrine reduced blood flow, but did not induce testicular injury [14]. PGA inhibits androgen synthesis *in vitro* [1]. Similarly, PG's $F_{1\alpha}$, E_1 and E_2 react with cytochrome P_{450} [82] indicating a possible P_{450} role in biosynthesis or metabolism of PG's. It is evident, however, that cholesterol-fatty acid esters can serve as precursors of androgen synthesis [6]. Moreover, PG's apparently can inhibit androgen synthesis by inhibiting the cholesterol esterase [6, 83]. Once cleaved from the cholesterol, the PUFA's may provide energy for steroidogenesis [84] or be used directly for PG synthesis (Fig. 4).

Thus, PG's may be synthesized in physiological quantities from cholesterol-PUFA esters, while pathological quantities may arise from membrane phospholipids through the action of lysosomal phospholipase A_2.

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