Rat Testicular Phospholipase A₂ Activity:

pH Optima, Its Cellular and Subcellular Distribution in the Gonad, and Some Factors That May Modulate Its Activity

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A sensitive radiometric assay was developed for detecting phospholipase A_1 and A_2 (PLA_1 and A_2) activity. Four to five times more PLA_2 activity was observed in rat testes than PLA_1 activity. Two PLA_2 were observed in the testis, one with an acid (3.5) and one with an alkaline (7.5–8.0) pH optimum. The acid pH optimal PLA_2 was located in the interstitial and lysosomal fractions. The alkaline pH optimal PLA_2 was localized in the germinal elements of the seminiferous tubules and in the lysosomal-enriched and membrane-enriched fractions. Triton X-100 inhibited PLA_2 at 10^{-2} M and inhibited PLA_1 at 10^{-3} M. At 10^{-2} M, triton X-100 activated PLA_1. EGTA inhibited PLA_2 activity, whereas Ca^{++} at 10^{-2} to 10^{-3} M restored this activity. Corticosterone had no effect on PLA_2 activity, but progesterone, dihydrotestosterone, and testosterone all stimulated the enzyme at lower concentrations (10^{-9}–10^{-7} M), with testosterone giving maximum stimulation at a lower dosage (ie, 10^{-9} M compared to 10^{-7} M for dihydrotestosterone). Both androgens inhibited PLA_2 activity at higher concentrations.

Key words: rat testicular phospholipase A_2 activity, pH optima, localization, control.

Phospholipase A (PLA-EC 3.1.1.4) is of interest in the study of male reproduction because it can provide free fatty acids for energy and for the production of glycerophosphoryl choline in the epididymis (Bjerve and Revitan, 1978), the production by spermatozoa of prostaglandins in the epididymis and vas deferens associated with maturation of spermatozoa (Johnson and Ellis, 1978) and prostaglandin production in the testis (Ellis, 1977). Arachidonic acid, the precursor of the predominant prostaglandins ($PFG_2\alpha$ and PGE_2) in the testis (Ellis, 1977), has been identified as a major component of sperm lipids (Neil and Masters, 1972). Moreover, there are large losses of arachidonic acid from the spermatozoa as the gametes pass through the male reproductive tract (Poulous et al, 1973; Poulous et al, 1975; Arora et al, 1975). It has been postulated that the spermatozoa may be the source of seminal prostaglandins (Poulous et al, 1975; Arora et al, 1975; Marley and Smith, 1975), although in man and sheep the seminal vesicles also contribute to this pool of prostaglandins. Histochemical studies in the rat (Johnson and Ellis, 1978) corroborate this conclusion. Prostaglandins *per se* and processes involved in their synthesis are thought to play a functional role in the maturation process as the sperm cells pass through the excurrent ducts of the male reproductive system (Ellis, 1977; Nishikawa and Wade, 1952).

In the testis, prostaglandins are required for contractions of the seminiferous tubules (Buhrley

and Ellis, 1975) and the capsule (Hargrove et al, 1973), and they decrease sperm transit time in several species (rabbit and bull, Hafs et al, 1976; and boar, Hemsworth et al, 1977).

Although PLA activity has been observed in human seminal plasma (Kunze et al, 1974), rat epididymis (Bjerve and Revitan, 1978), and rat testes (Chaudhary et al, 1978), as of this writing no one has used lecithin labeled with arachidonic acid in the A$_2$ position as the substrate, nor has there been an attempt to characterize the enzyme as to its source in the testis or to ascertain what specific modulators may serve to regulate its activity. The present investigation was undertaken to develop an assay system for PLA$_1$ and A$_2$, using 1-L-palmitoyl-2-arachidonyl-1-^{14}C-phoshatidyl choline as the substrate, to ascertain what pH optima are present for PLA$_2$, what cell types in the testis contain the two enzymes, where in the cells of the testis the enzymes are located, and what chemicals might modulate PLA$_2$ activity *in vitro* as it would relate to prostaglandin synthesis.

Methods

Adult male rats (Holtzman strain) used in this study were bred and maintained in our small animal laboratory with light from a southern exposure. Temperature was maintained at 22 C with a relative humidity of 35%. The animals were housed in professional animal care units (4–6 animals to a cage, 34 × 40 cm in size) with commercial feed and water available *ad libitum*. The animals were sacrificed when 12 to 16 weeks old by decapitation. Both testes were quickly removed from each animal, chilled, decapsulated, and finely minced on a Teflon block with razor blades. Aliquots of the pooled, finely minced preparations were weighed and homogenized with an all-glass homogenizer (Potter-Elevejhem, Kontes) to give a 30% homogenate in buffer, using either an acetic acid (80 mM), sodium phosphate (80 mM), or a Tris (0.1 M) buffer at the pH specified for the individual assay. The brei was centrifuged at 1000 g in a preparative ultracentrifuge (Spinco Model L) for 10 minutes to remove nuclear material and cell debris. The resulting supernatant solution was assayed for PLA$_2$ activity as described below.

PLA$_1$ and A$_2$ Assays

PLA activity was measured using 1-L-palmitoyl-2-arachidonyl-1-^{14}C-phosphatidyl choline as the substrate (55 mCi/nmole, Applied Science). The purity of this preparation was checked with thin-layer chromatography and was found to be 99+%, as specified by the supplier. A 1-μCi aliquot of this substrate was subjected to snake venom PLA$_2$ (Sigma) to verify the position of label for the arachidonyl-1-^{14}C. A stock solution of this substrate was prepared to a concentration of 1.82 nmole

or 0.10 μCi/0.5 ml in a 4:1 mixture of benzene:ethanol. Aliquots of 0.5 ml from this stock solution were then transferred by pipette into 15-ml conical centrifuge tubes. Using dry N$_2$ gas and a 45 C water bath, the solvent was then evaporated to dryness.

Aliquots of 0.5 ml from the tissue preparations to be assayed for PLA activity were then transferred by pipette into the centrifuge tubes. After gently but thoroughly mixing the contents of the tubules with a vortex mixer, the samples were incubated at 32.5 C (scrotal temperature) in a water bath for the desired length of time.

The incubations were terminated by heating the tubes in a boiling mineral oil bath for approximately 5 seconds. After allowing the tubes to cool for 5 minutes, the lipids were extracted using the Bligh and Dyer (1959) method as described below. A monophasic solution was obtained by adding 1.5 ml of a 2:1 (v:v) methanol:chloroform mixture to the reaction tubes. The contents of tubes were mixed thoroughly with a vortex mixer and allowed to stand for at least 15 minutes. A biphasic solution was then obtained by the sequential addition of 0.5 ml of chloroform and 0.5 ml of distilled water to each tube. The phases were clarified by centrifugation at 500 g for 5 minutes. The bottom chloroform layers were transferred to clean 15-ml conical centrifuge tubes with transfer pipettes. To ensure efficient extraction, another 1-ml aliquot of chloroform was added to the reaction tubes that contained the methanol-water phase. The tubes were again mixed thoroughly and centrifuged. The bottom layers were combined with those from the first extraction and evaporated to dryness with dry N$_2$ gas in a 45 C water bath. The lipid residues were concentrated in the tips of the tubes by washing the walls of the tubes with a 2:1 (v:v) mixture of chloroform:methanol, and the solvent was evaporated.

The lipid residues obtained were quantitatively transferred to strips of chromatofilm (3 cm-wide strips of silica gel, Eastman Kodak) using a 2:1 (v:v) chloroform:methanol solution. The strips were developed in ascending chromatography tanks using chloroform:methanol:water (65:35:4) (v:v:v). The radioactivity was localized on the strips by scanning the chromatofilm with a 2π Geiger-Muller strip counter. Three radioactive peaks were routinely seen. The peak migrating with authentic carrier arachidonic acid (Applied Science) exhibited the least polarity (Rf = 0.80) and migrated just behind the solvent front. This radioactive compound resulted from the hydrolysis of arachidonic acid from the labeled phospholipid. With the substrate used, the amount of labeled arachidonic acid released from the phospholipid was directly related to the amount of PLA$_2$ activity present in the samples. The peak migrating the shortest distance from the origin migrated with the authentic carrier lysolecithin (Applied Science) (Rf + 0.16). This labeled compound represented the amount of PLA$_1$ activity present in the samples. A third peak migrated midway between the solvent front and the origin (Rf + 0.49). This represented the residual, unreacted, phospholipid substrate that migrated with authentic carrier lecithin (Applied Science). All three compounds were eluted from additional chromatograms and subjected to

gas chromatography (F&M Scientific Research Chromatograph model 5750) using a 10% Sp-2330 on 100/120 mesh Supelcoport column (Supelcoport) to test for purity and to further characterize the products.

PLA$_2$ activity was quantified by cutting out the radioactive peaks corresponding to arachidonic acid and scraping the silica gel off the thin-layer strips into scintillation vials. PLA$_1$ was quantified by cutting out the radioactive peaks corresponding to L-α-lysoarachidonyl-1-^{14}C phosphatidyl choline and scraping the silica gel into scintillation vials. The vials were then filled with 20 ml of scintillation fluid (0.3 g of POPOP and 12 g of PPO in 3 liters of toluene). The contents of the vials were then mixed vigorously with a vortex mixer to suspend the gel particles just prior to counting in a liquid scintillation counter (Packard, series 3000).

Linearity of Assay

The linearity of the assay was ascertained by incubating for 15, 30, 45, 60, and 90 minutes aliquots of pooled rat testes homogenized (30% w/v) in phosphate buffer (pH 7.5). PLA$_2$ activity was assayed as described above. All subsequent assays were incubated for 45 minutes, the midpart of the linear range, except for the hormone and PABA studies, which were incubated for 40 minutes.

Determination of pH Optimum

In this study variations in pH ranging from 2.5 to 9.0 were obtained with a sodium acetate buffer from pH 2.5 to 5.0 and sodium phosphate buffer from pH 5.5 to 9.0. The assay was as described above, except that aliquots of the finely minced testicular tissue were weighed out and homogenized in the respective buffer at the specified pH.

Interstitial Cells, Teased Tubules, and Triton X-100

Seminiferous tubules were teased from rat testes by the method of Christensen and Mason (1965). Additional seminiferous tubules were teased and manually stripped of their germinal elements with forceps, according to the method of Urry et al (1975). Tubular walls were segregated into one fraction while the germinal cells were recovered from the media by centrifugation at 600 g for 10 minutes. The interstitial cells were aggregated into a separate fraction by centrifugation at 250 g for 5 minutes. An aliquot of pooled whole testicular tissue was also saved. All fractions were stored at −20 C until assayed. At the time of assay, the pooled tissue was homogenized in phosphate buffer (pH 7.5). The teased seminiferous tubules, germinal elements, and interstitial cells were split into two fractions each. One fraction was homogenized in sodium acetate buffer (80 mM) (pH 3.5), and the second fraction was homogenized in sodium phosphate buffer (80 mM) (pH 7.5). The germinal cells that were extruded were saved for assay at pH 7.5. All of the above samples were assayed for PLA$_2$ activity as described above.

A dose-response curve (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M) for triton X-100 on alkaline pH optimal PLA$_1$ and A$_2$ activities was initiated using rat testicular homogenates. All of the samples were assayed for both PLA$_1$ and A$_2$ activities as described above.

Plasma Membrane Assay

The presence of PLA$_2$ activity in enriched fractions of plasma membranes from rat testes was ascertained by isolating the membranes according to the method of Coleman et al (1967), using a swinging bucket head and an ultracentrifuge (Spinco Model L). The purified membranes were resuspended in 4 ml of phosphate buffer (pH 7.5) and assayed for PLA$_2$ activity as described above.

Lysosomal Assay

A rat testicular enriched lysosomal fraction was isolated and purified according to the method of Ragab et al (1967). The lysosomes were resuspended in either a 0.3 M sucrose-sodium acetate buffer (80 mM) (pH 3.5) or a 0.3 M sucrose-sodium phosphate buffer (80 mM) (pH 7.5). Aliquots of these two fractions were assayed for PLA$_2$ activity at both pH 3.5 and 7.5, as described above.

EGTA and Calcium Assays

In the ethyleneglycol-bis-(β-aminoethyl ether) N, N'-tetraacetic acid (Sigma) (EGTA) study, a 60% (w/v) homogenate in 0.1 M Tris buffer (pH 7.5) was diluted with an equal volume of Tris buffer containing the amount of EGTA, to yield homogenates (30%) with concentrations of 0, 10^{-6}, 10^{-5}, and 10^{-4} M EGTA. Each treatment level was replicated five times. In the calcium−EGTA interaction study, an equal volume of Tris buffer containing the specified amounts of EGTA and CaCl$_2$ was added to aliquots of the homogenate to yield homogenates (30%) containing 0, 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M calcium. All of these aliquots had a 10^{-4} M concentration of EGTA except for the control group. Each treatment was replicated seven times.

PBPAB, Testosterone, Dihydrotestosterone, Corticosterone, and Progesterone Assays

Fresh 30% (w/v) homogenates were prepared for each of these assays. Specific amounts of test compounds were added to tubes containing 50 μl of propylene glycol by first dissolving the compounds in chloroform. The chloroform was evaporated from the tubes using N$_2$ gas. Two milliliters of the homogenate was then added to the tubes containing the propylene glycol and varying amounts of the test compound, resulting in homogenates containing the desired concentrations of these compounds studied. These tubes were then mixed and incubated in an ice bath for 45 minutes before assaying for PLA$_2$ activity. Each specified concentration was replicated six times for each authentic compound (Sigma).

Statistics

A one-way analysis of variance with Tukey's HSD procedure (Neter and Wasserman, 1974) was used to ascertain statistical significance when more than one sample mean was compared to another. A t test was used when only one mean was compared against another.

Results

Arachidonic acid, lysolecithin, and lecithin obtained from incubations containing the radioactively labeled lecithin were judged to be these compounds on the basis of their co-migration with authentic carrier lipids by thin-layer chromatography and by the same retention times with gas chromatography. Arachidonic acid-1-^{14}C was judged to be in the sn-2 position, since less than 3% of the label was not released from the substrate by snake venom PLA$_2$. Moreover, arachidonic acid was observed to be released from endogenous substrates when samples with no exogenous substrate were added, which is indicative of a role for PLA$_2$ in testicular tissue.

Initial studies with the assay system indicated that some enzyme activity persisted after removing the samples from the incubator, so that when a number of samples were incubated, those that were extracted last had more activity than those that were processed immediately after the incubation. In order to circumvent this problem, attempts were made to find a suitable method for inactivating the enzyme. Acidification of the incubation media was rejected because it activated the acid pH optimal PLA$_2$ enzyme activity, it increased slightly the radioactivity in the nonincubated control samples, and it increased the variability of samples within a specific treatment group. Heating the samples for 5 seconds in a mineral oil bath was found to give low blanks and variability among samples, and it did not activate the acid pH optimal PLA$_2$ activity.

Four main sources of error or variability of assay response were noted: 1) differences in technique from investigator to investigator; 2) sample variability for samples of a given treatment group; 3) animal variability; and 4) season of year. The first source of variation was controlled by having the technicians practice quantitative chemistry techniques (eg, quantitative transfer of samples, etc). The second source of variability was controlled by using a minimum of five to seven samples per

treatment group. Animal to animal variability was controlled by using pooled homogenates from three to five animals for *in vitro* studies. With studies involving *in vivo* treatment of animals, seven animals per treatment group was found to be optimal. We can now attribute seasonal variability to be due to gonadotropic hormone control (unpublished data).

Time Linearity of PLA$_2$ Assay

The release of ^{14}C-labeled arachidonic acid from 1-L-palmitoyl-2-arachidnoyl-1-^{14}C-phosphatidyl choline was linear and directly related to incubation time (Fig. 1) throughout the 90-minute incubation period. Approximately 35% of the substrate was hydrolyzed at the longest time interval.

pH Optima of PLA$_2$ Activity

Two pH optima were found for PLA$_2$ activity: one at roughly pH 3.5 and the second with a plateau at pH 7.5 to 8.0, with no significant decrease in activity observed through pH 9.0 (Fig. 2).

Distribution of PLA$_2$ Activity in Rat Testicular Tissue

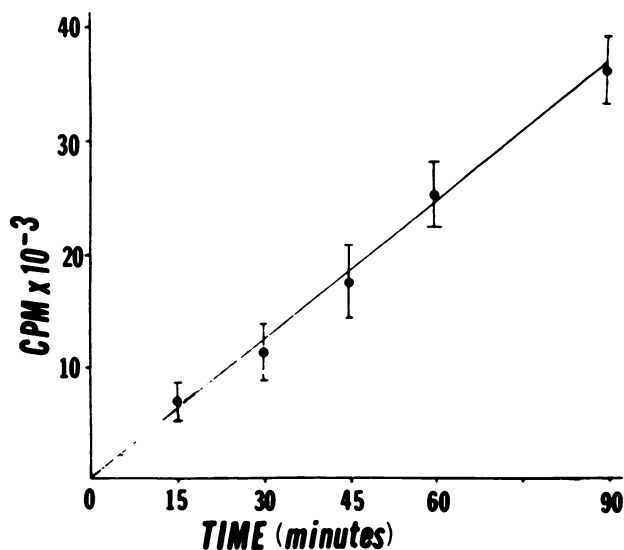Considerable alkaline pH optimal PLA$_2$ activity was observed in the whole homogenate of rat

Fig. 1. The time-linear relationship of PLA$_2$ activity of the rat testicular homogenate. The plotted line corresponds to the amount of ^{14}C-arachidonic acid released from the substrate by PLA$_2$ with respect to the time of incubation. Each bracketed point represents the mean ± standard error of five values.
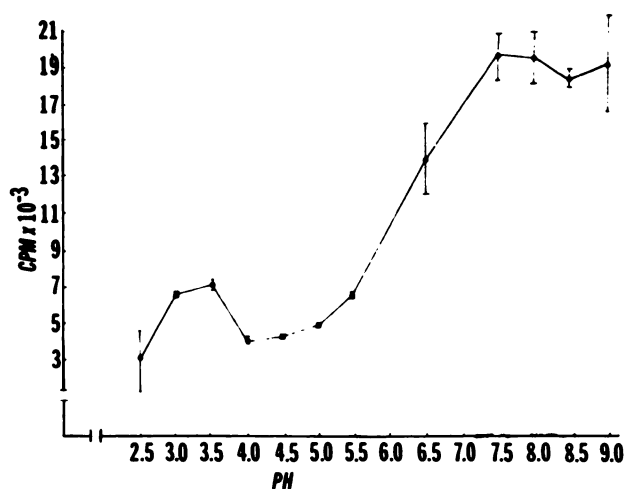
Fig. 2. The pH dependence of PLA_2 activity of rat testicular homogenate *in vitro*. The plotted line represents the amount of ^{14}C-arachidonic acid liberated from the substrate by PLA_2 with respect to pH. Each bracketed point represents the mean ± standard error of four values.

testes (Table 1). Interstitial cell homogenates contained more than three times as much acid as alkaline pH optimal PLA_2 activity. Teased tubular homogenates contained over twice as much alkaline pH optimal as acid pH optimal PLA_2 activity. Homogenates of tubular walls contained over three times as much alkaline pH optimal as acid pH optimal PLA_2 activity. Due to the small amount of material obtained, acid pH otpimal activity was not run on the plasma membrane-enriched fraction. The lysosomal-enriched fraction contained significantly more acid than alkaline pH optimal PLA_2 activity. The membrane-enriched

fraction also demonstrated high alkaline pH optimal PLA_2 activity.

When the data were corrected for the differences in amount of tissue present (per gram of tissue basis, Table 1) in an attempt to look at the relative specific activity of the enzyme, the lysosomes were observed to contain both more alkaline and more acid pH optimal PLA_2 activity than any of the other subcellular preparations. The interstitial cells were second in the amount of acid pH optimal activity that was exhibited by the various cellular fractions, but were relatively low in alkaline pH optimal activity. Alkaline pH optimal PLA_2 activity was concentrated in the teased tubules, with the germinal elements having a much higher activity than the tubular walls ($P \ll 0.001$).

Control of PLA_2 Activity

Alkaline pH optimal PLA_2 activity was approximately 4.4 times greater than that of PLA_1 in normal rat testes (Table 2). The fact that triton X-100 did not significantly increase PLA_2 activity at any tested concentration suggests that solubility of the substrate was not a limiting problem in the assay. Triton X-100 activated PLA_1 at 10^{-2} M, concomitant with a decrease in PLA_2 activity. At 10^{-3} M, PLA_1 was diminished, with the loss in activity disappearing as the concentration was decreased from 10^{-4} to 10^{-6} M. Only at the highest and lowest levels did triton X-100 significantly increase PLA_1 activity.

EGTA, a Ca^{++} chelator, diminished PLA_2 activity at 10^{-5} and 10^{-4} M concentration (Fig. 3). Ca^{++} at either 10^{-3} or 10^{-2} M reversed the inhibition ob-

TABLE 1. Phospholipase A_2 (PLA_2) Activity of Various Fractions of Rat Testicular Tissue *In Vitro*

Preparation	Amount of Tissue in Homogenate (g/ml)	No. of Samples	pH of Assay	PLA_2 Activity (cpm, mean ± SEM)	PLA_2 Activity (cpm/g tissue, mean ± SE)
Whole	0.41	6	7.5	15,089 ± 914	36,817 ± 2230
Interstitial cells	0.03	6	7.5	388 ± 69	13,867 ± 2466
Interstitial cells	0.03	6	3.5	1396 ± 261*	49,893 ± 9328*
Teased tubules	0.41	6	7.5	8658 ± 307	21,125 ± 749
Teased tubules	0.41	7	3.5	3820 ± 572	9321 ± 1396
Tubular walls	0.31	6	7.5	1814 ± 297	5859 ± 959
Tubular walls	0.31	7	3.5	459 ± 77*	1481 ± 248*
Germinal elements	0.18	5	7.5	4162 ± 191	23,140 ± 1061
Germinal elements	0.18	6	3.5	1208 ± 232*	6716 ± 1289*
Plasma membrane	0.43‡	10	7.5	6647 ± 376	15,488 ± 876
Lysosome	0.02	7	7.5	2146 ± 77	107,300 ± 3850
Lysosome	0.02	7	3.5	3349 ± 210*	167,450 ± 10,500*

* Probability values were computed for the mean immediately above where the value is placed and represent a value of $P < 0.01$.
† Plasma membrane preparation (1.5 ml) plus 3.5 ml of buffer to give 5.0 ml total homogenate.

TABLE 2. Alterations of Alkaline pH Optimum Phospholipase A_1 and A_2 (PLA_1, A_2) Activities of Rat Testicular Tissue by Triton X-100 *In Vitro*

Treatment	No. of Samples	PLA_1 Activity (cpm)	PLA_2 Activity (cpm)
Control	6	5746 ± 1060*	25,277 ± 3864
Triton X-100			
10^{-2} M	6	11,839 ± 486†	14,815 ± 529‡
10^{-3} M	6	1271 ± 139§	23,199 ± 1315§
10^{-4} M	6	5258 ± 212§	25,828 ± 367§
10^{-5} M	6	7390 ± 656§	30,540 ± 444§
10^{-6} M	6	9946 ± 540‡	29,467 ± 1813§

* Mean ± SEM.
† $P < 0.001$ when compared with the control groups.
‡ $P < 0.02$ when compared with the control groups.
§ $P < 0.001$ when compared with the 10^{-2} M group.

served with 10^{-4} M EGTA (Fig. 4). Testosterone stimulated PLA_2 activity at 10^{-9} through 10^{-7} M, but at 10^{-6} M the activity was diminished to essentially the control level (Fig. 5). Dihydrotestosterone increased PLA_2 activity at 10^{-9} M, attaining maximal activity at 10^{-7} M, (Fig. 6). At 10^{-6} M, there was a reduction in activity to essentially the control level. Corticosterone failed to modulate PLA_2 activity at concentrations ranging from 10^{-9} to 10^{-5} M (data not shown). Progesterone stimulated PLA_2 activity at concentra-

Fig. 4. Activation of rat testicular alkaline pH optimal PLA_2 activity by calcium. The bars represent the amount of ¹⁴C-arachidonic acid released from the substrate by PLA_2 activity in response to the treatments listed. Each bracketed bar represents the mean ± standard error of five values. The activity of the 10^{-4} M EGTA group was significantly lower ($P < 0.05$) than that of the control group. The 10^{-4} M EGTA groups containing 10^{-3} and 10^{-2} M $CaCl_2$ were significantly elevated ($P < 0.05$) when compared to the group containing only 10^{-4} M EGTA. The group containing 10^{-2} M $CaCl_2$ approached significance ($P < 0.10$) when compared to the control group.
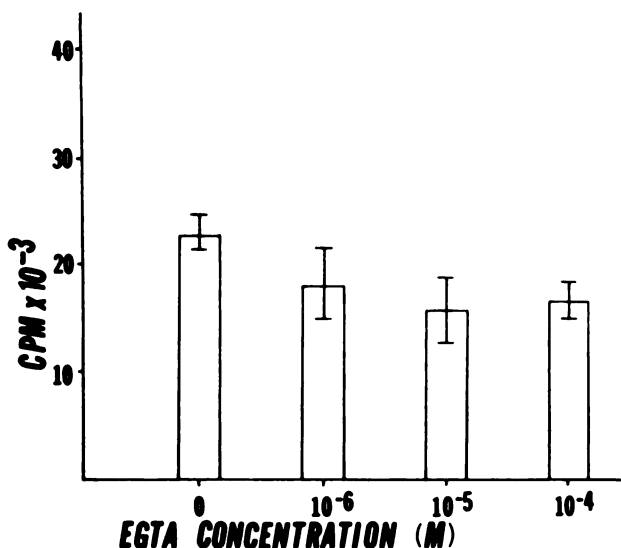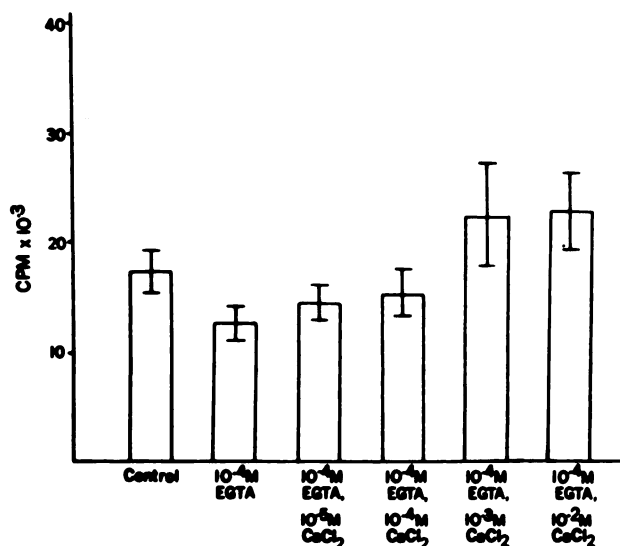
Fig. 3. Inhibition of rat testicular alkaline pH optimal PLA_2 activity by EGTA. The bars represent the amount of ¹⁴C-arachidonic acid released from the substrate by PLA_2 with respect to varying concentrations of EGTA. Each bracketed bar represents the mean ± standard error of five values. The 10^{-5} and 10^{-4} M groups were significantly lower than the control group ($P < 0.05$ and 0.025, respectively).
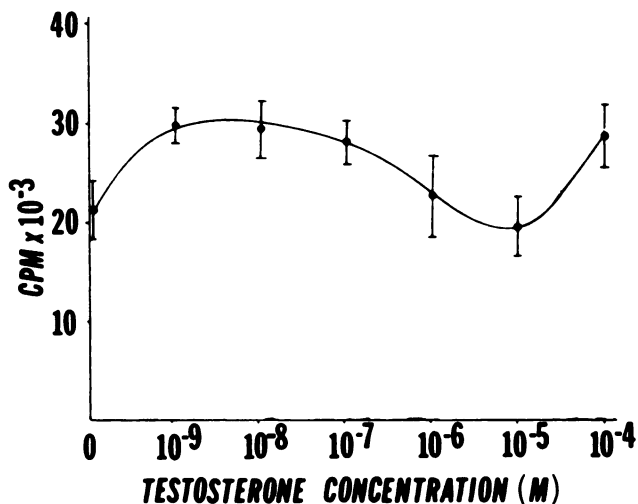
Fig. 5. Testosterone activation of rat testicular alkaline pH optimal PLA_2 activity. The plotted line represents the amount of ¹⁴C-arachidonic acid released from the substrate by PLA_2 activity with increasing concentrations of testosterone. Each bracketed point represents the mean ± standard error of six values. The 10^{-9} M group was significantly higher ($P < 0.05$) than the control group, whereas the 10^{-5} M group was significantly lower ($P < 0.05$) than the 10^{-7} M group. The 10^{-4} M group was significantly higher ($P < 0.05$) than the 10^{-5} M group.
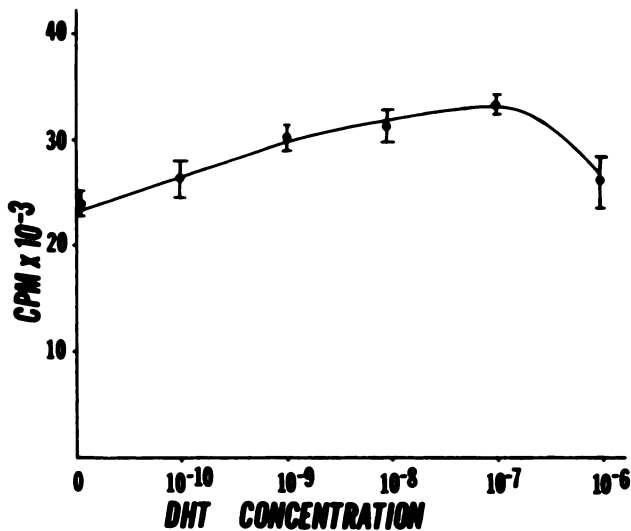
Fig. 6. 5-Dihydrotestosterone activation of rat testicular alkaline pH optimal PLA_2 activity. The plotted line corresponds to the amount of ^{14}C-arachidonic acid released from the substrate by PLA_2 activity in response to increasing concentrations of 5-dihydrotestosterone. Each bracketed point represents the mean ± standard error of six values. The 10^{-9} to 10^{-7} M groups were significantly higher ($P < 0.01$) than the control group. The 10^{-6} M group was significantly lower ($P < 0.025$) than the 10^{-7} M group.

Fig. 7. Activation of rat testicular alkaline pH optimal PLA_2 activity by progesterone. The plotted line corresponds to the amount of ^{14}C-arachidonic acid released from the substrate by PLA_2 activity in response to increasing concentrations of progesterone. Each bracketed point represents the mean ± standard error of six values. The 10^{-8} and 10^{-6} M groups were significantly higher ($P < 0.05$ and 0.01, respectively) than the control group. The 10^{-5} M group was significantly less ($P < 0.025$) than the 10^{-6} M group.

tions of 10^{-8} through 10^{-6} M, with maximal activity noted at the higher concentration (Fig. 7). PLA_2 activity was diminished at 10^{-5} M to essentially the control level.

Discussion

The first step in the synthesis of prostaglandins is the release of precursor unsaturated fatty acids, primarily arachidonic acid, from membrane phospholipids (Vogt et al, 1966; Lands and Samuelsson, 1968, Vonkeman and van Dorp, 1968). Our data show that testes contain both an alkaline and an acid pH optimal PLA_2 capable of releasing arachidonic acid from the sn-2 position of phosphatidyl choline. In this respect, Chaudhary (1976) reported both acid pH optimal and alkaline pH optimal PLA_1 and A_2 activities in rat testes, using 1-acyl-2[1-^{14}C]-oleolyl-sn-glycero-3-phosphocholine as the substrate.

An incubation temperature of 32.5 C, or scrotal temperature, was used in this investigation, since recent studies indicate that lysosomal enzymes are released above scrotal temperatures (Ellis, 1977), and this could markedly increase the acid pH optimal enzyme activity as measured in these experiments. Preliminary observations in this
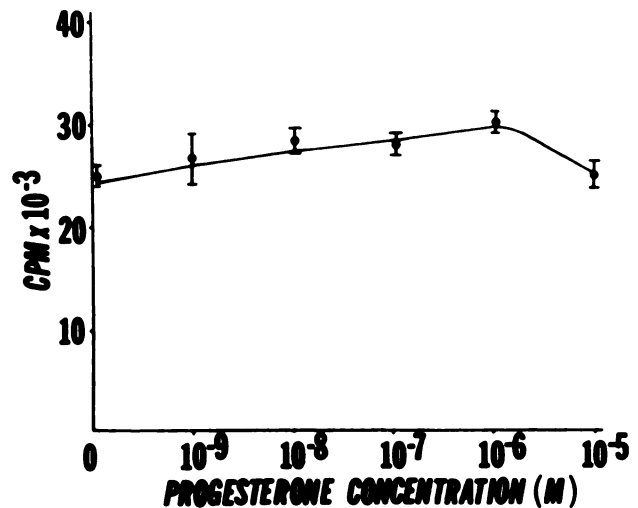
laboratory indicate that above-scrotal temperatures do increase rat testicular PLA_2 activity (unpublished data).

The decrease in PLA_2 by EGTA and the subsequent activation by Ca^{++} is consistent with the observations of other workers (Kunze et al, 1974) for PLA_2 activity in human seminal plasma. These workers reported that phosphatidylcholine was not a good substrate for PLA_2, but they used linoleic acid at the sn-2 acyl moiety instead of arachidonic acid. In this respect, sheep erythrocyte PLA_2 preferentially attacks uncharged phospholipids (phosphatidyl choline and phosphatidyl ethanolamine) in preference to phosphatidyl serine and releases long-chain unsaturated fatty acids in the sn-2 position (Jimeno-Abendano and Zahler, 1979), indicating that the substrate used by Kunze et al (1974) was not a preferred substrate for PLA_2.

Of importance is the observation that the interstitial cells contained primarily acid pH optimal PLA_2 activity, whereas the teased tubules and germinal cells contained more alkaline pH optimal activity. The higher acid pH optiaml PLA_2 activity in the interstitial cells could well be due to the lysosome, since this organelle is high in acid PLA_2 activity. The fact that the lysosomal fraction con-

tained more acid than alkaline pH optimal PLA_2 activity is consistent with observations of other workers for liver preparations (Mellors and Tappel, 1967; Rahman and Verhagen, 1969; Stoffel and Trabert, 1969). The high levels of membrane-bound alkaline pH optimal PLA_2 activity in the germ cells are consistent with the concept that a PLA_2 enzyme is needed for prostaglandin synthesis by the spermatozoa in the epididymis for the normal maturation of the germ cells as they pass through the epididymis (Johnson and Ellis, 1978; Ellis, 1977; Neil and Masters, 1972; Poulous et al, 1973; Poulous et al, 1975; Arora et al, 1975; Nishikawa and Wade, 1952). This indicates that PLA_2 activity in the germ cells arises during differentiation of the sperm cells in the testis. The fact that the potential for high PLA_2 activity is greater in the testis, where prostaglandin levels are low, points to the need for a mechanism for either a control of prostaglandin synthesis or a rapid metabolism of the prostaglandin in the testis. The activation of PLA_2 activity by testosterone and 5-dihydrotestosterone indicates a functional role for these two hormones with respect to PLA_2 activity and prostaglandin synthesis by the germ cells.

This conclusion is corroborated by independent observations (Farr and Ellis, 1980; Urry et al, 1977) that testosterone at physiologic concentrations ($10^{-9}-10^{-8}$ M), both *in vivo* and *in vitro*, stimulated seminiferous tubule contractions, whereas high concentrations (10^{-7} and 10^{-6} M) inhibited them (Farr and Ellis, 1980). Moreover, it has been found that 2,4'-dibromoacetophenone (a PLA inhibitor) inhibited seminiferous tubule contractions (Farr and Ellis, 1980) and that prostaglandins were needed for seminiferous tubule contractions *in vitro* (Buhrley and Ellis, 1975). Normally, prostaglandin synthesis by spermatozoa is low in the testis (Johnson and Ellis, 1978), where testosterone and dihydrotesterone would be high. Androgen binding protein (ABP) in the seminiferous tubules could serve the functional role of preventing the premature activation of prostaglandin synthesis by preventing the testosterone from activating spermatozoal PLA_2. Indeed, recent observations suggest that testosterone can be displaced from ABP by a Sertoli cell cytosol macromolecule (Rommerts et al, 1979), so that the androgen could react with Sertoli receptors once it enters the cell as a complex with ABP. Since ABP increases in the tubule in response to testosterone (Weddington et

al, 1976), this would further implicate this protein in a protective role in the male reproductive tract. This is important, since prostaglandins at high concentrations are deleterious to male fertility (Ericsson, 1973).

Recently, an androgen-sensitive PLA enzyme has been observed in the epididymis of castrate rats (Bjerve and Revitan, 1978), but this enzyme has been associated with the synthesis of glycerophosphocholine. Moreover, the enzyme remained high in the epididymides of animals receiving exogenous testosterone, a condition in which testosterone would prolong the life of residual spermatozoa in the epididymis. Thus, at least part of this enzyme could have originated from spermatozoa, as indicated by this investigation. The presence of both PLA_1 and PLA_2 in this investigation suggests that the spermatozoa could also contribute to the formation of glycerophosphocholine in the epididymis.

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