EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ ON THE REPRODUCTIVE SYSTEM OF THE MALE RAT

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Prostaglandins have been found to occur in the semen of man (see Samuelsson, 1963a, b) and ram (Bygdeman & Holmberg, 1966), in various male accessory sex organs (Euler, 1936; Bergström & Sjövall, 1960; Jouvenaz *et al.*, 1970) and in the testes of the rat (Carpenter & Wiseman, 1970) and boar (Michael, 1973), and may also be synthesized in the rat testis (Carpenter & Wiseman, 1970; Ellis *et al.*, 1972). Comparatively few studies, however, have been carried out to determine their effects, particularly on the seminiferous tubules. Ericsson (1972) administered very high doses of PGE_1 and PGE_2 (2.0 mg/kg body weight) subcutaneously to rats and obtained a marked inhibition of spermatogenesis. Since this was also accompanied by severe general body stress resulting in the death of several animals, it was not known how the effect on the tubules was caused. Memon (1973) injected rats intratesticularly with similar dose levels of $PGF_{2\alpha}$ and PGE_2 and reported a significant loss in testicular weight which again suggests that spermatogenesis was disrupted. Hargrove *et al.* (1971) immersed whole rabbit testes in PGE_1 at concentrations ranging from 3.6 nM to 360 nM and measured contractions using a sensitive myograph transducer. The rate of contractions decreased at 18 nM and was completely effaced at 360 nM. In a similar study with $PGF_{1\alpha}$, Johnson *et al.* (1971) reported that, in the range 3.6 to 60 nM, the rate of contractions doubled; from 71 to 213 nM, there was an increase in rate and tone but a decrease in amplitude. They attributed these effects to the testicular capsule and considered that they might be an important adjunct to the myoepithelial activity of the seminiferous tubules and hence to the propulsion of spermatozoa and fluid along the tubules.

The present work was carried out in order to obtain more information about the effect of $PGF_{2\alpha}$ on the testes of the rat, with particular reference to the tubules. In the first instance, it was decided to examine the effect of doses approximately intermediate between those used above and to apply the compound locally *in vivo*.

Male Wistar rats, aged between 10 and 12 weeks, were allocated to three groups (I, II and III) each consisting of at least eight animals. The animals were anaesthetized with an intraperitoneal injection of 0.2 ml Nembutal and a small incision (3 mm) was made in the right scrotum to the level of the tunica vaginalis. Prostaglandin $F_{2\alpha}$ -tromethamine salt ($PGF_{2\alpha}$), dissolved in sterile saline, was injected beneath the tunica vaginalis and hence over the right testis and epididymis of animals in Group I (50 μ g in 0.3 ml) and Group II (5 μ g in

0.3 ml). The rats in Group III were injected with saline (0.3 ml) only. The animals were killed 4 hr later and the right testes and epididymides were fixed in Bouin's fluid, embedded in paraffin wax and subsequently sectioned and stained.

Compared with the controls, the main effect appeared to be an increase in the number of immature germ cells seen within the lumen of the seminiferous tubules. In order to verify this, attention was focussed on the head of the testis (including the rete testis) and the caput epididymidis. Only in the rete testis of rats treated with prostaglandins were relatively large numbers of immature germ cells observed (Pl. 1, Fig. 1).

In none of the control animals were immature germ cells observed in the lumen of the caput epididymidis, but they were present in 9/14 animals of Group I and 6/8 in Group II. In Group I rats, the immature cells within the caput epididymidis were either spermatocytes in pachytene or young (incompletely differentiated) spermatids (Pl. 1, Fig. 2). In Group II rats, the latter type of cells predominated and more immature stages were rare (Pl. 1, Fig. 3). To compare the general effect in the two groups of rats, the area occupied by the various types of immature germ cells was measured and expressed as a proportion of the total luminal area of the duct per representative section from six sections/animal.

Almost half the total luminal area ($45.714 \pm 4.708\%$, mean $\pm$ S.E.) was occupied by immature cells in rats which received the higher dose of $\text{PGF}_{2\alpha}$ and between about 5 and 10% ($6.844 \pm 2.987\%$, mean $\pm$ S.E.) in those which received the lower dose.

It is well known that in normal rats a small proportion of germ cells apparently fail to develop normally and migrate to the lumen of the tubules where they are eventually sloughed. Under the experimental conditions of the above work, it appears likely that the prostaglandins promoted their release and progression along the tubules, into the rete testis and then into the caput epididymidis. The lowest dose affected immature cells lying closest to the lumen while the highest dose affected these cells and those lying deeper within the germinal epithelium.

It appeared to us that the above effect might be due to stimulation of processes normally involved in the release and transport of cells and fluid along the

EXPLANATION OF PLATE 1

FIG. 1. Various types of immature germ cells in the testis of a rat treated with a single dose of $50 \mu\text{g}$ $\text{PGF}_{2\alpha}$. $\times 400$.

FIG. 2. Various types of immature germ cells in the lumen of the caput epididymidis of a rat treated with a single dose of $50 \mu\text{g}$ $\text{PGF}_{2\alpha}$. $\times 400$.

FIG. 3. Young spermatids in the lumen of the caput epididymidis of a rat treated with a single dose of $5 \mu\text{g}$ $\text{PGF}_{2\alpha}$. $\times 400$.

FIG. 4. Part of a dissected seminiferous tubule in Tyrode's solution in which cells in the lumen were being actively propelled in the direction indicated by the white arrow. The lumen is constricted by a contraction of the boundary tissue (black arrow) which is at an oblique angle to the direction of movement. Note that cells of the germinal epithelium are also inclined towards the direction of the movement. $\times 200$.

FIG. 5. Photograph of an entire rat testis and one from which the contents have been removed in order to obtain an entire preparation of the tunica albuginea for eventual use with the transducer as described in the text.

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tubules. In this connection it has been variously suggested that the myoepithelial cells of the boundary tissue (Clermont, 1958; Leeson & Leeson, 1963; Niemi & Kormano, 1965; Suvanto & Kormano, 1970) and smooth muscle cells of the tunica albuginea (Langford & Davis, 1970; Hargrove *et al.*, 1971) might be involved. In order to study this further, an attempt was made to examine the contractile properties of isolated tubules *in vitro* with a transducer. The fragile nature of the tubules presented difficulties and so a small modification of the technique used by Suvanto & Kormano (1970) was employed. The same procedure was also used with strips of the tunica albuginea in order to compare the response of the two components under similar conditions. Briefly, small rectangular troughs of dental wax were constructed on the surface of glass slides. Opposite ends of the rectangle were ridged so as to provide two ledges below the upper level of the trough. The tissue was then suspended in the trough with

Table 1. Rate of individual contractions of the boundary tissue of seminiferous tubules of rat testis in different concentrations of $\text{PGF}_{2\alpha}$ *in vitro*

No. of tubules	Rate in Tyrode's solution only	Conc. $\text{PGF}_{2\alpha}$ ($\mu\text{g/ml}$)	Rate in $\text{PGF}_{2\alpha}$	Change in rate
3	6.78 ± 1.084	0.05	8.95 ± 0.846	$+1.82 \pm 0.240$
9	7.56 ± 0.504	0.05	7.6 ± 0.518	$+0.11 \pm 0.1552$
2	$5.93 \dagger$	0.5	7.72	$+1.79$
10	7.42 ± 0.569	0.5	5.84 ± 0.7751	-0.83 ± 0.3803
12	7.203 ± 0.404	5	10.75 ± 0.5889	$+3.72 \pm 0.431^*$
2	$8.41 \dagger$	5	7.035	-1.375
12	5.95 ± 0.301	50	10.68 ± 0.297	$+4.73 \pm 0.60^*$
2	$6.7 \dagger$	50	6.75	0.05

Values expressed as Means $\pm$ S.E.

* $P < 0.001$.

† Average values.

the two ends resting on the ledges where they were held in place with a small weight. The trough was then filled either with Tyrode's solution only or with the addition of $\text{PGF}_{2\alpha}$ at 33°C (scrotal temperature), a large cover-slip was placed on top and the tissue was studied under a microscope fitted with a stage incubator. Tubules that were devoid of interstitial tissue as well as those still covered by it were examined. In all cases, the same tubule (or strip of tunica) was first examined in Tyrode only and then in Tyrode containing $\text{PGF}_{2\alpha}$; a period of about 2 min was allowed to elapse for the tissue to become adjusted to changes in its environment.

In Tyrode's solution only, small contractions of the tubules were visible as local undulations of the wall (see Suvanto & Kormano, 1970). These undulations were generally accompanied by small oscillations of any cells within the lumen. Occasionally, and notably where spermatozoa were in the lumen, the contents refluxed and then moved in one direction only, a process which was repeated so that there was a definite flow from one end of the tubule to the other. In these circumstances, the distance between contractions on opposite sides of the wall was quite short so that the lumen was constricted at an angle to the

direction of movement (Pl. 1, Fig. 4). It seems possible, therefore, that when the contents of the lumen are being very actively moved in one direction, the contractions may be either oblique or spiral. This seems consistent with the almost lattice-like arrangement of the connective tissue fibres which are also present in the boundary tissue (Lacy & Rotblat, 1960).

To examine the effects of prostaglandins on the contractions of the boundary tissue, a particular region was identified in each tubule, e.g. by the presence of clumps of cells within the lumen. Undulations of the wall in this region, and on one side only, were then counted over a period of 2 min.

No difference was found in the rate of individual contractions of the boundary tissue in tubules with and without interstitial tissue. With $0.05 \mu\text{g PGF}_{2\alpha}/\text{ml}$ a small increase was obtained in 3/12 tubules examined; a similar result was obtained at a concentration of $0.5 \mu\text{g}/\text{ml}$ (Table 1). At $5 \mu\text{g}$ and $50 \mu\text{g}/\text{ml}$ the rate of contraction was significantly increased in 12/14 tubules ($P < 0.001$). Possibly due to the difficulties of accurately determining small changes in amplitude, a definite effect was only obtained at a dose of $250 \mu\text{g}/\text{ml}$ when the depth was increased between two to five times. In Tyrode's solution only and in the presence of $\text{PGF}_{2\alpha}$ at concentrations of $0.5 \mu\text{g}/\text{ml}$ to $250 \mu\text{g}/\text{ml}$, no contractions of the tunica albuginea were obtained.

In view of the results obtained by Johnson *et al.* (1971) on whole rabbit testis, two further experiments were carried out. In one of these, six entire testes (from six different rats) were used while in the second experiment the tunica albuginea only was used, the tissue being dissected as a whole from six further testes from an additional group of rats (see Pl. 1, Fig. 5). In all cases, the material was immersed in a bath and attached to an Isotonic Step-Wedge transducer set at 250 mmHg with the 'Damp' setting at range 2 and a recorder speed of 0.1 mm/sec. The work was carried out with the bath containing Tyrode's solution at 33°C or with the addition of $\text{PGF}_{2\alpha}$ at concentrations ranging from $0.005 \mu\text{g}/\text{ml}$ to $5 \mu\text{g}/\text{ml}$ which, in the case of the whole testes, was increased to $50 \mu\text{g}/\text{ml}$.

With the entire testes, no contractions were registered under any of the above conditions. With the tunica albuginea, no contractions were obtained at $0.005 \mu\text{g}/\text{ml}$ but, at $0.05 \mu\text{g}/\text{ml}$, a response was registered reaching a maximum after about 2 min; at $0.5 \mu\text{g}/\text{ml}$, a stronger response was obtained reaching a maximum after about 3.5 min; at $5 \mu\text{g}/\text{ml}$, the contractions were even more marked reaching a maximum after about 8 min (Text-fig. 1).

The concentration of $\text{PGF}_{2\alpha}$ which produced a measurable response in the isolated tunica albuginea (i.e. $0.05 \mu\text{g}/\text{ml}$) fell within the range of values of $\text{PGF}_{1\alpha}$ which Johnson *et al.* (1971) found to be effective in stimulating whole rabbit testes (approximately 0.0035 to $0.07 \mu\text{g}/\text{ml}$ or about 10 to 200 nM) using a very sensitive transducer. Since such relatively small amounts seem to be effective *in vitro*, it would appear that the sloughing of the cells in the tubules *in vivo* might be due to contractions of the tunica albuginea. A comparison of the results obtained with the tunica by the microscopical technique with those obtained with the transducer shows, however, that the latter procedure is much more sensitive, probably of the order of at least $\times 1000$. It could be argued, therefore, that since a positive result was obtained with the isolated tubules by

the microscopical technique but not with the tunica, the tubules may be much more sensitive than the tunica. It is also difficult to understand why even very high concentrations ($50 \mu\text{g/ml}$) of $\text{PGF}_{2\alpha}$ had no apparent effect on whole testes when the transducer was used. Thus, even though the isolated tunica may be highly sensitive, this may be misleading in terms of the effects observed *in vivo* within the seminiferous tubules. Carpenter (1974) has recently estimated

TEXT-FIG. 1. Response of entire testes (a and b) and isolated tunica albuginea (c to f) to $\text{PGF}_{2\alpha}$ at various concentrations. The vertical arrows indicate the time at which $\text{PGF}_{2\alpha}$ was introduced. No response is shown by the entire testis even at very high concentrations whereas the isolated tunica shows a response at $0.005 \mu\text{g/ml}$ and above. Each of the smallest divisions represents a movement of $1 \text{ mm}/10 \text{ sec}$ on the chart.

that the amount of $\text{PGF}_{2\alpha}$ in the testis of the adult rat is 0.03 to $0.16 \mu\text{g/g}$ which, taking into account the limitations of the microscopical technique, seems reasonably close to the concentration of $\text{PGF}_{2\alpha}$ which produced a response in the isolated tubules. However, it is apparent that, in order to clarify the results

obtained in the tubules as compared with the tunica, a much more sensitive procedure for studying the response of the tubules *in vitro* is needed and work on this problem is in progress.

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