Reduction of testicular blood flow and focal degeneration of tissue in the rat after administration of human chorionic gonadotrophin

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ABSTRACT

The effect of 100 IU human chorionic gonadotrophin (hCG) on testicular capillary blood flow was studied in adult male rats using a $^{133}$Xe clearance method and a radioactive microsphere technique. To investigate the role of Leydig cells in regulation of testicular blood flow after treatment with hCG, rats were pretreated with ethane dimethylsulphonate (EDS) which selectively destroys mature Leydig cells. Six hours after treatment with hCG, testicular blood flow decreased in control and hypophysectomized rats to 25–50% of normal values, but not in EDS-pretreated animals. Prostaglandin E$_2$ levels were also determined 6 h after an injection of hCG. A 300-fold increase in the concentration of prostaglandin E$_2$ occurred in normal testis tissue. This rise was markedly inhibited if EDS was given 3 days before administration of hCG. Furthermore, 6 h after administration of hCG, the filling of the testicular capillary bed with methylacrylate was decreased, while in control rats and rats treated with EDS and hCG, complete filling of the capillaries was seen. Cell degeneration in some subcapsular seminiferous tubules was observed 6–10 days after treatment with hCG.

The results suggest that the hCG-induced precapillary vasoconstriction, probably mediated (in part) by prostaglandins, causes reduction in testicular blood flow 6 h after administration of hCG, and may result in cell damage.


INTRODUCTION

The hormonal control of testicular blood flow in the rat has been studied by several investigators. Setchell & Sharpe (1981) described an increase in testicular blood flow 16 h after injection of 200 IU human chorionic gonadotrophin (hCG). An increase in testicular blood flow was also found 24 h after the administration of various doses of hCG (Punjabi, van Hoecke, Verdonck & Vermeulen, 1984) or after repeated injections of low doses of hCG (Daehlin, Damber, Selstam & Bergman, 1985). Damber, Bergh & Daehlin (1985) reported an increase in testicular blood flow 8 h after administration of hCG, while testis blood flow was found to be decreased 2 h after hCG treatment (Wang, Gu, Qian & Jing, 1984). After a 20-min constant-rate intra-arterial infusion of luteinizing hormone (LH), testis blood flow was found to be slightly increased (Damber & Janson, 1978). In the ovary, an increase in blood flow was observed within 20 min of injection of LH in the rat (Wurtman, 1964; Varga, Horvath, Folly & Stark, 1985) and rabbit (Janson, 1975). A decrease in capillary blood flow in the guinea-pig ovary occurs during luteal regression (Azmi & O'Shea, 1985). This reduction in blood flow was attributed to the vasoconstrictive prostaglandin F$_{2\alpha}$, which is known to have a luteolytic effect (Blatchley & Donovan, 1972; Horton & Poyser, 1976).

In the testis, prostaglandin F$_{2\alpha}$ and E$_2$ can be produced by Leydig cells. The concentrations of these compounds rise markedly 2–12 h after stimulation with hCG and, with isolated Leydig cells, production of prostaglandin F$_{2\alpha}$ and E$_2$ was maximal 6 h after
injection of hCG in vivo (Haour, Kouznetzova, Dray & Saez, 1979). Until now, no relationship between prostaglandin levels and testicular capillary blood flow after hCG treatment has been reported.

The possible roles of Leydig cells and prostaglandins in the regulation of the testicular blood flow were therefore studied.

**MATERIALS AND METHODS**

**Animals**

Adult male Wistar rats, weighing between 250 and 350 g, were used. They were kept in a controlled environment with water and food available ad libitum.

Human chorionic gonadotrophin (100 IU; Pregnyl; Organon, Oss, The Netherlands) was injected s.c. Clearance of $^{133}$Xe and radioactive microsphere measurements, the determination of the prostaglandin concentrations and the methylacrylate cast experiments were carried out 6 h after administration of hCG. One group of rats was hypophysectomized and used for the $^{133}$Xe clearance experiments 1 or 3 days later. Another group was hypophysectomized and used for the radioactive microsphere experiments 5 days later. Some of the rats were treated with ethane dimethylsulphonate (EDS; 30 mg/ml in dimethyl sulfoxide: $H_2$O, 1:3), administered in one i.p. injection at a dose of 75 mg/kg body weight, 3 days before the hCG injection in order to destroy Leydig cells selectively (Molenaar, de Rooij, Rommers et al. 1985).

**Clearance of $^{133}$Xenon**

The technique for intratesticular clearance of $^{133}$Xe was used to investigate the effect of hCG on testicular blood flow in 16 hCG-treated rats (nine intact and seven hypophysectomized) and in 11 control rats (eight intact and three hypophysectomized). The animals were anaesthetized with sodium pentobarbital (Nembutal; Ceva, Paris, France) in a dose of 60 mg/kg i.p. as a single injection.

About 1–300 µCi $^{133}$Xe (5–10 mCi/ml; Amersham International plc, Amersham, Bucks), dissolved in about 0·02 ml physiological saline, was injected through a small 25-gauge needle directly into the left testis. $^{133}$Xenon rapidly diffuses in the testicular parenchyma and is gradually removed by blood and lymph. The animals were kept supine with their heads in a Perspex container from which the expired air was withdrawn to prevent recirculation of the xenon. A collimated scintillation detector was placed directly over the testis and the remaining activity measured. Gamma-emission was measured over periods of 12 s, with 15 s between each measurement. The measured data were plotted semi-logarithmically from 0 to 15 min. Testicular flow was calculated using the formula: $F = 0·693/t_1 \times \tau \times 100$ (Thorburn, Kopald, Herd et al. 1963) in which $F$ is the flow in ml/100 g per min, $t_1$ is the half-time in min determined from the graph, and $\tau$ is the partition coefficient between tissue and blood (0·82 according to Fritjofsson, Persson & Pettersson, 1969).

**Microspheres**

The radioactive microsphere technique described by Heyman, Payne, Hoffman & Rudolf (1977) and Schamhardt, Verdouw, van der Hock & Saxena (1979) was used to estimate testicular blood flow in 15 hCG-treated (four intact, seven hypophysectomized and four hypophysectomized rats pretreated with EDS) and in 13 untreated rats (seven intact and six hypophysectomized rats). Microspheres with a diameter of 15 µm were injected directly into the left ventricle from where they were distributed throughout the body according to blood flow. The amount of trapped microspheres in the capillary bed within an organ is correlated to the blood flow through that organ.

**Prostaglandins**

Testes were stored at $-20^\circ$C for several days. Concentrations of prostaglandin $E_2$ were determined by radioimmunoassay as described previously by Zijlstra & Vincent (1984).

**Methylacrylate casts**

Six control rats, six hCG-treated rats, two EDS-treated rats and three hCG-treated rats pretreated with EDS were used to obtain casts of the arterial vascular bed of the testis. After the rats were anaesthetized with sodium pentobarbital, the ribs were cut from the xiphoid process on both sides of the sternum. Heparin (Tromboliquine; Organon Technika, Oss, The Netherlands; 2500 IU) was injected intracardially. A polythene catheter was introduced through a hole punctured in the left ventricle and pushed up into the aorta where it was fixed with a sling around the aorta. Perfusion was carried out through this catheter with 250 ml lukewarm (about $35^\circ$C) physiological saline containing heparin (50 IU/ml). The right atrium was opened for exsanguination and subsequent removal of erythrocytes. Coloured methylacrylate (Mercox; Japan Vilenote Company Ltd, Tokyo), which can pass through microvessels (Takayama & Tomoyoshi, 1981), was injected until it appeared in the right atrium. The specimens were stored for 24 h at $4^\circ$C. Thereafter, the testes with their spermatocords were extirpated and the soft tissue was dissolved by immersion in 20% KOH. Finally, the remaining vascular casts were washed in gently running warm ($50–55^\circ$C) water.
**Microscopic examination**

Eleven untreated animals (seven intact and four hypophysectomized) and 27 hCG-treated animals (sixteen intact and eleven hypophysectomized) were killed and the testes removed. The time interval between hCG administration and removal of the testes was 6–10 days. In three hypophysectomized rats pretreated with EDS, the testes were removed 1 day after hCG administration. Thirty-six rats which received daily injections of hCG for periods of 2–10 days were also studied. The testes of these animals were removed 24 h after the last injection. The medial parts of the testes were fixed in Bouin's solution, embedded in methylacrylate, sectioned at 5 μm and stained with haematoxylin–periodic acid Schiff. The percentage of tubular cross-sections with degeneration was determined.

**Statistics**

The results of the blood flow measurements were expressed as means ± s.e.m. and analysed for statistical significance using the Mann–Whitney U test. Differences were considered to be statistically significant if $P<0.05$. For evaluating the testicular degeneration percentages, Chi-square tests of goodness of fit were used.

**RESULTS**

**Clearance of $^{133}$Xenon**

Following intratesticular injection of $^{133}$Xe, the labelled gas diffuses in the testicular tissue and is gradually removed by flowing blood and lymph. A marked difference in shape was found between the $^{133}$Xe wash-out curves of control and hCG-treated rats (Text-Fig. 1). In the 11 untreated rats, a biphasic wash-out curve was seen with a rapid and a slow phase, while in all 16 hCG-treated rats the $^{133}$Xe wash-out took place in a single exponential manner. In the hCG-treated rats, determination of the halftime could be performed easily using the single exponential curves. In the control rats, different slopes could be determined in the biphasic curves. For calculation of the flow values in the control rats, the slope between 2.5 and 7.5 min was considered to be constant over the whole period of the experiment. A comparison between the values for testicular blood flow of control and hCG-treated rats revealed that testicular flow after hCG treatment was approximately 50% of the normal value (Table 1).

**Microspheres**

Results from the measurement of blood flow by the microsphere technique are shown in Table 2. Six
TABLE 1. Testicular blood flow estimated by the $^{133}$Xe clearance method 6 h after injection of 100 IU human chorionic gonadotrophin (hCG) in intact and hypophysectomized rats. Only the left testes were used. Values are given as means ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment of rats</th>
<th>Number of animals</th>
<th>Testicular blood flow (ml/100 g per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>14.7±1.63</td>
</tr>
<tr>
<td>hCG-treated</td>
<td>9</td>
<td>7.4±0.71*</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>3</td>
<td>14.0±0.55*</td>
</tr>
<tr>
<td>Hypophysectomized + hCG</td>
<td>7</td>
<td>6.1±0.86*</td>
</tr>
</tbody>
</table>

*P<0.05 compared with corresponding untreated group (Mann–Whitney U test).

TABLE 2. Testicular blood flow estimated by the radioactive microsphere technique 6 h after injection of 100 IU human chorionic gonadotrophin (hCG) in intact and hypophysectomized rats with or without pretreatment with ethane dimethylsulphonate (EDS). Both testes of each animal were used. Values are given as means ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment of rats</th>
<th>Number of animals</th>
<th>Testicular blood flow (ml/100 g per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>14.4±1.95</td>
</tr>
<tr>
<td>hCG-treated</td>
<td>4</td>
<td>3.3±0.16*</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>6</td>
<td>6.7±1.15*</td>
</tr>
<tr>
<td>Hypophysectomized + hCG</td>
<td>7</td>
<td>2.1±0.32*</td>
</tr>
<tr>
<td>Hypophysectomized + EDS + hCG</td>
<td>4</td>
<td>7.3±0.88*</td>
</tr>
</tbody>
</table>

*P<0.05 compared with corresponding untreated group (Mann–Whitney U test).

TABLE 3. Effect of administration of human chorionic gonadotrophin (hCG) on the concentration of prostaglandin E$_2$ in testicular tissue in normal rats and rats pretreated with ethane dimethylsulphonate (EDS). Both testes of each animal were used. Values are given as means ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment of rats</th>
<th>Number of animals</th>
<th>Prostaglandin E$_2$ (ng/testis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>2.3±0.10</td>
</tr>
<tr>
<td>hCG-treated</td>
<td>3</td>
<td>6.0±0.85</td>
</tr>
<tr>
<td>EDS-treated</td>
<td>3</td>
<td>3.7±0.32</td>
</tr>
<tr>
<td>EDS + hCG</td>
<td>3</td>
<td>21.2±5.74</td>
</tr>
</tbody>
</table>

Blood flow was measured by $^{133}$Xe clearance and radioactive microsphere technique. The absolute flow values obtained by the xenon method depend largely on the interpretation of the curve. However, when the methodology as proposed by Wax (1971) was used for calculation of absolute values, good correlations were obtained between the xenon and microsphere technique. Similar findings were reported by Damber & Janson (1977) who found good agreement between the testicular blood flow values obtained with the $^{133}$Xe clearance and the radioactive microsphere method (17.8±3.5 and 19.9±5.5 ml/100 g per min respectively). The biphasic xenon wash-out curve for the untreated rats has been described earlier for rats and dogs (Wax, 1971) and man (Fritjofsson, Persson & Pettersson, 1969). In normal rams, the xenon wash-out curves, as described by Setchell, Waites & Thorburn (1966) and van Vliet, de Ruiter-Bootsma, Oei et al. (1987), show similarities with those obtained from hCG-treated rats. There is no explanation for the differences observed in the shape of the wash-out curves in these species.

The marked reduction in blood flow after administration of hCG could be prevented completely when the rats were pretreated with EDS, which selectively destroys Leydig cells in mature rats (Molenaar et al.).

DISCUSSION

The results of the present investigation show that 6 h after one injection of 100 IU hCG, testicular blood flow decreased to 25–50% of the normal values in intact and hypophysectomized rats.

This indicates that Leydig cells stimulated by high doses of hCG can regulate testicular blood flow. It is not known whether Leydig cells also play an important role in the regulation of vascular properties under normal physiological conditions. They do not appear to be essential for the maintenance of blood flow since no changes in blood flow were observed after destruction of Leydig cells with EDS.

In hypophysectomized rats without treatment with hCG, a reduction of blood flow was observed when the microsphere technique was applied but this was not found with the xenon technique. This discrepancy could not be explained. In the literature there are no indications that the absence of pituitary hormones leads to major changes in testicular blood flow. Csernay, Laszlo & Kovacs (1968) found no changes in testicular blood flow at least 1 month after hypophysectomy.

In the present investigation, the inhibitory effect of hCG on testicular blood flow in hypophysectomized rats was the same as in intact animals. The inhibitory effects of hCG on blood flow in intact and hypophysectomized rats were also demonstrated with the methacrylate casts. The absence of a capillary bed in the methacrylate casts obtained from hCG-treated rats indicates that the diameter of the capillaries had decreased to such an extent that the methacrylate could not penetrate. The decrease in diameter of the capillaries could explain the decrease in testicular flow.

The decrease in testicular blood flow after hCG administration occurred at about the same time as capillary permeability increased. This hCG-induced change in vascular properties results in increased interstitial fluid formation as has been reported by various investigators (Setchell & Sharpe, 1981; Sharpe, 1984; Damber et al. 1985; Bergh, Widmark, Damber & Cajander, 1986; Widmark, Damber & Bergh, 1986). This effect could be mediated by intratesticular prostaglandin E2 which rises markedly after hCG administration and which can be produced by Leydig cells as shown by Haour et al. (1979). Furthermore, we found that following destruction of Leydig cells by EDS, the prostaglandin response to hCG was greatly diminished and no change in blood flow was observed. Similarly, Setchell & Rommerts (1985) found that the effects of hCG on testicular permeability were absent after treatment with EDS. Although our data suggest that prostaglandins could play an important role in the regulation of the vascular system, the effects of other compounds have not been measured and pertinent conclusions cannot therefore be drawn.

Moreover, in the literature there is disagreement about the role of prostaglandins in connection with testicular blood flow. It has been reported that prostaglandins can reduce testicular blood flow immediately after injection in the testicular artery (Free & Jaffe, 1972; Einer-Jensen & Soofi, 1974). On the other hand, a direct role of prostaglandins in the increase in interstitial fluid formation after injection of hCG was doubted by Veijola & Rajaniemi (1985) and Sowerbutts, Jarvis & Setchell (1986), as they found that indomethacin, a prostaglandin synthesis inhibitor, had no effect on the increase in vascular permeability after hCG treatment. However, it may be possible that the hormone-stimulated prostaglandin synthesis was only partly inhibited. Our observation that hCG treatment reduced testicular blood flow is supported by the preliminary results of J.-E. Damber (personal communication) who showed that 6 h after administration of LH, a significant decrease in testicular blood flow coincided with an increase in the volume of interstitial fluid.

### Table 4. Percentages of tubular cross-sections with testicular degeneration after one or daily (for 2–10 days) injections of 100 IU human chorionic gonadotrophin (hCG) to normal and hypophysectomized rats with or without pretreatment with ethane dimethanesulphonate (EDS). Values are given as means ± s.e.m.; the range is given in parentheses

<table>
<thead>
<tr>
<th>Treatment of rats</th>
<th>Number of animals</th>
<th>Number of tests examined</th>
<th>% of tubular cross-sections with degeneration</th>
<th>Number of tests with degeneration</th>
<th>Average number of tubules examined per testis with degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>hCG-treated</td>
<td>16</td>
<td>30</td>
<td>2.5 ± 1.13 (0–3.04)</td>
<td>8</td>
<td>526</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>11</td>
<td>18</td>
<td>28.3 ± 6.94 (0–88.9)</td>
<td>13</td>
<td>627</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>+ EDS + hCG</td>
<td>36</td>
<td>36</td>
<td>4.1 ± 1.38 (0–32.3)</td>
<td>11</td>
<td>507</td>
</tr>
<tr>
<td>Daily injections</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2–10) of hCG</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
The microscopic examination of testis tissue revealed focal necrosis of subcapsular seminiferous tubules in hCG-treated rats, while none of the controls showed any tubular damage. In hypophysectomized rats this effect of hCG was more pronounced than in intact rats. Daily injections of hCG did not further increase the damage. The damaged tissue was always located in the region just beneath the testicular capsule. This can be explained by the particular vascularization of the testis, in which the peripheral region is supplied last by recurrent branches of the centripetal arteries (Hundeiker, 1971). Degenerative changes of seminiferous tubules in testes of intact rats within 24 h after treatment with an LH-releasing hormone (LHRH) agonist have been described earlier by Habenicht, El Etreby & Neumann (1985). They ascribed this tubular damage to a direct vascular effect of the LHRH agonist. However, since the present study indicates that Leydig cells are the mediators in the reduction of blood flow, the action of LHRH may be effected through Leydig cells either directly or indirectly via pituitary LH. Indications of damaged testicular tissue were also reported by Sandow, von Reichenberg, Engelbart et al. (1985) 24 h after hCG treatment, and in hypophysectomized rats by Kerr & Sharpe (1986) after daily treatment with LH alone or in combination with an LHRH agonist. These data and those of the present study show that a hormone-induced transient reduction in blood flow can be so severe that focal degeneration of testicular cells can occur.

Further investigations are necessary to explain the mechanisms involved and the relevance of these LH/hCG effects for the regulation of blood flow under physiological conditions.

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The authors are grateful to Dr H. Y. Oei and Mr A. Hoekstra of the Department of Nuclear Medicine, University Hospital Utrecht for their advice and help with the xenon experiments. Many thanks are also due to Mr F. Zijlstra for the determination of the prostaglandin levels, to Mr J. Heiligers for carrying out the microsphere measurements, to Mrs A. de Wildt-Wernik for technical assistance, to Mr M. K. Niekerk and Mr H. Otter for preparing the illustrations and to Mrs L. Michielsen for typing the manuscript.

REFERENCES


Sowerbutts, S. F., Jarvis, L. G. & Setchell, B. P. (1986). The increase in testicular vascular permeability induced by human chorionic gonadotrophin involves 5-hydroxytryptamine and possibly oestrogens, but not testosterone, prostaglandins, histamine or bradykinin. *Australian Journal of Experimental Biology and Medical Science* 64, 137–147.


DESCRIPTION OF PLATES

Plate 1
Methylacrylate casts of the arterial vasculature of (left) control rat testis and (right) testis after treatment with human chorionic gonadotrophin (hCG). Note the complete lack of methylacrylate in the capillaries of the testis of the hCG-treated rat.

Plate 2

FIGURE 1. Section of a testis of an intact rat 10 days after injection of 100 IU hCG. Degeneration of the seminiferous epithelium can be observed in the area between the tunica albuginea and the black dots. (× 30.)

FIGURE 2. Cross-sections of seminiferous tubules of an intact rat 10 days after injection of 100 IU hCG. In some tubules (marked by asterisks) only Sertoli cells are present. In other tubules, only part of the seminiferous epithelium has disappeared (arrows). (× 100.)

FIGURE 3. Severe degeneration of testicular tissue in the testis of a hypophysectomized rat 6 days after injection of 100 IU hCG. To the right of the figure, Sertoli cells and some germ cells can still be seen in some of the tubules. In the middle of the figure, some tubules are devoid of both germ cells and Sertoli cells but the interstitial tissue appears normal, while interstitial tissue is also degenerating in the left of the figure. (× 100.)