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Terbutaline treatment inhibits the hCG-induced increase in venular permeability in the rat testis

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Summary. Treatment with hCG results in an increase in venular permeability in the rat testis. This change in vascular permeability can be detected by the carbon-labelling technique, by measurement of the volume of interstitial fluid and by quantification of the leucocyte migration into the interstitial space. Carbon-labelling, interstitial fluid volume and leucocyte migration were all reduced in rats treated with hCG + terbutaline compared to the values in animals given hCG only. However, terbutaline treatment did not influence the hCG-induced increase in testosterone secretion. These observations suggest that the hCG-induced increase in vascular permeability in the testis can be reduced by a β-adrenergic agonist.

Introduction

Blood flow and vascular permeability are of importance for testicular function but the mechanisms regulating these aspects of testicular physiology are largely unknown. Treatment of adult rats with hCG causes increases in vascular permeability and in testicular interstitial fluid volume (Sharpe, 1984). The increase in interstitial fluid is preceded by intravascular accumulation of polymorphonuclear leucocytes (PMNLs) and is accompanied by the migration of PMNLs into the interstitial space (Bergh *et al.*, 1986). Interendothelial cell junctions in post-capillary venules are opened, and through this route macromolecules leak into the interstitial space (Bergh *et al.*, 1987). Treatment with hCG also results in dilatation of precapillary sphincters, probably raising the hydrostatic pressure in venules (Damber *et al.*, 1986; Widmark *et al.*, 1986). These hCG-induced microvascular changes in the testis are very similar to those that occur in acute inflammation (Williams, 1985) and we have suggested that they could be mediated by similar mechanisms (Bergh *et al.*, 1987; Bergh & Damber, 1987). PMNLs evidently play a crucial role, since the hCG-induced increase in venular permeability does not occur in leukopenic rats (Bergh & Damber, 1987).

The physiological role of the hCG-induced changes in testicular microcirculation is, however, unknown. To study this further we have attempted to find a drug that inhibits the hCG-induced increase in permeability. Venular permeability is inhibited by beta stimulatory drugs such as terbutaline in various models of experimental inflammation by direct effects on endothelial cells (Northover & Northover, 1969; for reviews, see Svensjö & Roempke, 1984; Persson & Svensjö, 1985). We have therefore investigated the influence of terbutaline on the hCG-induced increase in venular permeability in the rat testis.

Materials and Methods

Adult male Sprague–Dawley rats (300–350 g) were injected subcutaneously (s.c.) with 50 i.u. hCG (Pregnyl: Organon, Oss, The Netherlands) alone or in combination with 0·3 mg terbutaline/kg body weight (Bricanyl: Draco, Lund, Sweden). Control rats were injected with saline (9 g NaCl/l) or with terbutaline (0·3 mg/kg).

One group of rats was killed 4 h after treatment, and the testes were removed. One testis was placed in 10 ml ethanol and the other was fixed in Bouin's solution. Testicular testosterone concentration was determined in the

ethanol extract using a radioimmunoassay as previously described (Damber & Janson, 1978; Damber & Bergh, 1980). The sensivity of the assay was 10 pg/tube. The intra- and interassay coefficients of variation were 9% and 11%, respectively. The fixed testes were dehydrated and embedded in methacrylate plastic (Histo-Resin: LKB, Stockholm, Sweden). The volume density of PMNLs in testicular blood vessels and in the interstitial space was determined in 2 μm thick sections stained with haematoxylin and eosin, using morphometric techniques as described previously (Bergh *et al.*, 1986).

At 4 h after treatment another group of rats was anaesthetized with pentobarbitone sodium (40 mg/kg body weight) and the tail artery was cannulated. A solution of colloidal carbon (Pelikan Drawing Ink: Pelikan Werke, Hannover, West Germany; 1 ml/kg body weight) was injected intra-arterially and, after 1 h, the testes were removed and fixed by immersion in 4% formaldehyde, 3% glutaraldehyde and 0·05% picric acid in 0·1 M-cacodylate buffer (for details see Bergh *et al.*, 1987). By this technique, leaking blood vessels are stained by carbon particles (the carbon particles penetrate open endothelial cell gaps, but are stopped by the underlying basement membrane). This technique has been used extensively in studies on inflammation (Cotran & Majno, 1964) and it can also be used to label leaking blood vessels in the testis after hCG treatment (Bergh *et al.*, 1987). Carbon-stained vascular segments could be seen when the fixed testes were studied under a dissection microscope (at × 50 magnification). Randomly chosen areas of the testicular surface were photographed and the proportion of small blood vessels stained with carbon was measured (length of carbon-stained vessels/total length of vessels), using an MOP-Video plan image analyser (Kontron, Munich, West Germany). Several blood vessel segments (diameter < 75 μm) with a total length of 10 000 μm were examined per testis. Parts of the testicular tissue were then post-fixed in 1% OsO_4 and embedded in Epon. The morphology of testicular blood vessels was studied in 1 μm thick sections using a light microscope and in 80 nm thick sections using a Philips 300 electronmicroscope (for details see Bergh *et al.*, 1987).

Treatment with hCG does not result in an increase in interstitial fluid volume until 6–8 h after treatment (Sharpe, 1984) and the anti-permeability effect of a single injection of terbutaline is rather short (Svensjö & Roempke, 1984). The effect of terbutaline on hCG-induced increase in interstitial fluid volume was therefore tested by a s.c. injection of terbutaline (0·3 mg/kg body weight) 3 h after treatment with 50 i.u. hCG s.c. The interstitial fluid volume was measured 3 h later using the method described by Sharpe & Cooper (1983) and Widmark *et al.* (1986) and the results were compared to those in rats given only hCG (6 h before experiment) or only terbutaline (3 h before experiment).

Results

Testosterone secretion

The testicular testosterone concentration was increased 4 h after hCG treatment but addition of terbutaline did not influence the testosterone response. Terbutaline treatment alone did not influence testicular testosterone concentration compared to saline (Table 1).

Table 1. Testis weight, leucocyte concentration and testicular testosterone in rats treated with hCG, hCG + terbutaline or terbutaline alone

	Control (N = 5)	Terbutaline (N = 5)	hCG + terbutaline (N = 6)	hCG (N = 5)
Testicular wt (g)	1·76 ± 0·04	1·89 ± 0·06	1·71 ± 0·04	1·75 ± 0·04
Volume density of PMNLs in testicular blood vessels (% of testis volume)	0·68 ± 0·08	0·63 ± 0·05	3·0 ± 0·7	5·7 ± 0·3
Volume density of PMNLs in the interstitial space ($\times 10^{-5}$)	0	0	0·1 ± 0·1†	2·6 ± 0·9*
Testicular testosterone (ng/testis)	156·0 ± 10·8	146·6 ± 29·7	753·0 ± 48·3*	576·6 ± 18·5*

Values are mean ± s.e.m.
*Significantly larger than basal value, $P < 0·05$ (Mann–Whitney U-test).
†Significantly lower than after treatment with hCG only, $P < 0·05$.

Leucocyte accumulation

At 4 h after hCG treatment there was a 6-fold increase in the volume density of PMNLs in testicular blood vessels and some leucocytes were observed in the interstitial space (Table 1; Fig. 1). In control rats, treated with saline or with terbutaline alone, interstitial leucocytes were not

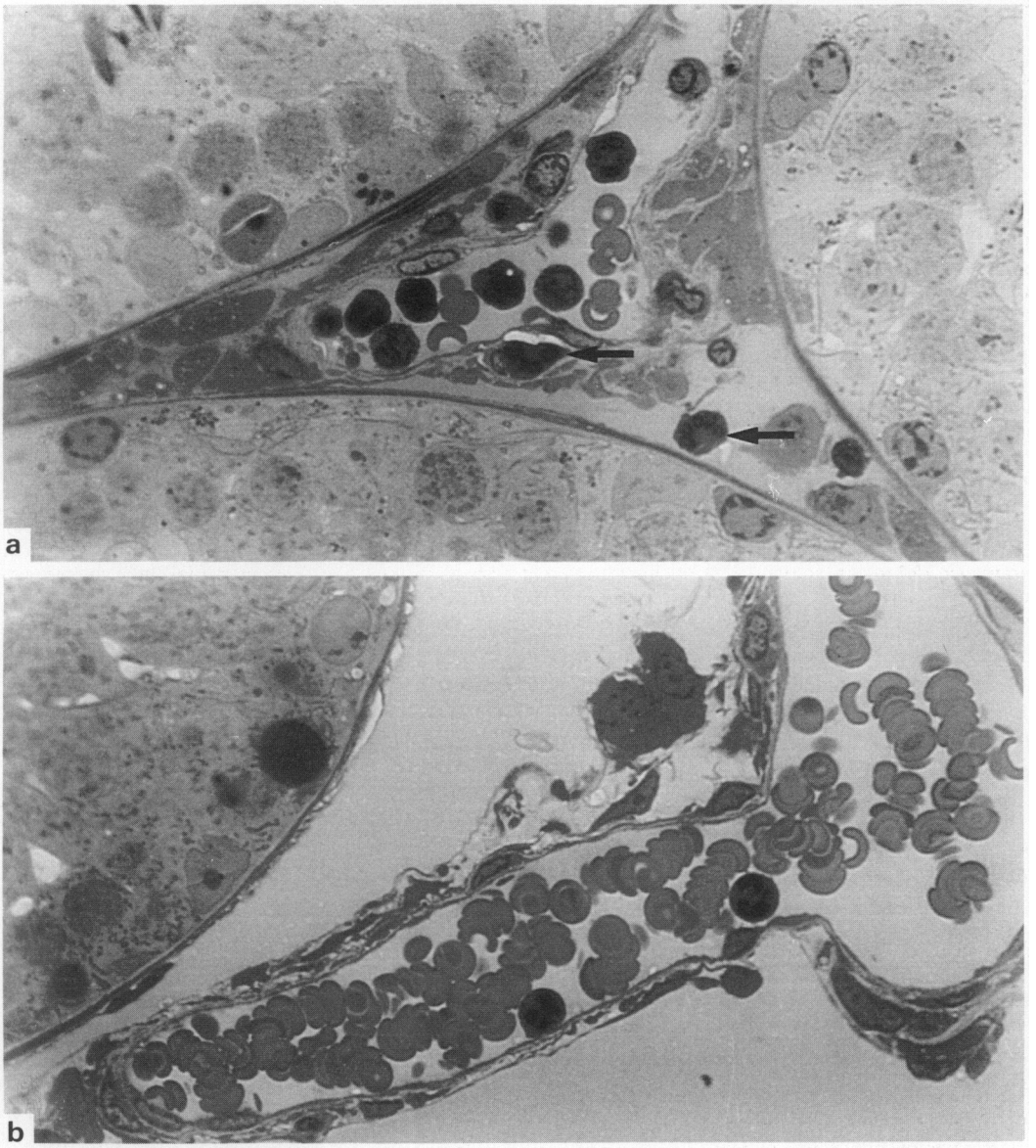

Fig. 1. Light micrographs of testicular sections 4 h after treatment with (a) hCG and (b) hCG + terbutaline. In the hCG-treated animal, a PMNL is observed migrating through the venular wall (arrow). Another PMNL is observed in the interstitial space (arrow). In the hCG + terbutaline-treated animal, PMNLs are seen in the blood vessels, but migration into the interstitium was not observed. × 1000.

observed. Terbutaline treatment did not affect the intravascular concentration of PMNLs compared to saline treatment (Table 1). In rats treated with hCG plus terbutaline, the volume density of intravascular leucocytes was increased like that after treatment with hCG alone. In the hCG + terbutaline group migration of leucocytes into the interstitial space was not observed in 5 of 6 of the testes examined and very few migrating PMNLs were observed in the remaining testis (Table 1; Fig. 1).

Carbon labelling

Using a dissection microscope, carbon-labelled venules could be observed under the testicular capsule in hCG-treated animals. Morphological examination in the light and the electron microscope revealed that the black staining was caused by subendothelial deposits of carbon and only post-capillary venules were labelled. Adhering and migrating PMNLs were often observed in these venular segments (as earlier described in detail, see Bergh *et al.*, 1987; and Fig. 1). In rats given hCG plus terbutaline, carbon labelling was only occasionally observed in the dissection microscope and very few carbon-stained venules were observed in the tissue sections (see below and Fig. 1). In saline- or terbutaline-treated animals, carbon deposits were never observed.

When measured in the dissection microscope, 6·4 ± 0·4% (mean ± s.e.m., 6 testes for each group) of the total length of small vessels was stained by carbon in hCG-treated animals, but only 0·30 ± 0·16% was stained in hCG + terbutaline-treated animals. In control animals (saline or terbutaline), carbon-stained vessels could not be observed in the dissection microscope. Randomly chosen cross-sections of post-capillary venules (diameter 10–75 μm) were examined for the presence of subendothelial carbon deposits using the light microscope at ×1000 magnification: in hCG-treated animals, 17 ± 3% (mean ± s.e.m.) of the 50 venular sections were stained but in hCG + terbutaline-treated rats, none of the 50 venules examined was stained.

Interstitial fluid volume

Testicular interstitial fluid volume was significantly larger than in controls 6 h after hCG treatment (105·4 ± 6·4 *vs* 76·6 ± 5·6 μl/g testis, mean ± s.e.m., $n = 5$–6; $P < 0·05$, Mann–Whitney U-test). In rats treated with hCG + terbutaline interstitial fluid volume was significantly lower than after treatment with hCG (77·3 ± 5·0 *vs* 105·4 ± 6·4 μl/g testis, n = 5–6, $P < 0·05$). Terbutaline treatment alone did not influence interstitial fluid volume compared to controls (80·7 ± 8·1 *vs* 76·6 ± 5·6 μl/g testis, $n = 5$ respectively).

Discussion

Leucocytes accumulate in testicular blood vessels 4 h after hCG treatment. They adhere to the endothelium in post-capillary venules and migrate to the interstitium through open interendothelial cell junctions. Simultaneously, the venular permeability for macromolecules is increased (Bergh *et al.*, 1986, 1987). The mechanisms mediating these inflammation-like changes in testicular microcirculation are unknown, but leucocytes apparently play a crucial role since the hCG-induced increase in venular permeability and interstitial fluid volume does not occur in leukopenic rats (Bergh & Damber, 1987). It is not known why leucocytes accumulate and adhere to the endothelium after hCG treatment, but one possibility is that the testis secretes a leucotactic factor in response to hCG.

Adhering leucocytes, by mechanisms not fully understood, are able to open inter-endothelial cell junctions (Williams, 1985). In this way, leucocytes play an important role in regulating the supply of macromolecules to the inflamed tissue (Williams, 1985). In various types of experimental inflammation, the formation of endothelial cell gaps in post-capillary venules (which is partly caused by endothelial cell contraction; Majno *et al.*, 1967), can be blocked by β-adrenergic agonists such as terbutaline (in the same dose as in the present study), probably by direct action on the endothelial cell (for references see Svensjö & Roempke, 1984; Persson & Svensjö, 1985). Consequently, beta agonists, despite their vasodilatory effect, reduce inflammation-induced tissue oedema (Persson & Svensjö, 1985). The present observation that the hCG-induced venule leakage in the testis was reduced by terbutaline (monitored by decreased carbon-labelling, interstitial fluid volume and leucocyte migration) can probably be explained by the antipermeability effect of this drug. Leydig cells have β-receptors, and β-adrenergic stimulants are known to increase testosterone secretion *in vitro* (Cooke *et al.*, 1982) and *in vivo* (Eik-Nes, 1969). It is, however, not very likely that

the anti-permeability effect observed in the present study is mediated by action on the Leydig cells, since neither Leydig cell testosterone secretion nor the secretion of the factor attracting leucocytes to testicular blood vessels appeared to be influenced by terbutaline treatment. Moreover, if Leydig cells are affected by terbutaline, a stimulatory effect is likely (see above) and this should increase rather than reduce permeability, since stimulation of Leydig cells by hCG, LH or LHRH increases interstitial fluid volume whereas inhibition with anti-LH serum decreases interstitial fluid volume (Sharpe, 1984).

The physiological role of the hCG-induced changes in testicular microcirculation are unknown. Previous studies indicate that they may occur only during maximal hormonal stimulation. Doses of hCG giving a submaximal testosterone response neither increase interstitial fluid volume (Widmark *et al.*, 1986) nor result in testicular leucocyte accumulation (Bergh *et al.*, 1986). The present observation that the hCG-induced increase in venular permeability can be reduced by terbutaline, without apparent effects on Leydig cell function, suggests that this drug may be used in experiments designed to elucidate the functional significance of hCG-induced changes in vascular permeability.

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