HCG Treatment Increases Intratesticular Pressure in the Abdominal Testis of Unilaterally Cryptorchid Rats

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Adult, unilaterally cryptorchid rats were given a single subcutaneous injection of hCG. HCG treatment of 100 I.U. (but not 10 I.U.) resulted in a marked increase in intratesticular pressure (approximately 40 mm Hg) in the abdominal testis that was maximal 24 hours after treatment. This increase in pressure is caused by increased vascular permeability coupled with insufficient lymph drainage. In the scrotal testis, hCG treatment resulted in increased vascular permeability and lymph flow, but this did not result in a marked increase in testicular pressure. No morphologic signs of hCG-induced damage were observed in either the abdominal or scrotal testis 10 days after hCG treatment. Testicular microcirculation, as studied by laser doppler flowmetry, was abnormal in the abdominal testis, but hCG treatment inhibited vasomotion in both the abdominal and scrotal testis.

Key words: testis, cryptorchidism, hCG, vascular permeability.

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Experimental cryptorchidism results in morphologic and functional changes in all the main cell types in the testis. Germ cells degenerate (van Demark and Free, 1970), Sertoli cell function (Hagenäs et al, 1978; Au et al, 1983; Bergh et al, 1984) and morphology (Kerr et al, 1979a; Bergh, 1981) are altered, and Leydig cell testosterone secretion is decreased *in vivo* (Bergh and Damber, 1978; Kerr et al, 1979b; Risbridger et al, 1981; de Kretser, 1982, Damber et al, 1985). The effect on Leydig cell morphology is more controversial, since both decreases (Bergh and Damber, 1978) and increases (Risbridger et al, 1981) in Leydig cell size have been reported.

Recent studies indicate that testicular blood flow and vascular permeability are influenced by experimental cryptorchidism (Sharpe, 1984, Damber et al, 1985). Morphologic changes in the blood vessels have also been described in the congenitally ectopic rat testis (Chung et al, 1984) and in human cryptorchidism (Francavilla et al, 1979). Vascular permeability is subnormal in the abdominal testis (Sharpe, 1984, Damber et al, 1985), but is markedly increased after hCG treatment (Sharpe, 1984, Damber et al, 1985). HCG treatment results in the doubling of testicular blood flow in the scrotal testis, while there is no

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increase in the unilateral abdominal testis (Damber et al, 1985). However, the mechanisms behind these vascular changes in the abdominal testis and the subsequent effects on tubular and Leydig cell function are not known. The present study was performed to study further the vascular effects of hCG treatment in the unilaterally cryptorchid rat. Such studies are of clinical importance, since hCG treatment is one of the current methods used in the treatment of cryptorchid boys.

Materials and Methods

Testicular descent was prevented unilaterally in newborn Sprague-Dawley rats by cutting the gubernaculum testis as described previously (Bergh et al, 1978). The rats were raised under normal laboratory conditions and used in experiments when adult (400 to 500 g body weight). The animals were injected subcutaneously with 100 I.U. human chorionic gonadotropin (hCG, Pregnyl, Organon, Oss, The Netherlands). After 0, 8, 24, 48, or 240 hours the rats were sedated with Mebumal (40 mg/kg), the abdominal cavity was opened and testicular pressure was measured using a Schiotz tonometer, an instrument designed to estimate intra-occular pressure in humans. In principal, a metal plunger is placed on the surface of the object under study, resulting in an indentation in the surface. The degree of this indentation, which is related to organ pressure, is quantified. Measurements have an accuracy of approximately 3 mm Hg and pressures below 7 mm Hg fall outside of the reference range (Kolker and Hetherington, 1976). Similar tonometers have been used to estimate intratesticular pressure in bulls and humans (Hahn et al, 1969, Lewis et al, 1985).

After estimating intratesticular pressure, each testis was removed, weighed and fixed in Bouin's solution. The testicular tissue was embedded in metacrylateplastic (Histo-Resin, LKB, Stockholm) and its morphology was studied on 2-μm thick sections stained with hematoxylin and eosin. During the course of this study, we observed that hCG treatment (100 I.U.) resulted in a marked increase in intratesticular pressure in the abdominal testis. To determine whether such increases were related to this particular type of experimental cryptorchidism, adult rats (300 to 400 g body weight) were sedated with Mebumal and one testis was moved to the abdomen of each rat and sutured to the dorso-lateral abdominal wall as described earlier (Bergh and Damber, 1984). After 8 weeks, these unilaterally cryptorchid rats were stimulated with 100 I.U. hCG s.c. and testicular pressure and morphology were examined 24 hours later as described above. Since the increase in intratesticular pressure in the abdominal testis might be related to the size of the organ, immature normal rats (27 to 28 days old with testis weights similar to those of the abdominal testis in the cryptorchid animals) were given 100 I.U hCG s.c. and the intratesticular pressure was measured 24 hours later. In previous studies we have observed that large doses of hCG (doses giving a maximal testosterone response, approx. 50 I.U. or more) are needed to increase testicular vascular permeability (Bergh et al, 1986, Widmark et al, 1986). To test whether the observed increase in testicular pressure in the abdominal testis could be induced by lower doses of hCG, unilaterally cryptorchid rats (neonatal operation) were given 10 I.U. hCG s.c. and the intratesticular pressure was measured after 24 hours.

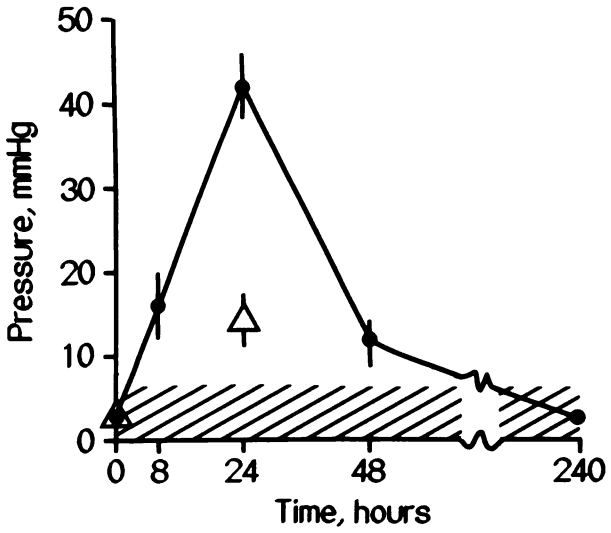

Fig. 1. Testicular pressure in the testis (circles) of unilaterally cryptorchid rats at different times after a single s.c. injection of hCG (each point represents the mean ± SEM of observations in 6 to 8 organs). The shaded area indicates the pressure range where pressures cannot be determined with the Schoitz tonometer. The pressure in the contralateral scrotal testis was always below 7 mm Hg. Basal and hCG-stimulated (after 24 hours) testicular pressure was also measured in rats made cryptorchid as adults (mean ± SEM, N = 5). The pressure in the abdominal testis of these adult rats is marked with triangles; their scrotal pressures were below 7 mm Hg.

Testicular blood flow was studied in adult, unilaterally cryptorchid rats (operated at birth) using laser doppler flowmetry as described earlier (Damber et al, 1986). The blood flow was measured both under basal conditions and 8 hours after treatment with 100 I.U. hCG.

Testicular lymph flow was estimated semi-quantitatively by injecting 0.1 or 0.04 ml of a 2% solution of trypan blue in saline into the scrotal and abdominal testis using a 27-G needle (the volume injected was directly proportional to the volume of the interstitial space, see Bergh and Helander, 1978). After some time, blue staining was noted in lymph vessels in the testicular cord and the time for the dye to reach lymph vessels close to the aorta was recorded. Lymph flow was estimated in adult, unilaterally cryptorchid rats (operated at birth) treated with 100 I.U. hCG 8 hours previously and compared with that in untreated, adult unilaterally cryptorchid rats.

Results

Treatment with 100 I.U. of hCG resulted in a marked increase in intratesticular pressure in the abdominal testis that was maximal 24 hours after treatment (Fig. 1). A similar but less pronounced

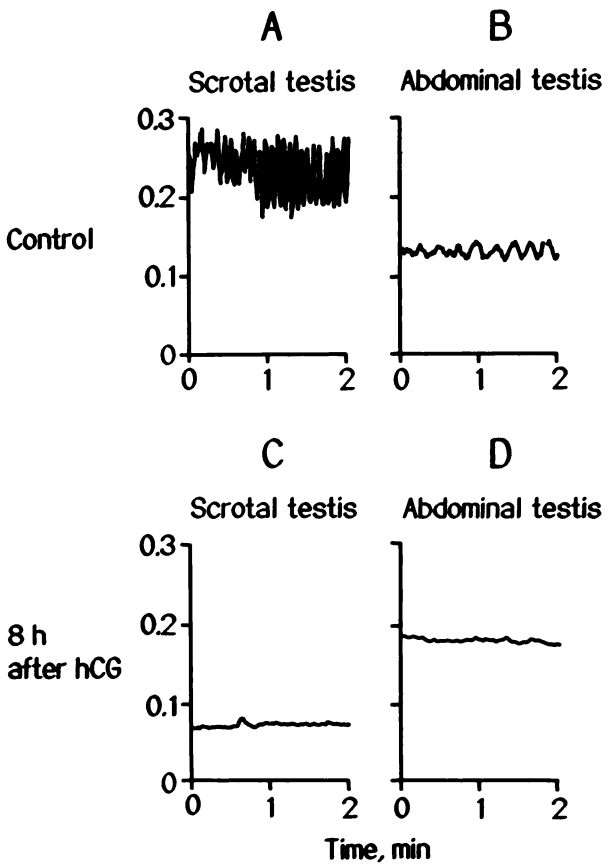

Fig. 2. Typical recordings of the blood flow signal using laser doppler flowmetry in the testis of unilaterally cryptorchid rats before (A, B) and 8 hours after hCG treatment (C, D). An abnormal blood flow pattern was found in the abdominal testis. HCG treatment abolished vasomotion in both types of testis.

increase in intratesticular pressure was also noted in the abdominal testis in rats made cryptorchid as adults (Fig. 1). The increased organ pressure was already clearly evident upon palpation. HCG treatment also resulted in an increase in testicular weight (Table 1), which previous studies have shown to be principally the result of an increased volume of interstitial fluid (Damber et al, 1985). There was also a slight increase in testicular weight (Table 1) and interstitial fluid volume (Damber et al, 1985) in the scrotal testis, but this apparently does not result in an increase in intratesticular pressure that can be detected with this type of tonometer. Twenty-four hours after the treatment of unilaterally cryptorchid rats (neonatal operation) with 10 I.U. hCG, the intratesticular pressure was below 7 mm Hg in both the scrotal and abdominal testis. Intratesticular pressure was below 7 mm Hg in the hCG-treated immature rats.

Although polymorphonuclear leukocytes are normally not observed in the interstitial space in either the scrotal or abdominal testis, some polymorphonuclear leukocytes were present in the interstitial space in the scrotal testis 8 and 24 hours after treatment, as earlier described (Bergh et al, 1986), and similarily were observed in the abdominal testis 8, 24 and 48 hours after hCG treatment. Ten days after hCG treatment, the weight of both the abdominal and scrotal testis was similar to that prior to treatment (Table 1). The average tubule diameter in the abdominal testis was 148 ± 7 before and 144 ± 5 μm 10 days after hCG treatment (mean ± SEM, N = 6 to 8 rats and 20 tubules examined per testis). The average number of germ cells per tubular cross-section was not affected (3.2 ± 1.9 and 5.2 ± 1.8, respectively), and the morphologic appearance of the hCG-treated abdominal testis was not different from that prior to treatment. Morphologic examination of the scrotal testis 10 days after treatment did not reveal any signs of hCG-induced alterations.

In the scrotal testis, the laser doppler flow signal showed rhythmical variations (8 to 10 per minute) as previously described in the normal scrotal testis (Damber et al, 1986), but the amplitude and frequency (3 to 5 per min) were much lower in the abdominal testis (Fig. 2). Eight hours after hCG treatment, the blood flow signal was constant in both types of testis (Fig. 2). In rats made cryptorchid as adults, vasomotion had disappeared in the abdominal testis.

After injection of trypan blue, stained lymph vessels were observed near the aorta after 102 ± 35 seconds on the scrotal side, but not until 714 ± 96 seconds (mean ± SEM, N = 6) on the abdominal side.

TABLE 1. Testicular Weight (g) of Adult Unilaterally Cryptorchid Rats at Different Times after Treatment with a Single dose of HCG*

	0 h (N = 6)	8 h (N = 6)	24 h (N = 6)	48 h (N = 6)	240 h (N = 8)
Scrotal testis	2.33 ± 0.10	2.56 ± 0.12	2.70 ± 0.15†	2.30 ± 0.07	2.24 ± 0.06
Abdominal testis	0.32 ± 0.04	0.30 ± 0.06	0.47 ± 0.08†	0.42 ± 0.03†	0.25 ± 0.02

*Mean ± SEM.

†Significantly different from corresponding basal value, $P < 0.05$ (Mann-Whitney U-test).

Eight hours after hCG treatment, lymph flow was apparently increased on the scrotal side since staining was already observed after 55 ± 14 seconds. On the abdominal side, the increase in lymph flow was marginal and staining was noted after 678 ± 144 seconds (mean ± SEM, N = 6).

Discussion

Testicular microcirculation normally shows large rhythmical variations with periods of high erythrocyte velocity alternating with periods of very slow or no flow, which can easily be monitored using laser doppler flowmetry (Damber et al, 1986). This phenomenon, called vasomotion, has been observed in several organs and is probably due to spontaneous myogenic activity in the precapillary sphincters (Funk and Intaglietta, 1983). Vasomotion is of considerable importance for transvascular exchange (Intaglietta, 1981). It is inhibited by hCG treatment, resulting in a continuous flow, as previously observed (Damber et al, 1986), but the mechanism behind this inhibition remains unknown. In the present study, we found that vasomotion was greatly reduced in the abdominal testis. Although the reason for it is not known, the finding of a change in blood flow pattern is consistent with previous observations of an impaired vascular permeability and altered vascular morphology in the abdominal testis (Francavilla et al, 1979, Sharpe, 1984, Chung et al, 1984, Damber et al, 1985).

HCG treatment in high doses results in an increase in vascular permeability, principally located in postcapillary venules (Bergh et al, 1987), and in the interstitial fluid volume in the rat testis (Sharpe, 1984). An intravascular leukocyte accumulation precedes increased vascular permeability and, simultaneously, leukocytes migrate into the interstitial space (Bergh et al, 1986). Leukocytes are probably involved in mediating increased venule permeability, since pretreatment with anti-leukocyte serum prevents the hCG-induced increase in interstitial fluid volume (Bergh and Damber, 1987, Widmark et al, 1987). We have previously observed that hCG treatment in high doses results in a marked increase in vascular permeability and interstitial fluid volume in the unilateral abdominal testis (Damber et al, 1985). The present study suggests that this is probably related to the accumulation of leukocytes, as in the scrotal testis. The present study, although using a crude method, suggests that the lymph flow from the abdominal testis is slow. Increased vascular permeability coupled with insufficient lymph drainage could explain the marked increase in organ pressure in the abdominal testis after hCG treatment. Blood flow is only marginally increased in the unilateral abdominal testis 8 hours after hCG treatment and returns to basal values 24 hours after treatment (Damber et al, 1985). We speculated that this could be due to the lack of a tubular factor (present in the scrotal testis) that stimulates testicular blood flow (Damber et al, 1985). The present study offers another explanation. Blood flow cannot be increased further in the abdominal testis because of the high intratesticular pressure.

The venous pressure in most organs is low and an increased organ pressure of the magnitude observed in the abdominal testis might impair microcirculation by increasing venous resistance. In the eye, an increased pressure of this magnitude would clearly be diagnosed as glaucoma and treatment would be necessary to prevent damage (Kolker and Hetherington, 1976). In the present study, however, we were unable to observe any signs of an hCG-induced damage to the abdominal testis, although this testis is already severely degenerated prior to hCG treatment. Therefore, additional damage may be difficult to detect. Some studies using multiple hCG doses do, however, describe hCG-induced tubule damage and interstitial edema in the experimentally positioned abdominal rat testis (Eisenstaedt et al, 1940; Heine et al, 1973). Focal tubular necrosis has been described in the normal scrotal testis after hCG treatment but such changes were only observed in a minority of the treated animals (van Vliet et al, 1986). In our study, such morphologic alterations were not found.

Multiple doses of hCG are currently used in the treatment of prepubertal cryptorchidism. The doses employed are lower than those that induced changes in this rat model, and most investigators have not been able to detect hCG-induced testicular damage in cryptorchid boys (Läckgren 1983). Interestingly, however, Läckgren (1983) reported that the undescended testis is unusually firm some days after hCG treatment, and Charny (1960) observed intratesticular edema. One common side-effect of hCG treatment is a feeling of warmth and swelling in the region of the undescended testis. These observations, together with the present results (obtained in an experimental model mimicking the congenital condition by preventing testicular descent), indicate the necessity of studies on intratesticular pressure after hCG-treatment in cryptorchid boys.

In conclusion, this study, together with that of Damber et al (1985), clearly indicates disturbances in blood flow, microcirculation and vascular permeabil-

ity in the unilateral abdominal rat testis under basal conditions. A glaucoma-like increase in testicular pressure is noted after a high dose hCG treatment. It remains to be shown whether similar changes can be observed after hCG treatment in cryptorchid boys.

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