

Control of testicular vasomotion by testosterone and tubular factors in rats

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Testicular vasomotion (rhythmical variations in testicular blood flow) was studied in adult rats using laser Doppler flowmetry. Vasomotion was not present in testes in which the Leydig cells had been destroyed, but it could be induced by a low dose of testosterone. Transposition of a scrotal testis into the abdominal cavity inhibited vasomotion and this was apparently not caused by Leydig cell malfunction. Depletion of specific germ cells (by unilateral X-irradiation induced killing of spermatogonia and maturation depletion of germ cells) did not abolish vasomotion in the testis. It is suggested that testicular vasomotion is influenced by testosterone and by factors from Sertoli cells.

Introduction

Vasomotion, spontaneous rhythmical variations in arteriolar blood flow, is observed in several tissues (Intaglietta, 1988) including rat testes (Damber *et al.*, 1982, 1986). Vasomotion is probably caused by spontaneous myogenic activity in arterioles. This activity can be modulated by hormonal and neuronal factors, but the role and physiological control of vasomotion is largely unknown (Funk and Intaglietta, 1983; Intaglietta, 1988). Transvascular fluid exchange and vascular resistance can be modulated by influencing vasomotion. During periods of high flow, plasma is filtered out from the vasculature and returns during periods of slow flow. Vascular resistance is decreased when vasomotion amplitude is increased (Intaglietta, 1988).

In the rat testis, vasomotion is developmentally and hormonally controlled (Damber *et al.*, 1986, 1990; Widmark *et al.*, 1986, 1989). It is not present in prepubertal animals, and it is inhibited by treatment with large doses of luteinizing hormone (LH) or human chorionic gonadotrophin (hCG) and by local administration of catecholamines (Damber *et al.*, 1982). Vasomotion disappears if the Leydig cells are destroyed by ethane dimethyl sulfonate (EDS) treatment and it can be restored by high doses of testosterone (Damber *et al.*, 1992), but it is not known whether local factors other than testosterone are of importance. The physiological role of vasomotion in the testis is unknown, but the relative haematocrit in testicular capillaries fluctuates in phase with vasomotion (Damber *et al.*, 1986) and inhibition of vasomotion with LH or hCG is associated with an increase in the amount of interstitial fluid in the organ (Widmark *et al.*, 1986, 1989) indicating that it may influence transvascular fluid exchange as in other organs (Intaglietta, 1988). The protein concentration is similar in testicular interstitial fluid and plasma and, thus, there is almost no oncotic osmotic pressure opposing capillary pressure. Transvascular fluid exchange must therefore be particularly influenced by changes in pressure in the testis (Setchell, 1990; Sweeney *et al.*, 1991).

The aim of this study was therefore to elucidate the mechanisms involved in the control of testicular vasomotion.

Materials and Methods

Experiment 1

Adult male Sprague–Dawley rats (350–450 g) were given a single intraperitoneal injection of ethane dimethane sulfonate (EDS; 75 mg kg⁻¹ body weight) as described by Damber *et al.* (1987). EDS treatment results in the destruction of the Leydig cells in the testis, but other testicular cells are initially unaffected (Sharpe *et al.*, 1990). On day 6, the rats were assigned to five groups. Testosterone esters (Sustanon; kindly donated by Organon, Oss, The Netherlands) were injected subcutaneously at four different doses (0.1, 1, 25 or 250 mg); the control group received vehicle (arachis oil) only. Twenty-four hours later the animals were anaesthetized with pentobarbital (40 mg kg⁻¹). A scrotal incision was made to expose the testes. Testicular blood flow was studied in two different areas (devoid of large vessels) on the testicular surface using laser Doppler flowmetry (Periflux 3; Perimed, Stockholm) as described by Damber *et al.* (1986). The blood flow signal (volts) was recorded on an electrostatic recorder for at least 5 min on each surface area, and average flow level, vasomotion amplitude and frequency were later analysed. The testes were removed, weighed and cut into two pieces. One piece was placed in ethanol and intratesticular testosterone concentration was analysed by a radioimmunoassay as described by Damber and Bergh (1980). The sensitivity of the assay was 10 pg when applying the 95% confidence limit. The other piece was fixed in Bouin's solution, embedded in metacrylate resin (Histo-Resin; LKB, Stockholm) and testicular morphology examined using 2 µm thick sections stained with haematoxylin–eosin.

Experiment 2

Adult male Sprague–Dawley rats (350–450 g) were anaesthetized with pentobarbital (40 mg kg⁻¹ body weight) and the left

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Table 1. Effect of testosterone in rats in which the Leydig cells have been destroyed

	Dose of testosterone esters (mg)				
	0	0.1	1	25	250
Number of animals	6	8	6	5	7
Testis weight (g)	1.54 ± 0.16	1.44 ± 0.33	1.42 ± 0.07	1.53 ± 0.10	1.48 ± 0.07
Concentration of testicular testosterone (ng g ⁻¹)	nd	4.6 ± 0.8	8.1 ± 1.1 ^b	31.7 ± 4.6 ^c	81.0 ± 4.2 ^d
Flow (V)	4.1 ± 0.3	3.8 ± 0.4	6.0 ± 0.9 ^e	7.5 ± 1.6 ^e	5.2 ± 0.1 ^e
Amplitude (V)	0	0.49 ± 0.4 ^a	1.3 ± 0.2 ^e	2.0 ± 0.6 ^e	1.6 ± 0.1 ^e
Frequency (peaks min ⁻¹)	0	6.3 ± 0.6 ^a	7.2 ± 0.4	8.5 ± 0.7	8.4 ± 0.7

Values are means ± SEM.

nd: not detectable.

^aSignificantly different from value in rats not given testosterone, $P < 0.05$.

^bSignificantly different from value in the 0, 25, 250 mg groups, $P < 0.05$.

^cSignificantly different from value in 0, 0.1, 1, 250 mg group, $P < 0.05$.

^dSignificantly different from 0, 0.1, 1 and 25 mg group, $P < 0.05$.

^eSignificantly different from 0 and 0.1 mg groups $P < 0.05$ but not different from the other values with the same superscript.

testis was placed in the abdominal cavity and the inguinal canal closed by sutures as described by Bergh and Damber (1984). Six days later the animals were assigned to four groups. Group 1 was injected with vehicle (arachis oil) only and group 2 with 25 mg testosterone esters (Sustanon) subcutaneously. Groups 3 and 4 had been injected intraperitoneally with EDS (as described above) on the day of operation. Six days after the operation, group 3 received an injection of vehicle only and group 4 was injected subcutaneously with 25 mg testosterone esters. At day 7 (24 h after injections), the animals were anaesthetized with pentobarbital (40 mg kg⁻¹ body weight) and testicular blood flow, testosterone concentration and testicular morphology were examined in the scrotal and abdominal testes as described in Expt 1.

Experiment 3

The left testis of adult male Sprague–Dawley rats (350–450 g) was irradiated with a dose of 3 Gy using 4 MeV X-rays produced by a linear accelerator, as described by Kangasniemi *et al.* (1990). The right testis was protected by a lead shield and served as a control. X-irradiation of the testis at this dose results in a selective, transient depletion of cycling spermatogonia. If the testes are studied at different intervals after irradiation, they lack a particular type of germ cell as a result of maturation depletion (Kangasniemi *et al.*, 1990). At 7, 17, 28, 45 and 56 days after irradiation, the animals were anaesthetized with pentobarbital and testicular blood flow, testosterone and morphology were examined as described above.

Statistical analysis

Comparisons among groups were made using the Kruskal–Wallis one-way analysis of variance; for comparisons between groups, the Mann–Whitney U test and the Wilcoxon test for paired observations were used. Correlation was expressed by the Spearman rank correlation coefficient. A P value less than 0.05 was considered statistically significant.

Results

Experiment 1. Role of testosterone in vasomotion

Light microscopy confirmed that Leydig cells were not present in EDS- and vehicle-treated animals. The concentration of testicular testosterone was below the detection limit of the assay. Testosterone ester treatment resulted in a dose-dependent increase in intratesticular testosterone concentration (Table 1) and values equivalent to those in intact animals (see Damber and Bergh, 1980) were reached with the highest dose. There was a strong linear correlation between testosterone ester dose and intratesticular testosterone concentration ($r_s = 0.94$, $P < 0.0001$). A discrete increase in the number of degenerating germ cells, particularly in tubular stage VII was noted in all treatment groups, but otherwise testicular morphology was similar to that of intact scrotal rat testes.

Vasomotion was absent in rats receiving EDS only (Fig. 1). In all rats given testosterone esters in doses from 1 to 250 mg, an almost identical vasomotion pattern (similar flow level, amplitude and frequency) was found (Table 1, Fig. 1). In rats given 0.1 mg testosterone esters, the average blood flow signal was lower than that in rats receiving the higher doses, but vasomotion with a normal frequency but small amplitude was present (Fig. 1).

Experiment 2. Effect of cryptorchidism on vasomotion

No Leydig cells were present in the EDS-treated scrotal and abdominal testes. Cryptorchidism resulted in degenerative changes in the seminiferous epithelium in testes with or without Leydig cells and in those receiving testosterone supplement, and testicular weights were reduced (Table 2, Fig. 2). Normal vasomotion was present in scrotal testes, but in the contralateral abdominal testes a continuous flow was observed (Fig. 3).

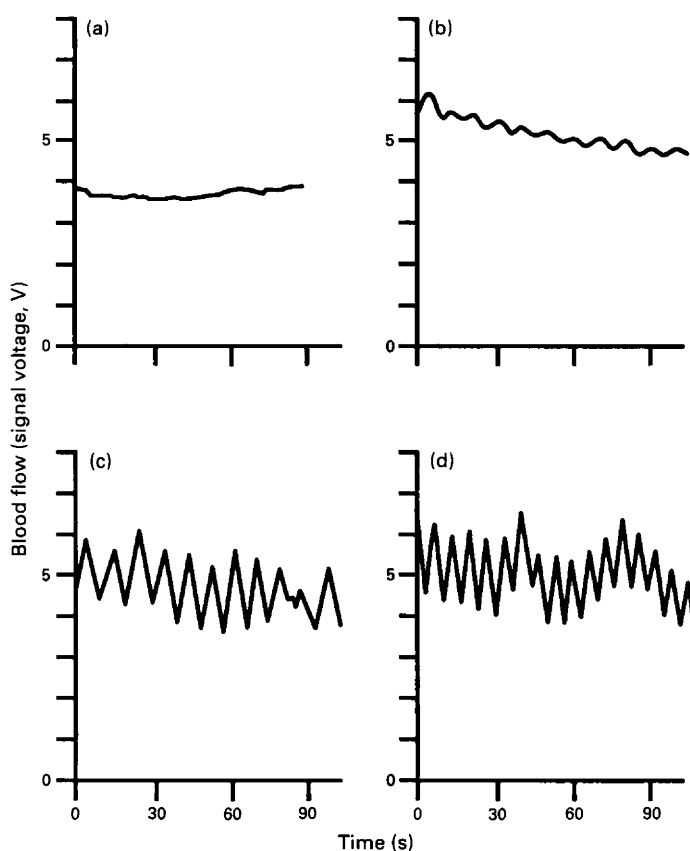


Fig. 1. Representative laser Doppler recordings of testicular blood flow in rats in which the Leydig cells have been destroyed and which had been treated with different doses of testosterone esters (TE) 24 h earlier (a) vehicle only; (b) 0.1 mg TE; (c) 1 mg TE; (d) 250 mg TE.

Table 2. Effects of surgically induced unilateral cryptorchidism in adult rats 7 days after operation

Treatment	Position of testis	Testis weight (g)	Concentration of testicular testosterone (ng g ⁻¹)	Flow (V)	Vasomotion amplitude (V)	Vasomotion (peaks min ⁻¹)
Vehicle only (n = 5)	Scrotal	1.96 ± 0.04	150 ± 86	4.0 ± 0.8	0.8 ± 0.2	7.4 ± 0.4
	Abdominal	0.89 ± 0.05 ^a	56 ± 12	4.8 ± 1.4	0	0
Testosterone esters (n = 5)	Scrotal	1.82 ± 0.11	64 ± 20	4.8 ± 1.1	1.1 ± 0.2	7.4 ± 0.4
	Abdominal	0.90 ± 0.11 ^a	77 ± 23	5.0 ± 0.9	0	0
EDS (n = 5)	Scrotal	1.52 ± 0.24	nd	3.6 ± 0.7	0	0
	Abdominal	0.69 ± 0.08 ^a	nd	5.2 ± 1.8	0	0
EDS + testosterone esters (n = 5)	Scrotal	1.54 ± 0.14	40 ± 10	4.9 ± 1.6	1.0 ± 0.3	6.5 ± 0.5
	Abdominal	0.75 ± 0.09 ^a	80 ± 32	5.9 ± 2.0	0	0

Values are means ± SEM.

nd: not detectable.

n = number of animals; EDS: ethane methyl sulfonate.

^aSignificantly different from the value in the scrotal testis, $P < 0.05$.

Treatment with testosterone esters did not influence the blood flow pattern in scrotal or in abdominal testes. EDS treatment inhibited vasomotion in the scrotal testes, but it could be res-

tored with testosterone ester treatment (Fig. 3). Blood flow was continuous in abdominal testes of EDS-treated animals and this was not influenced by testosterone supplementation (Fig. 3).

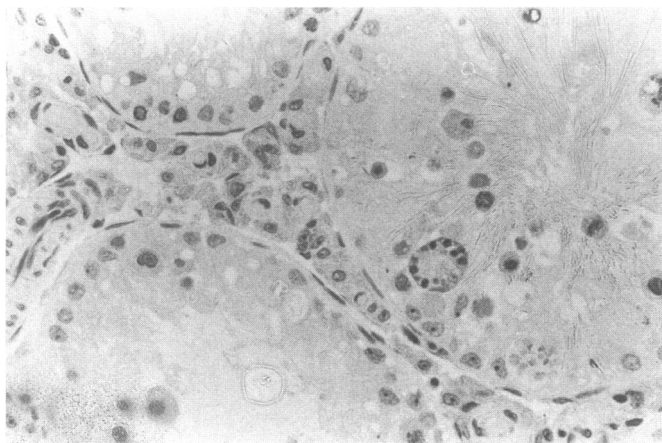


Fig. 2. Section from the abdominal testis of a unilaterally cryptorchid rat ($\times 270$). The seminiferous tubules have an abnormal morphology; some lack differentiated germ cells and others contain abnormal and giant spermatids. No apparent morphological changes are observed in blood vessels or in the Leydig cells. Vasomotion was not present in this testis.

Experiment 3. Role of different types of germ cell in vasomotion

Local irradiation of the left testis resulted in depletion of spermatogonia but spermatogenesis in the contralateral testis was unaffected.

At different times after treatment the irradiated testes showed tubules where different types of germ cell were missing (summarized in Table 3, Fig. 4) and as described in detail previously by Kangasniemi *et al.* (1990). Testicular testosterone concentrations were similar in the control and irradiated testes at all study points except at 56 days (Table 3). Depletion of germ cells by irradiation did not at any time induce clear change in testicular vasomotion compared with that in control testes (Table 3, Fig. 5). At 7, 17 and 28 days, the blood flow recordings were identical in the irradiated and in the contralateral control testes in all animals. At 38, 45 and 56 days, the irradiated testes showed vasomotion but the pattern was more irregular than in the contralateral normal testes in 4 of 9, 2 of 5, 4 of 6 of the examined animals, respectively (Fig. 5). In the other animals examined at 38, 45 or 56 days, normal vasomotion was seen in the irradiated testes. This difference in response could not be explained by the morphology of the individual testes: they all showed a similar extent of germ cell depletion.

Discussion

This study confirms that a high dose of testosterone can restore normal vasomotion to the testes of rats in which the Leydig cells have been destroyed (Damber *et al.*, 1992). However, considerably less testosterone is apparently needed, since vasomotion was restored, although with a smaller amplitude, when the concentration of testicular testosterone was about 5 ng g^{-1} , that is about 5% of the normal value in adult rats (Damber and Bergh, 1980). An apparently normal flow can be induced by a dose of 1 mg testosterone esters. This dose is known to normalize serum testosterone, but it does not normalize intratesticular

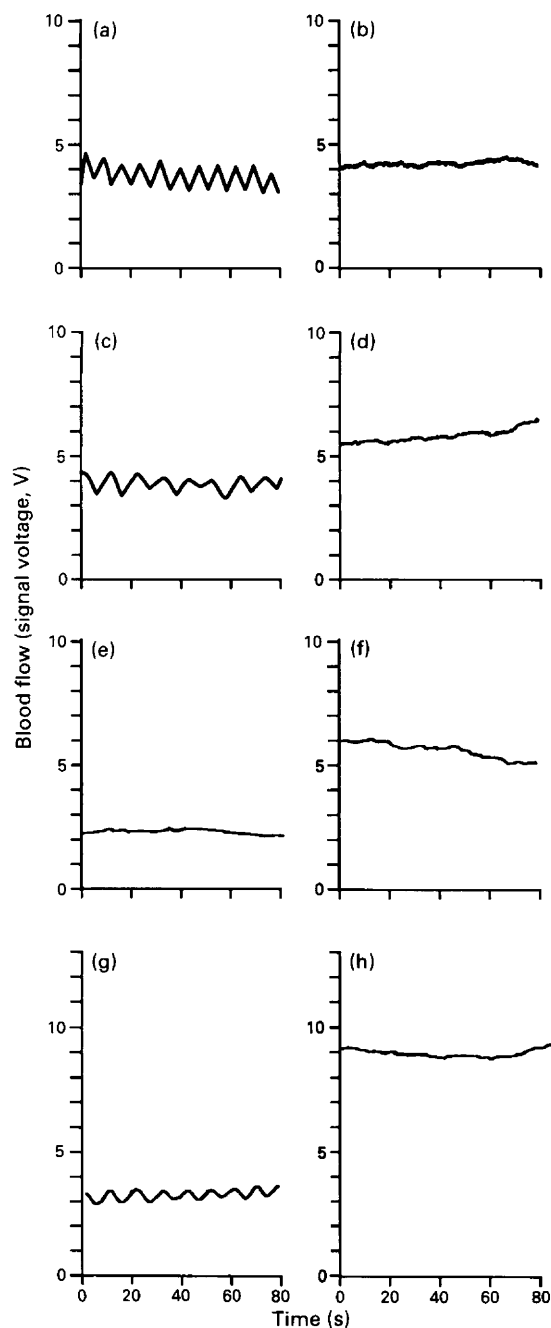


Fig. 3. Representative laser Doppler recordings of testicular blood flow in scrotal (a, c, e, g) and abdominal testes (b, d, f, h) in rats that have been unilaterally cryptorchid for 7 days and that received the following additional treatments. (a, b) No further treatment; (c, d) 25 mg testosterone esters on day 6; (e, f) ethane methyl sulfonate (EDS) on day 0; (g, h) EDS on day 0 and 25 mg testosterone esters on day 6.

testosterone or spermatogenesis (Sharpe *et al.*, 1990). The mechanism by which testosterone may induce vasomotion (myogenic activity in small arterioles) is unknown (see below). This study suggests that vasomotion, when induced by low concentrations of testosterone in testes in which the Leydig cells have been destroyed, is of the same frequency as in intact

Table 3. Effects of unilateral testicular irradiation in rats

Time after irradiation (days)	Testis	Number of animals	Weight of testis (g)	Testosterone concentration (ng g ⁻¹)	Germ cells depleted	Flow (V)	Vasomotion amplitude (V)	Vasomotion frequency (peaks min ⁻¹)
7	c	5	2.00 ± 0.09	129 ± 47	none	5.9 ± 0.4	0.9 ± 0.1	10.0 ± 0.4
	i		1.94 ± 0.01 ^a	120 ± 30	spermatogonia	4.9 ± 0.6	1.1 ± 0.3	9.4 ± 0.4
17	c	5	2.01 ± 0.06	117 ± 21	none	6.6 ± 0.7	1.1 ± 0.2	9.6 ± 0.8
	i		1.79 ± 0.05 ^a	99 ± 23	spermatogonia, pachytene spermatocytes	5.6 ± 0.5	1.1 ± 0.2	9.0 ± 0.4
28	c	5	1.94 ± 0.06	129 ± 20	none	6.5 ± 0.5	1.1 ± 0.1	8.8 ± 0.4
	i		1.49 ± 0.05 ^a	148 ± 26	primary spermatocytes, round spermatids	5.2 ± 0.6	1.1 ± 0.2	7.4 ± 0.9
38	c	9	1.97 ± 0.05	85 ± 10	none	5.3 ± 0.2	0.8 ± 0.1	9.7 ± 0.6
	i		1.15 ± 0.05 ^a	80 ± 14	pachytene spermatocytes, round spermatids	5.2 ± 0.2	0.7 ± 0.1	9.2 ± 0.3
45	c	5	2.05 ± 0.03	216 ± 84	none	7.3 ± 0.4	1.3 ± 0.1	9.0 ± 0.3
	i		1.12 ± 0.05 ^a	184 ± 47	round mature spermatids	7.1 ± 1.0	1.5 ± 0.5	7.4 ± 0.7 ^a
56	c	6	1.93 ± 0.05	88 ± 21	none	6.3 ± 0.3	1.5 ± 0.1	9.6 ± 0.2
	i		1.29 ± 0.06 ^a	107 ± 18 ^a	mature spermatids	6.2 ± 0.7	1.4 ± 0.2	10.0 ± 0.3

Values are means ± SEM.

c: control; i: irradiated testis.

^aSignificantly different from that in the contralateral testis, $P < 0.05$.

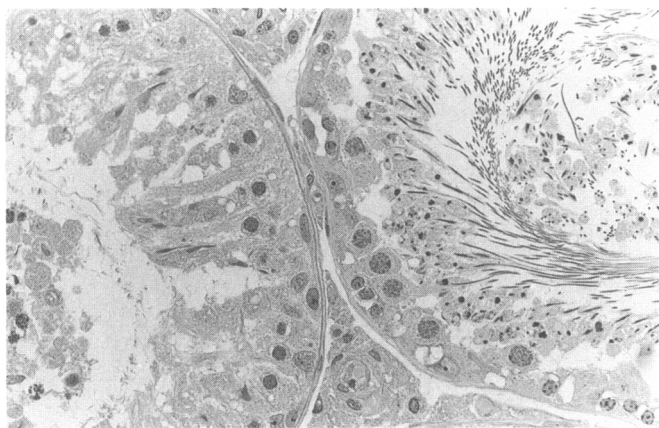


Fig. 4. Section from a testes irradiated 38 days earlier ($\times 270$). The numbers of round spermatids and pachytene spermatocytes are reduced. There are no apparent morphological changes in the blood vessels or in the Leydig cells. Vasomotion was normal in this testis.

testes. It appears that slightly more testosterone is required to normalize vasomotion amplitude than vasomotion frequency. The observation that an apparently normal flow can be induced by very low concentrations of testosterone suggests that testicular vasomotion is not regulated by moderate physiological reductions in testosterone concentration in the testis.

We have previously demonstrated that vasomotion is not present in 20-day-old prepubertal animals (Damber *et al.*, 1990).

At this age, the concentration of testicular testosterone is about 15 ng g⁻¹ and scrotal development is not complete (Bergh *et al.*, 1987). Vasomotion is inhibited in abdominal testes in this study, and we showed that this is not related to Leydig cell dysfunction or reduced intratesticular testosterone concentration. Vasomotion does not disappear until several days after transposition of an adult testis into the abdomen (Hjertkvist *et al.*, 1988). Long-term cryptorchidism results in altered vascular morphology (see Bergh, 1989 for review), but a low amplitude, low frequency vasomotion is still present in adult testes that have been lying in the abdomen since birth (Hjertkvist *et al.*, 1988). Thus, the lack of vasomotion in prepubertal and the 7-day abdominal testes in this study cannot be explained by insufficient testosterone or by direct effect of temperature or cryptorchidism on the vasculature. Other factors must therefore be important.

One possible source for a factor modulating testicular vasomotion would be the seminiferous tubules. When testosterone is given to rats with testes without Leydig cells, it probably influences the seminiferous tubules, since androgen receptors are known to be present on peritubular and Sertoli cells (Sharpe *et al.*, 1990, see below). A general stimulation of tubule metabolism, or the induction of a specific testosterone-dependent factor, are possible reasons for the vascular effects of testosterone in these animals. It is, however, not known to what extent an extremely low concentration of intratesticular testosterone (hardly detectable) influences tubule metabolism. Using immunohistochemistry, we have recently observed androgen receptors in the

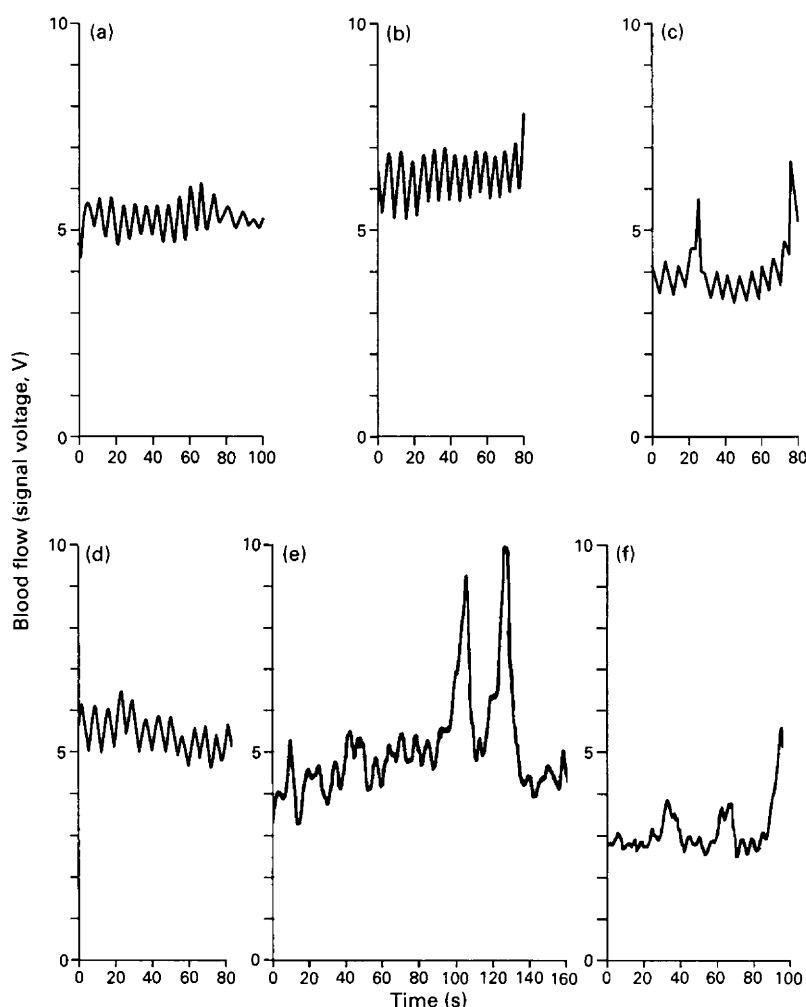


Fig. 5. Representative laser Doppler recordings of testicular blood flow in testes irradiated at different times prior to study (a) 7 days; (b) 17 days; (c) 38 days; (d) 45 days; (e) 45 days; (f) 56 days.

muscular layer of small arteries in scrotal and abdominal testes (Bergh and Damberg, 1992). The present observation that a very low dose of systemically administered testosterone esters induces vasomotion suggests a direct effect of androgens on blood vessels. However, testosterone did not induce vasomotion in testes with tubule damage induced by cryptorchidism. Cryptorchidism results in altered Sertoli cell function and in degeneration of primary spermatocytes and spermatids; some studies suggest that the primary effect of increased temperature is Sertoli cell malfunction. There are as yet no indications of altered vascular morphology after 7 days of cryptorchidism (see Bergh, 1989 for review). The inhibited vasomotion in 7-day cryptorchid testes could thus suggest that Sertoli cells or germ cells are involved in modulating testicular vasomotion. The data from the irradiation experiments indicate that a particular germ cell type is not critically necessary for normal vasomotion, although an irregular pattern was often observed after depletion of spermatids. Sertoli cell function is influenced by adjacent germ cells; some germ cells stimulate, whereas others inhibit, Sertoli cells (Jegou *et al.*, 1988; Allenby *et al.*, 1991). Sharpe *et al.* (1991) showed that selective depletion of late spermatids

resulted in an increase in the volume of interstitial fluid in the testis (volume of interstitial fluid is influenced by changes in blood flow and vascular permeability, see Bergh *et al.*, 1988) and suggested that the altered function of Sertoli cells could via paracrine effects influence the vasculature. Collectively, available data thus indicate that the lack of vasomotion in cryptorchid testes could be related to impaired Sertoli cell function, and that these Sertoli cells cannot secrete the factors necessary for normal vasomotion. However, direct cryptorchidism or temperature-induced effects on the vasculature cannot be ruled out.

Previous studies have shown that Leydig cells play a key role in the regulation of testicular blood flow and vasomotion (Bergh *et al.*, 1988). We suggest that part of this effect could be mediated directly via vascular androgen receptors but that testosterone-dependent Sertoli cell products are also involved. The nature of these postulated products of Sertoli cells are unknown.

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