5-Hydroxytryptamine – a local regulator of testicular blood flow and vasomotion in rats

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The effects of 5-hydroxytryptamine (5-HT), 5-HT₂ receptor antagonists (ritanserin and ketanserin), histamine and substance 48/80 on testicular blood flow and microcirculation were studied in adult rats. The substances were administered by topical application on the testicular surface and by intratesticular injections, and blood flow was measured by radioactive microspheres and with a laser Doppler flowmeter. Blood flow was decreased by 5-HT in a dose-dependent manner and vasomotion in the testis was inhibited, suggesting that it could be involved in the physiological regulation of the testicular vasculature. The 5-HT antagonists did not significantly influence flow or vasomotion in intact testes. Histamine did not cause any major effects on testicular blood flow. Substance 48/80 caused degranulation of testicular mast cells, and reduced testicular blood flow and vasomotion suggesting that testicular mast cells, possibly by releasing 5-HT, could be involved in the local control of the testicular vasculature.

Introduction

Total blood flow, vasomotion (spontaneous rhythmical variations in arteriolar blood flow) and vascular permeability in the testis are apparently influenced by factors secreted by Leydig cells and seminiferous tubules (for references see Bergh and Damber, 1993), but the nature of these factors and their physiological and pathophysiological importance is largely unknown. Other cell types in the testis could also be involved in the local regulation of the testicular vasculature. Mast cells, which are a major source of vasoactive substances, and inflammation mediators like 5-hydroxytryptamine (5-HT) and histamine, are present around the subcapsular part of the main testicular artery (Sowerbutts *et al.*, 1986), and mast cells are probably involved in the control of blood flow in other steroid hormone producing organs like the ovary (Krishna *et al.*, 1989) and the adrenal cortex (Hinson *et al.*, 1989). Treatment with hCG causes mast cell degranulation (Morales and Heyn, 1992), and hCG influences blood flow, vasomotion, and vascular permeability in rat testes. Polymorphonuclear (PMN) leucocytes accumulate in testicular venules, and PMNs migrate and macromolecules leak into the interstitial space after hCG/LH administration (Bergh and Damber, 1993). The testis apparently secretes an inflammation mediator in response to hCG or LH, but the cellular source and the nature of this factor(s) is unknown.

Against this background, we examined the role of mast cells and mast cell products in testicular blood flow. 5-HT is secreted by testicular mast cells (Lombard-des Couttes *et al.*, 1974), testicular nerves (Campos *et al.*, 1990), and Leydig cells in

response to hCG stimulation (Tinajero *et al.*, 1993a, b). Ketanserin (a 5-HT antagonist) inhibits the hCG-induced increase in testicular interstitial fluid volume (Sowerbutts *et al.*, 1986). In addition, 5-HT is known to induce vasoconstriction in the main testicular artery (Free, 1977; Davis, 1992) and high doses cause testicular ischaemia and atrophy (Free, 1977). In addition, it has been demonstrated that 5-HT is an important autocrine or paracrine regulator of Leydig cell function (Dufau *et al.*, 1993; Tinajero *et al.*, 1993a, b) and that Leydig cells play a key role in regulating testicular blood flow (Bergh and Damber, 1993).

Materials and Methods

Materials

Radioactive microspheres (16.5 ± 0.1 µm in diameter) labelled with Cerium-141 were purchased from New England Nuclear Products (Boston, MA). 5-Hydroxytryptamine (5-HT, H-7752), histamine dihydrochloride (H-7250) and Compound 48/80 (C-4257) were purchased from Sigma Chemical Co., St Louis, MO; ritanserin (R 55667) and ketanserin (R 49945) were kindly provided by Jansen Research Foundation (Beerse). All substances were dissolved in sterile saline.

Animals

Adult male Sprague–Dawley rats (300–350 g) were kept in a controlled laboratory environment. Food and water were available *ad libitum*. All animals were anaesthetized with pentobarbital (40 mg kg⁻¹), given as a single i.p. injection. During each experiment the animals were kept supine on a heating pad.

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Revised manuscript received 1 September 1995.

Table 1. Blood flow (ml (100 g)⁻¹ min⁻¹) to testes and various control organs during topical application of drugs to the surface of the left testis over the main testicular artery in rats

Treatment	Testes		Epididymis		Kidneys		Psoas	
	Right	Left	Right	Left	Right	Left	Right	Left
48/80 1000 µg (n = 6)	54.5 ± 11.6	25.8 ± 8.6*	31.9 ± 6.2	29.3 ± 6.0	681.5 ± 114.3	651.0 ± 114.0	17.2 ± 2.2	16.7 ± 2.9
100 µg (n = 6)	34.3 ± 5.5	31.8 ± 2.0	17.9 ± 2.0	17.8 ± 2.5	396.5 ± 97.0	420.7 ± 115.0	11.6 ± 3.5	11.3 ± 2.9
Histamine 100 µg (n = 6)	26.8 ± 6.6	26.1 ± 6.0	18.9 ± 2.0	19.6 ± 3.9	500.4 ± 128.1	509.0 ± 8.4	17.6 ± 7.2	16.7 ± 4.9
5-HT 10 µg (n = 6)	28.9 ± 7.7	4.0 ± 1.6*	25.8 ± 3.8	23.7 ± 2.9	396.7 ± 175.7	410.6 ± 124.8	22.6 ± 6.3	18.9 ± 6.0

Values are means ± SEM.

*Significantly different from that in the contralateral testis $P < 0.03$.

Measurement of testicular blood flow using radioactive microspheres

Rats were anaesthetized and the testes were exposed through a scrotal incision. A piece of filter paper (5 mm × 10 mm) was placed over the straight subcapsular part of the main artery on both testes. The test substances, histamine (100 µg), substance 48/80 (100 µg or 1000 µg), or 5-HT (10 µg in 0.1 ml saline) were applied to the filter paper on the left testis and 0.1 ml saline to the right testis. After 30 min, blood flow to various organs was measured using radioactive labelled microspheres as described by Damber and Janson (1977) and Damber (1978). After subsequent collection of a blood sample from the tail artery, the testes, epididymides, kidneys and psoas muscles were dissected out, weighed, and the radioactivity in the samples measured. Blood flow values are given as ml (100 g tissue)⁻¹ min⁻¹.

Testes exposed to substance 48/80 were also fixed in 4% formaldehyde, 3% (v/v) glutaraldehyde and 0.05% (w/v) picric acid in 0.1 mol cacodylate buffer l⁻¹; postfixed in 1% (w/v) OsO₄; dehydrated and embedded in Epon (Bergh and Söder, 1990). The morphology of testicular mast cells adjacent to the testicular artery was examined on 1 µm toluidine blue stained sections.

Measurement of testicular blood flow and vasomotion using laser Doppler

Rats were anaesthetized and one testis was exposed via a scrotal incision. The testis was mobilized into a plastic holder and embedded in 3% (w/v) agar as described by Hinton *et al.* (1979) and Setchell *et al.* (1995). A laser Doppler probe (PF 412, Perimed, Stockholm) was placed over the middle part of the testis not covered by agar and testicular blood flow was measured using a laser Doppler flowmeter (PF 4, Perimed) as described by Damber *et al.* (1986). The agar was used to immobilize the testis and in this way avoid artefacts in the blood flow signal owing to movements (Setchell *et al.*, 1995). After a stable blood flow signal with normal vasomotion was observed for 5 min, the testis was injected with test substance in 0.1 ml sterile saline. The injection was made using a 27 gauge needle connected to a 1 ml syringe and took place at the caudal pole of the testis. According to Russel *et al.* (1987), 50 µl of trypan blue injected into the caudal pole of the testis spread

1 cm from the injection site in 2 min. Blood flow was then recorded for at least 40 min. In control rats, one testis was injected with 0.1 ml sterile saline.

The following substances and doses were examined:

Substance 48/80 1 ng, 10 ng, 100 ng, 1 µg, 10 µg, 100 µg. In these rats testicular morphology was examined as described above.

Histamine 1 µg, 10 µg.

5-Hydroxytryptamine 0.6 ng, 6 ng, 25 ng, 50 ng.

Ketanserine (a 5-HT₂ antagonist) 50 ng, 500 ng, 5 µg, 50 µg.

Ritanserine (a 5-HT₂ antagonist) 0.1 µg, 1 µg, 10 µg, 100 µg. Ritanserine (10 µg) was also injected together with 50 ng 5-HT.

Statistical analyses

Changes in blood flow after treatment were analysed using the Wilcoxon non parametric test for paired observations and linear correlation. Differences between groups were analysed using the Mann-Whitney U test. The level of significance was set at $P < 0.05$.

Results

Effects of topical application of histamine, substance 48/80 and 5-hydroxytryptamine on the testicular surface over the testicular artery

Substance 48/80 (1 mg) caused a significant decrease in blood flow, but 100 µg substance 48/80 was without effect (Table 1; it seems as though the contralateral testes in the animals given 1 mg substance 48/80 have an increased flow but this could be related to generally higher organ blood flows in this group). Morphological examination showed that the mast cells were degenerated by both doses (not shown). Topical application of 100 µg histamine did not influence testicular blood flow, but application of 10 µg 5-HT caused a major decrease in testicular blood flow (Table 1).

Table 2. The percentage decrease in blood flow as measured with a laser Doppler flowmeter and its duration after local injection of different doses of 5-HT in adult rat testes (values are means $\pm$ SEM, $n = 4-5$ animals in each group)

5-HT (ng)	Percentage decrease in flow	Duration of response (min)
0	1 $\pm$ 1	3 $\pm$ 1
0.6	13 $\pm$ 2*	2 $\pm$ 1
6.0	23 $\pm$ 2*	13 $\pm$ 2*
25	28 $\pm$ 1*	35 $\pm$ 2*
50	33 $\pm$ 2*	36 $\pm$ 6*
50 + 10 μ g ritanserin	32 $\pm$ 2*	7 $\pm$ 2*†

*Significantly different from before treatment ($P < 0.05$; Wilcoxon's non-parametric test for paired observations) and after injections of saline ($P < 0.05$; Mann-Whitney U test).

†Significantly different from after injection of 50 ng 5-HT alone ($P < 0.05$; Mann-Whitney U test).

Effects of intratesticular injections of saline, substance 48/80, histamine, 5-HT and 5-HT antagonists

Saline. Local injection of saline did not significantly influence testicular blood flow (Table 2).

Substance 48/80. Local injection of 100 μ g substance 48/80 abolished vasomotion and decreased blood flow by $22 \pm 4\%$ (mean $\pm$ SEM; $n = 2$) for more than 40 min. Injection of 10 μ g substance 48/80 made the vasomotion pattern irregular and the blood flow was significantly decreased by $11 \pm 2\%$ ($P < 0.05$; mean $\pm$ SEM; $n = 5$) for 16 ± 4 min (mean $\pm$ SEM; $n = 5$, Fig. 1). The lower doses tested were without effects. Morphological examination revealed that the mast cells were totally degranulated by 100 μ g substance 48/80, most were degranulated by 10 μ g substance 48/80, but only a few were partially degranulated by 1 μ g substance 48/80 (Fig. 2).

Histamine. After local injection of 10 μ g the blood flow was significantly raised by $15 \pm 2\%$ ($P < 0.05$; mean $\pm$ SEM; $n = 5$) during 40 ± 7 min (mean $\pm$ SEM, $n = 5$), but the vasomotion pattern was essentially unaffected. One microgram of substance 48/80 increased flow by $6 \pm 0.5\%$ (mean $\pm$ SEM; $n = 3$) and vasomotion was unchanged.

5-Hydroxytryptamine. Local injection of 5-HT caused a dose-dependent decrease in blood flow and an inhibition of vasomotion. The duration of the response was also dose dependent (Table 2). Vasomotion was inhibited during the time when flow was reduced (Fig. 3). The linear correlation coefficients between dose of 5-HT and change in blood flow and duration of the response were 0.70 ($P < 0.002$) and 0.76 ($P < 0.001$), respectively.

5-Hydroxytryptamine antagonist (ritanserin, ketanserin). Neither of the 5-HT₂ antagonists showed any significant effect on the testicular blood flow or vasomotion when injected in doses up to 50 μ g. A very large dose of ritanserin (100 μ g) resulted

Fig. 1. Blood flow recording (in arbitrary perfusion units, PFU) (a) in a rat testis locally injected with 10 μ g substance 48/80, and (b) in a rat testis injected with saline. After injection of substance 48/80, blood flow is reduced and vasomotion is irregular. Saline injection did not influence blood flow.

in a slightly decreased blood flow and an irregular vasomotion. However, an injection of 50 ng 5-HT, after the testis had received 10 μ g ritanserin, caused a similar decrease in blood flow to that without ritanserin, but the time for restoration of normal blood flow was markedly reduced (Table 2), demonstrating that the 5-HT antagonist was effective.

Discussion

In this study we used two different methods to deliver substances to the testis. Considerably higher doses were apparently needed to induce effects after topical than after intratesticular administration. This indicates that most of the topically applied substance does not reach the testis and for this reason conclusions are based mainly on the experiments in which the test substances were injected into the testis.

One aim of this study was to evaluate the role of testicular mast cells in the local regulation of testicular blood flow and microcirculation. Degranulation of testicular mast cells with substance 48/80 (Irman-Florjanc and Erjavec, 1983) results in a moderate decrease in blood flow and an inhibition of vasomotion. Rat mast cells contain several factors that may influence blood flow, including histamine and 5-HT (Lombard des-Couttes *et al.*, 1974). Histamine apparently does not have a pronounced influence on testicular blood flow (only very high doses caused a slight increase). In contrast, 5-HT caused a dose-dependent decrease in blood flow. It is therefore not unlikely that the effect of substance 48/80 could be mediated by release of 5-HT from testicular mast cells. The physiological regulation of testicular mast cell secretions is unknown, but their numbers are increased in infertile testes (Nagai *et al.*, 1992). Morales and Heyn (1992) suggested that hCG treatment causes degranulation of testicular mast cells 6 h and 24 h after

Fig. 2. Sections showing the tissue adjacent to the subcapsular part of the rat testicular artery (A), in (a) a control animal and (b) 20 min after local injection of 10 μ g substance 48/80. Several mast cells with numerous granules are observed in the control testis (arrowheads) but only degranulated (small arrowheads) or degranulating cells (large arrowheads) were observed after administration of substance 48/80. Scale bars represent 25 μ m.

treatment, a response apparently analogous to the adreno-corticotrophic hormone- and LH-induced mast cell degranulation in the adrenal cortex (Hinson *et al.*, 1989) and ovary (Krishna *et al.*, 1989), respectively. Testicular blood flow is decreased 6 h after LH/hCG treatment (van Vliet *et al.*, 1988; Widmark *et al.*, 1989), but whether this is related to release of 5-HT remains unknown. Treatment with LH/hCG also induces an inflammation-like increase in vascular permeability in the testis (Bergh and Damber, 1993), but this response is probably not related to release of mast cell products like 5-HT and histamine, since local injection of these substances into the testis does not influence vascular permeability (Bergh and Söder, 1990; Setchell *et al.*, 1988).

The concentration of 5-HT in rat plasma is reported to be 3.9 ng ml⁻¹ and in testicular interstitial fluid to 23 ng ml⁻¹ (Campos *et al.*, 1990). The high content in interstitial fluid could be an overestimation, as 5-HT could have been released from cells during the collection of interstitial fluid (Maddocks and Setchell, 1988). Testicular 5-HT probably originates from at least three different sources: testicular mast cells (Lombard-des Couttes *et al.*, 1974), testicular nerves (Campos *et al.*, 1990) and Leydig cells (Tinajero *et al.*, 1993b). The relative contributions from these sources are unknown, and there is no information available on whether intratesticular 5-HT concentration *in vivo* is influenced by physiological regulators of testicular function. The only information available is that denervation reduced intratesticular 5-HT by 34% (Campos *et al.*, 1990). It has been

shown, however, that 5-HT could be an important autocrine or paracrine regulator of Leydig cell function (Dufau *et al.*, 1993; Tinajero *et al.*, 1993a, b). It is secreted by Leydig cells in response to hCG stimulation *in vitro*; Leydig cells have 5-HT receptors and 5-HT, by increasing Leydig cell corticotrophin releasing factor secretion, inhibits Leydig cell testosterone secretion (Tinajero *et al.*, 1993). In addition, 5-HT could be an important regulator of testicular blood flow. Local injection of 5-HT, in doses similar to the testis content, decreased in a dose-dependent way testicular blood flow and inhibited vasomotion. Local injection of the 5-HT antagonist into intact testes did not, however, induce any significant changes in blood flow, perhaps suggesting that, under basal conditions, 5-HT is stored in cells and is only released when needed. Studies have shown that 5-HT causes constriction of the subcapsular testicular artery (Free, 1977; Davis, 1992; Setchell *et al.*, 1988), and we have confirmed this observation *in vivo* with microscopy (O. Collin, J-E. Damber and A. Bergh, unpublished). The present observation that 5-HT inhibits vasomotion indicates that it also influences smaller intra-testicular arterioles.

Indirect evidence suggests that the testis could be particularly susceptible to reductions in blood flow. The oxygen concentration in the seminiferous tubules is low and owing to the long and rather narrow artery, capillary pressure in the testis is only marginally higher than venous pressure (Setchell, 1990; Sweeney *et al.*, 1991). Moderate reductions in capillary

Fig. 3. Blood flow recording (in arbitrary perfusion units, PFU) (a) in a testis injected with 6 ng 5-HT, and (b) in a testis injected with saline. Almost immediately after 5-HT injection (—), flow is reduced and vasomotion inhibited. Saline injection did not influence blood flow.

pressure and inhibition of vasomotion (vasomotion facilitates transvascular fluid exchange; Intaglietta, 1988) could consequently have adverse effects on the testis (Bergh and Damber, 1993). The observations that the testis produces 5-HT and that it is particularly sensitive to this vasoconstrictor could therefore be of importance for testicular pathophysiology. 5-HT is released during stress; it is increased in the testis in pathological conditions such as varicocele and during treatment with drugs like propranolol (Dufau *et al.*, 1993; Tinajero *et al.*, 1993b) and previous studies have demonstrated testicular atrophy after administration in high doses (see Free, 1977). The testis also produces another highly potent vasoconstrictor, endothelin-1, in response to hCG stimulation (Collin *et al.*, unpublished) and in other vascular beds endothelin-1 and 5-HT have synergistic effects (Yang *et al.*, 1990, 1992). Stimulation of rat testis with high doses of hCG may induce focal degeneration of seminiferous tubules (van Vliet *et al.*, 1988), but it remains to be shown whether this could be caused by an hCG-induced secretion of locally produced vasoconstrictors.

This study was supported by grants from the Swedish Medical Research Council (project no. 5935) and the Maud and Birger Gustavsson Foundation. The 5-HT antagonists were kindly donated by Jansen Research Foundation. Skilful technical assistance was provided by S. Kilter.

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