Testicular blood flow and testosterone concentrations in spermatic venous blood of anaesthetized rats

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Summary. Testicular blood flow was measured by a radioactive microsphere technique. A significant correlation, $r_s = 0.82$, was found between testicular blood flow and testosterone outflow in the spermatic venous blood, indicating that factors which affect the testicular circulation may influence testicular endocrine function.

Introduction

Many factors are known to affect testicular blood flow. Infusion of epinephrine or norepinephrine into the spermatic artery of rats (Free & Jaffe, 1972a) or rams (Setchell, Waites & Thorburn, 1966) leads to rapid vasoconstriction with a marked transient decrease in blood flow through the testis, and isoproterenol causes vasodilatation and increased blood flow in rams. Prostaglandins E-2 and F-2 α have been reported to decrease testicular blood flow in conscious rats (Free & Jaffe, 1972b). Other agents which decrease testicular blood flow appear to be serotonin (Boccabella, Salgado & Alger, 1961) and cadmium (Setchell & Waites, 1970; Johnson & Turner, 1972), but the role of these compounds in the normal function of the testis remains obscure.

Eik-Nes (1964), using an in-situ perfusion model of the testis, found a positive correlation between perfusion rate and secretion of testosterone in the dog. This finding and the observations that epinephrine (Levin, Lloyd, Lobotsky & Friedrich, 1967) and prostaglandins (Saksena, Safoury & Bartke, 1973) depress plasma testosterone levels suggest that these effects may be mediated through an influence on the testicular vascular bed.

The main purpose of the present study therefore was to study *in vivo* the relationship between testicular blood flow and the output of testosterone in the spermatic vein.

Materials and Methods

Male rats of the Sprague–Dawley strain (Møllegaard-Hansen, Ejby, Denmark), weighing 300–400 g, were kept in a controlled environment (temperature: 25°C, light 05:00–19:00 h). Standard rat pellets and water were always available. The anaesthetic used was sodium pentobarbitone (Nembutal: Abbott) given i.p. as a single dose of 40 mg/kg. The animals were kept supine during each experiment, and body temperature was maintained by a heating pad.

Measurement of testicular blood flow

The use of radioactive microspheres has been shown to be suitable for testicular blood flow measurements in rats (Damber & Janson, 1977). Radioactive 'carbonized' microspheres with a diameter of 15 ± 5 (range) μm were purchased from 3M Co. (St. Paul, Minnesota, U.S.A.). The microspheres were labelled with ytterbium-169 (^{169}Yb) or scandium-46 (^{46}Sc). The initial specific activity of ^{169}Yb was 10.07 mCi/g, and that of ^{46}Sc was 8.63 mCi/g. Under anaesthesia catheters (0.6 mm i.d., 1 mm o.d., PE-50: Intramedic, Clay Adams, U.S.A.) were inserted into the right brachial artery, the tail artery and into the left ventricle or the ascending aorta via the left carotid artery. The catheters were filled with a solution (1:10 v/v) of heparin (500 i.u./ml) and 0.15 M-NaCl. The brachial arterial

catheter was connected to a Statham P23 AC transducer attached to a Grass Model 7 Polygraph. Approximately 50 000 spheres, suspended in 20% (w/v) dextran, were transferred to a glass chamber of a volume of 0.9 ml. The design of the chamber and the handling of it are described in detail by Rudolph & Heymann (1967). The chamber containing the sphere suspension was vigorously agitated with a high-frequency mechanical stirrer and the suspension was flushed into the heart for 30 sec with 1 ml 0.15 M-NaCl. As reference samples, blood was withdrawn at a constant rate (0.8 ml/min) from the tail artery catheter for 15 sec before, during and after the infusion of microspheres.

Experiment 1. Seventeen rats were treated with a single infusion of microspheres (^{169}Yb or ^{46}Sc). Blood from the right spermatic vein was collected into pre-weighed glass tubes for $2\frac{1}{2}$ min before and $2\frac{1}{2}$ min after the start of the infusion. The right internal spermatic vein was cannulated at midline laparotomy with a PE-50 catheter inserted at least 5 mm from the point of entry into the caval vein. The catheter, 3–4 cm in length, was fixed so that the open end was at the level of the caval vein. The total blood volume collected during 5 min was calculated by dividing the difference in tube weight, before and after blood collection, with the specific weight of rat whole blood (1.05; J.-E. Damberg, unpublished). The plasma volume was calculated from the haematocrit of rat blood (45%; J.-E. Damberg, unpublished). The outflow of testosterone in the spermatic vein was calculated by multiplying the venous plasma flow with the plasma concentration of testosterone. After blood collection, the rats were killed by an overdose of sodium pentobarbitone and dissected. Radioactivities of the 'reference' samples and individual organs and tissues were measured in an automatic well scintillation counter (Packard Auto-Gamma). Each sample was counted for 3 min. By counting standards of known number of microspheres from the same batches, the number of spheres in the various samples and tissues could be calculated. Blood flow (F) was calculated, as described by Rudolph & Heymann (1967), as $(Q_{\text{ref}} \times N_{\text{organ}})/N_{\text{ref}}$, where Q_{ref} = rate of withdrawal of the reference sample, N_{organ} = number of spheres present in the organ and N_{ref} = number of spheres present in the reference sample. The accuracy and precision of blood flow measurement by the microsphere technique were calculated as described by Buckberg *et al.* (1971).

Experiment 2. The effect of laparotomy on testicular blood flow was tested. In 6 rats testicular blood flow was measured by use of ^{169}Yb -labelled spheres, and, after laparotomy and exposure of the right spermatic vein, by using spheres labelled with ^{46}Sc .

Testosterone assay

Testosterone concentrations in spermatic venous plasma were measured by radioimmunoassay. The antiserum was raised against testosterone-3-oxime-BSA and had a cross-reaction of 40% with 5α -dihydrotestosterone. [$1,2,6,7\text{-}^3\text{H}$]Testosterone (sp. act. 110 Ci/mmol: New England Nuclear, Boston, Massachusetts, U.S.A.) was purified in the laboratory by paper chromatography (Bush, 1961) and used for assay and recovery reagent. Plasma (50 μl) was extracted 3 times with 5 ml diethyl ether. The testosterone antiserum was diluted 1:30 000 in borate buffer (0.05 M, pH 8.0) containing gelatin (1 mg/ml), human gamma-globulin (0.1 mg/ml) and 1.3×10^{-7} Ci [$1,2,6,7\text{-}^3\text{H}$]testosterone/ml. The antiserum was added to the diethyl ether fraction after evaporation of the solvent. The samples were incubated for 3 h at 20°C. Blanks and standard samples of testosterone up to 3 ng were assayed in duplicate together with the experimental samples. Separation of bound and unbound steroid was achieved by using saturated ammonium sulphate. Liquid scintillation counting was performed in polyethylene vials (New England Nuclear) containing 10 ml of a solution of 27.5 g Permablend (Packard, scintillation grade) dissolved in 5 litres toluene. The testosterone concentrations were determined from the standard curve which was fitted by the equation given by Leclercq, Täljedal & Wold (1971).

To test the influence of cross-reacting steroids in the assay a paper chromatographic step, as described by Carstensen & Bäckström (1976) was used ($n = 6$). Since no significant difference in testosterone concentration was obtained in comparison with a direct assay, the experimental samples were assayed without chromatographic separation. Water blank values were close to zero and were therefore not considered in the calculations. The recovery for the extraction of testosterone was $92.1 \pm 0.84\%$ and the coefficient of variation for between-assay duplicate samples was 5.7%. The

validity of the testosterone radioimmunoassay was further tested by adding known amounts of testosterone to charcoal-treated human female plasma: when 0.75, 1.0 and 1.5 ng testosterone were added, the mean recoveries were 0.74, 0.97 and 1.36 ng, respectively. The sensitivity of the assay was 0.2 ng/ml.

Results

The mean $\pm$ s.e.m. concentration of testosterone in spermatic vein plasma in Exp. 1 was 43 ± 1.9 ng/ml ($n = 17$).

Table 1. Organ blood flows (mean $\pm$ s.e.m., no. of observations in parentheses) in anaesthetized male rats prepared with right spermatic vein cannulation

Organ	Right testis	Left testis	Right epididymis	Left epididymis	Ventral prostate	Kidneys
Blood flow (ml/100 g min ⁻¹)	$6.0 \pm 0.8^*$ (17)	14.5 ± 1.8 (17)	7.8 ± 1.3 (7)	9.6 ± 1.9 (7)	21.6 ± 2.6 (14)	261.0 ± 25.5 (17)

Mean arterial blood pressure during the measurements was 78 ± 5 mmHg.

* Significantly different ($P < 0.01$, Wilcoxon's paired t test) from value for left testis.

Text-fig. 1. The correlation between testicular blood flow, measured by a radioactive microsphere technique, and the outflow of testosterone in the spermatic venous blood of anaesthetized rats.

Arterial blood pressure and heart rate were unaffected by the injections of microspheres. The blood flows to various organs are shown in Table 1. Although the blood flow of the right (cannulated) testis was lower than that of the left (intact) testis, no significant difference was observed between the right and left epididymis. Of the flow in the spermatic vein, 48.5 ± 3.4 (s.e.m.) % represented testicular flow. The relationship between testicular blood flow and outflow of testosterone (ng/min) in the spermatic vein is shown in Text-fig. 1, and the correlation ($r_s = 0.82$) was significant ($P < 0.005$, Spearman rank correlation coefficient). In Exp. 2, the mean ($\pm$ s.e.m.) testicular blood flow before laparotomy was 23.7 ± 4.2 ml/100 g min⁻¹ and was reduced after laparotomy to 11.6 ± 1.6 ml/100 g min⁻¹. The significant reduction ($P < 0.05$, Wilcoxon's paired *t*-test) of testicular blood flow (43.4 ± 11.2 %) was associated with a reduction in systemic blood pressure of 36.8 ± 5.0 %.

Discussion

In the present study blood flow was probably considerably below physiological levels. Two main factors may account for this. First, blood flow on the side with the spermatic vein cannula was lower than that on the other side. Second, blood flow was significantly reduced by laparotomy and this must also have affected the intact, non-cannulated testis. The reasons for the blood flow reduction, i.e. arterial spasm due to manipulation, increased venous pressure due to cannulation, and decreased systemic arterial pressure due to anaesthesia and laparotomy appear to be unavoidable factors with the present experimental approach. The approach is useful, however, for testicular blood flow measurements provided that the technique correctly reflects the true flow. From the present results, it appears probable that this is the case because the value of 50 ± 9.8 % measured by direct venous cannulation (Damber & Janson, 1977) compares well with that of 48.5 ± 3.4 % obtained for the testicular component of the outflow from the spermatic vein in the present study. The values for the blood flow from the 'cannulated' testis may therefore be considered to represent true flow.

The concentration of testosterone in spermatic venous plasma of the rat was in good agreement with the value reported by Knorr, Vanha-Perttula & Lipsett (1970), but was about 40 % higher than that reported by Free & Tillson (1973). However, the peripheral concentrations of testosterone found by Free & Tillson (1973) were only 10–20 % of values obtained in our laboratory (Carstensen, Marklund, Damber, Näsman & Lindgren, 1976) and those reported by others (Bartke, Steele, Musto & Caldwell, 1973; Ghanadian, Lewis & Chisholm, 1975).

The positive correlation between testicular blood flow and outflow of testosterone in the spermatic vein found in this study under in-vivo conditions confirms the tendency observed by Free & Tillson (1973), and indicates that factors which influence the tonus of the blood vessels of the testis may indirectly affect the synthesis and/or secretion of testosterone. Nguyen Duc Kien, Hamlin & Gomes (1976) found a parallel reduction of plasma testosterone concentration and testicular blood flow after infusion of prostaglandin F-2 α to rabbits and similar results were obtained for the rat by infusion of prostaglandin E-2 (Free & Tillson, 1973). One reason why Free & Tillson (1973) did not obtain the same strong correlation as in the present study may be because their material included conscious and anaesthetized animals. The conscious animals had a lower rate of testosterone secretion than the animals kept under anaesthesia, although there was no difference in testicular blood flow. Testosterone release is a passive phenomenon dependent on rapid diffusion and at high flow rates, like those obtained by Free & Tillson (1973), testosterone release may be diffusion-limited, while at lower flow rates, as in the present study, the release may be flow-limited.

It is known (Bartke *et al.*, 1973) that plasma testosterone concentrations normally fluctuate during the day with episodes as short as 30 min. Although the LH value is the main factor responsible for basal plasma testosterone concentrations, it is possible that circulatory variations may contribute to fluctuations in plasma testosterone concentrations.

Work is now in progress with this in-vivo experimental model to investigate the influence of substances such as hormones and vasoactive agents on testicular blood flow and the related testosterone concentrations.

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