Testicular Blood Flow in Young and Old Rats and Influence of hCG

U. PUNJABI, J. VAN HOECKE, L. VERDONCK, AND A. VERMEULEN

From the Academic Hospital, University of Ghent, Department of Endocrinology, Ghent, Belgium

To evaluate the influence of age, testicular capillary blood flow (TCBF) was measured, using the microsphere technique, in rats 3 to 24 months old, under basal conditions and after hCG stimulation (10 IU/d for two days). Despite a decline in plasma T levels, testicular capillary blood flow did not decrease with age, and hCG stimulation resulted in similar increases of approximately 50% ($P < 0.01$) in testicular capillary blood flow in all age groups. We concluded that the age-associated decrease in testosterone secretion in rats is not the consequence of a decreased testicular blood flow.

Key words: testis, blood flow, hCG, rat aging.

J Androl 1984; 5:223–226.

Sexual maturation, gonadotropic hormones, testicular blood flow, senescence and season are known to alter testosterone secretion in males (Setchell, 1978; Wong et al, 1983). In old rats there is an age-dependent decrease in testosterone secretion (Ghanadian et al, 1975; Chan et al, 1977). It has been reported that in contrast to the increase in LH levels that occur with age in man, LH levels in aging rats are slightly decreased (Gray, 1978; Pirke et al, 1978; Geisthövel et al, 1981).

Since it has been observed that LH as well as hCG increase testicular capillary blood flow (Janson, 1975; Damber and Janson, 1978 a; b; Pirke et al, 1979), we investigated whether aging in rats is accompanied by a decrease in testicular capillary blood flow as measured by the microsphere technique, and whether this is restored by hCG stimulation. Such a study was performed by Pirke et al (1979) using the Xenon-washout technique, but the values reported by these authors are much higher than those reported in the literature, probably because the method was not appropriate.

Supported by grant 3.0014.78 of the F.W.G.O.

Reprint requests: A. Vermeulen, Academic Hospital, Department of Endocrinology, University of Ghent, De Pintelaan 185, B-9000, Ghent, Belgium.

Submitted for publication August 22, 1983; accepted for publication November 17, 1983.

Materials and Methods

Male Wistar rats were obtained at ten weeks of age and all were kept at the animalarium under natural light-dark schedule, with water and food available *ad libitum*.

When the animals had reached the appropriate age (three, six, 12, and 21 to 24 months; 12–16 per group), testicular capillary blood flow was determined. In order to eliminate any effect of season on testicular capillary blood flow and plasma testosterone levels, a group of six-month-old rats was also studied simultaneously with the 12 and 21–24 months old rats. However, neither testicular capillary blood flow nor plasma T (obtained in different seasons) showed significant differences in the different groups of six-month-old rats.

Testicular capillary blood flow was measured using the microsphere technique (Damber and Janson, 1977). Microspheres of 15 ± 5 μm diameter, labelled with either Ce^{141}, Ru^{103} or Nb^{95}, were obtained from New England Nuclear, Germany.

Rats were anesthesized with Nembutal and the left cardiac ventricle was catheterized, via the left carotid artery, for injection of microspheres. The femoral artery was catheterized to record blood pressure via a Beckman dynograph Type RS-2. Blood was sampled from the tail artery. The catheters were filled with a solution of heparin and saline (1:10). Body temperature was kept constant with a thermal cushion. The solution, containing a drop of Tween 80 and the radioactive microspheres in 20% dextran (w/v), was agitated using a mechanical stirrer (Rudolph and Heyman, 1967); 25 μl of the suspension, together with 175 μl saline, were flushed into the heart within 10 seconds. Starting prior to, and continuing during and 1 minute after the microsphere injection, blood was withdrawn at a constant rate (R = 0.786 ml/min) via the tail arterial catheter. The testes were removed and weighed and radioactivity was determined. Cardiac output (C.O.) was obtained from the formula:

$$\text{C.O. ml/min} = \frac{I \times R}{B}$$

where I = radioactivity injected; R = rate of blood withdrawal; B = radioactivity in blood withdrawn and

$$\text{TCBF ml/min/gm} = \frac{\text{C.O.} \times T}{I}$$

where C.O. = cardiac output in ml/min; T = radioactivity in testes; I = radioactivity injected.

Blood flow values were rejected if a testis contained <400 microspheres, or if the blood pressure was not stable. Plasma testosterone was measured by RIA (Vermeulen, 1973). Significance of differences was assessed by Student's t-test.

Results

In the first experiment, the influence of hCG dose on testicular capillary blood flow and plasma T in anesthetized rats was studied. Since sodium pentobarbital (Nembutal®) is known to only slightly affect systemic and regional hemodynamics (Manders and Vatner, 1976), it was chosen as the anesthetic in this experiment, at an intraperitoneal dose of 0.25 ml (0.06%)/300 g body weight.

Five groups of eight to 16 adult (≥ six months old) rats were injected i.m. for two days with saline containing 0.2, 0.5, 10 or 100 I.U. hCG. Testicular capillary blood flow was determined 24 hours after the last injection. With the exception of the 0.5 I.U. dose, increasing doses of hCG caused a significant increase in blood flow (Fig. 1). Since there was no difference in testicular weight between hCG treated and untreated animals, the results are similar whether expressed in ml/g/min or in ml/testis/min. At the time of testicular capillary blood flow measurement, plasma testosterone levels were increased after 2, 10 and 200 I.U., respectively, but not 0.5 I.U. of hCG (24 hours post-injection) (Fig. 1). As 10 I.U. of hCG, administered twice with an interval of 24 hours, resulted in a clearcut increase in testicular capillary blood flow in young rats, the second experiment employed that dose to study the influence of age and the response of testicular capillary blood flow to hCG. In four groups of rats, aged three, six, 12, and 21–24 months, respectively, blood flow was measured under basal conditions and 24 hours after hCG treatment. As can be seen from Table I, testicular capillary blood flow expressed in ml/g/min *was similar at different ages;* plasma T levels, on the other hand, were significantly lower in the 12- and 21–24-month-old rats than in the three to six-month-old rats.

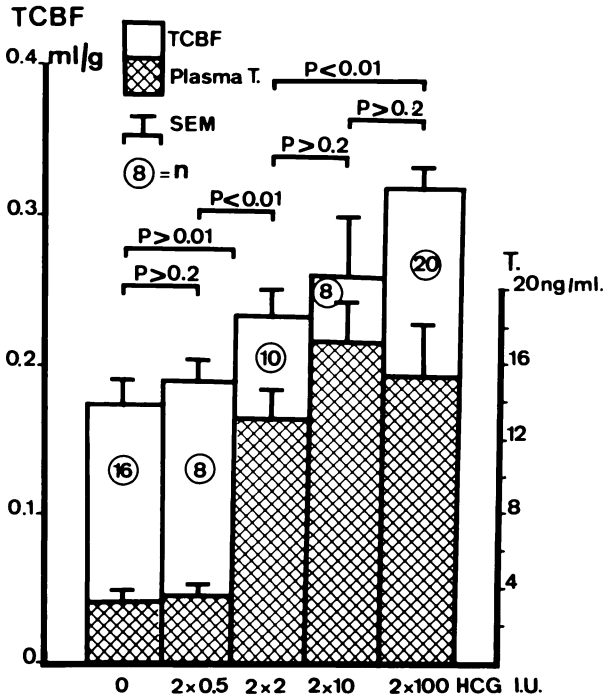

Fig. 1. Influence of increasing doses of hCG i.m. on testicular capillary blood flow (TCBF), measured by the radioactive microsphere technique, and plasma testosterone.

Testicular weight increased slightly with age (Table 1), but although the mean testicular capillary blood flow (ml/testis/min) in the eldest group was slightly higher than in the younger animals, this increase did not reach statistical significance ($P > 0.05$). After hCG stimulation (2 × 10 IU I.M.), a hightly significant ($P < 0.01$) increase in testicular capillary blood flow, of about 50% over basal values, was observed in all age groups (Fig. 2).

Discussion

Our values for testicular capillary blood flow in adult rats under basal conditions (0.190 ± 0.002 (SE) ml/g, n = 54) are in agreement with those reported by Damber and Janson (1977), using the same method, and with those reported by others using different methods (Waites and Setchell, 1966; Joffre and Joffre, 1971). Significantly higher values (0.27–0.37 ml/g/min) were obtained by Einer-Jensen and Soofi (1974) and by Pirke et al (1979) (0.505 ± 0.12 ml/g/min) using the inert gas clearance technique; in a more recent paper, however, Pirke et al (1982) reported a testicular capillary blood flow of 0.273 ± 0.038 (SEM) ml/g/min.

TABLE 1. Influence of Age on Mean Body Weight, Testicular Weight, TCBF* and Plasma T in Rats

Age (Months)	N	Body Weight ($\bar{M}$ ± SD)	Testicular Weight (g) ($\bar{M}$ ± SD)	TCBF† ml/g/min ($\bar{M}$ ± SEM)	TCBF† ml/Testis/min ($\bar{M}$ ± SEM)	Plasma T ng/ml ($\bar{M}$ ± SE)
3	12	383 ± 17	1.80 ± 0.05	0.195 ± 0.014	0.353 ± 0.029	269 ± 37
6	16	430 ± 22	1.89 ± 0.24	0.192 ± 0.007	0.362 ± 0.013	311 ± 16
12	14	524 ± 60	2.08 ± 0.30	0.196 ± 0.014	0.371 ± 0.033	157 ± 32
21–24	16	457 ± 26	2.03 ± 0.09	0.221 ± 0.016	0.426 ± 0.031	115 ± 12

* Testicular capillary blood flow.
† Measured by the radioactive microsphere technique using microspheres 15 ± 5 μm labelled with CE^{141}, Ru^{103} or Nb^{95}.

Direct measurement by cannulation of the spermatic vein yields much lower values than indirect methods, due to the fact that cannulation and laparatomy reduce testicular blood flow (Damber and Janson, 1978a). Furthermore, only 50% of spermatic vein blood represents testicular blood flow, the rest originating from the epididymis, and the fat and connective tissue along the vascular pedicle (Damber and Janson, 1977).

Up to 24 months, age did not influence testicular capillary blood flow in our study. Pirke et al (1979), on the other hand, observed that the capillary blood flow in 24-27-month-old rats was significantly lower than in three-month-old rats, but their method probably overestimates testicular blood flow. Although it has been suggested that aging in rats starts at 22–28 months (Geisthovel et al, 1981), and despite unchanged testicular capillary blood flow, plasma T levels in our 12, 21, and 24-month-old rats were significantly lower than in six-month-old rats. Decreased testicular capillary blood flow, therefore, cannot be responsible for decreased testosterone secretion, a conclusion also supported by the absence of signs of atherosclerosis in the testicular blood vessels of two-year-old rats, as reported by Pirke et al (1979). In agreement with the data in the literature (Pirke et al, 1979; Damber and Janson, 1978b; Damber et al, 1981; Setchell and Sharpe, 1981), we observed a significant increase of testicular capillary blood flow after hCG stimulation, the increase being significant at a dose of 2 I.U. I.M./day for two days.

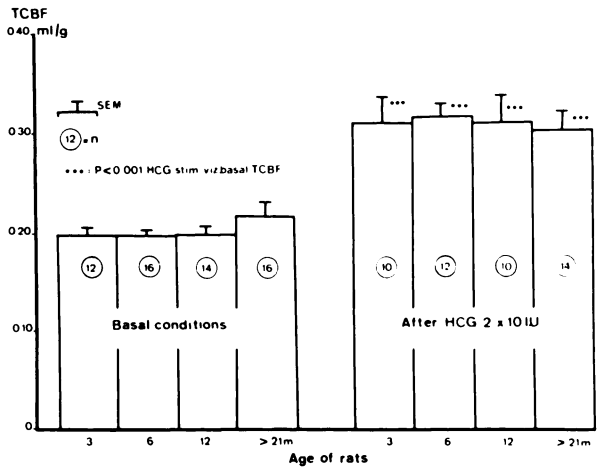

Fig. 2. Testicular capillary blood flow (TCBF) in rats of different ages (three, six, 12 and 21–24 months, respectively) under basal conditions and after hCG 2 × 10 I.U. intramuscularly, measured by the radioactive microsphere technique.

Damber et al (1981) reported an increase in testicular capillary blood flow from ±0.25 ml/g/min to ±0.65 ml/g/min 20–24 hours after 100 I.U. hCG, which is significantly higher than the blood flow observed in this study after 10 I.U. hCG. Surprisingly, in a recent paper Pirke et al (1982) did not observe an increase in testicular capillary blood flow after hCG, which contradicts the data from an earlier paper (Pirke et al, 1979). An even more important increase in rat ovarian blood flow after LH stimulation has been reported by Janson (1975). In other species, the immediate effects of LH on testicular blood flow could not be demonstrated (Janson, 1975; Nguyen Duc Kien, 1977).

As to the mechanism of this increase, Hartman et al (1950) suggested on morphological grounds that vasodilation occurs in the rat testis in response to hCG and Setchell and Sharpe (1981) reported that hCG increases capillary permeability to albumin in normal testes beginning before any change in blood flow.

Using the formula suggested by Damber and Janson (1978b):

$$\text{Vascular resistance} = \frac{\text{mean arterial BP (mmHg)}}{\text{TCBF (ml/100g/min)}}$$

we also observed a significant decrease in testicular vascular resistance after hCG treatment, from 7.87 ± 0.49 mm Hg/100g/min to 4.91 mm Hg/ml/100g/min.

Testicular capillary blood flow after hCG was similar in young and old animals, the increase over basal values being around 50%. Pirke et al (1979) similarly observed identical testicular capillary blood flow after hCG stimulation in 24–27-month-old rats and in younger rats, notwithstanding the significantly lower basal capillary blood flow in old rats. These authors therefore concluded that the decline in plasma T observed in old rats was due only to the lowered LH levels, which would cause the decrease in testicular capillary blood flow. Whereas extrapolating data obtained with a pharmacologic dose of hCG to the physiologic effects of LH in old rats is certainly hazardous, our data show conclusively that the decrease in plasma T in old rats is not the consequence of a decrease in testicular capillary blood flow. Indeed, there is a clearcut age-dependent decrease in plasma T levels, whereas testicular capillary blood flow does not change with age, either under basal conditions or after hCG stimulation.

Acknowledgment

The authors thank Professor Leusen, Head of the Department of Physiology, for permission to use the facilities of his laboratory for measurement of organ blood.

References

Chan SWC, Leathem JH, Esashi T. Testicular metabolism and serum testosterone in aging male rats. Endocrinology 1977; 101:128–133.

Damber JE, Janson PO. Methodological aspects of testicular blood flow measurements in rats. Acta Physiol Scand 1977; 101:278–285.

Damber JE, Janson PO. Testicular flow and testosterone concentrations in spermatic venous blood of anaesthesized rats. J Reprod Biol 1978a; 52:265–269.

Damber JE, Janson PO. The effects of LH, adrenaline and noradrenaline on testicular blood flow and plasma testosterone concentrations in anesthesized rats. Acta Endocrinol 1978b; 88:390–396.

Damber JE, Selstam G, Wang J. Inhibitory effect of estradiol-17β on human chorionic gonadotropin induced increment of testicular blood flow and plasma testosterone concentrations in rats. Biol Reprod 1981; 25:555–559.

Einer-Jensen N, Soofi G. Decreased blood flow through rat testes after intratesticular injection of PGF2. Prostaglandins 1974; 7:377–386.

Geisthövel F, Brabant G, Wilkings EJ, Nieschlag E. Changes in testicular hCG binding and Leydig cell function in rats throughout life. Horm Res 1981; 14:47–55.

Ghanadian R, Lewis YG, Chisholm GD. Serum testosterone and dihydrotestosterone changes with age in rats. Steroids 1975; 25:753–762.

Gray GD. Changes in levels of luteinizing hormone and testosterone in the circulation of ageing male rats. J Endocrinol 1978; 76:551–552.

Hartman CG, Millman N, Stavorski J. Vasodilation of the rat testis in response to human chorionic gonadotropin. Fertil Steril 1950; 1:443–453.

Janson PO. Effects of the luteinizing hormone on blood flow in the follicular rabbit ovary, as measured by radioactive microspheres. Acta Endocrinol 1975; 79:122–133.

Joffre M, Joffre J. Débit sanguin testiculaire chez le rat: mise en évidence sur les courbes de "clearance" du Xenon^{133} d'une composante liée aux graisses épididymaires. C R Hbd Séances Acad Sci 1971; 273:496–508.

Manders WT, Vatner SF. Effects of sodium pentobarbital anesthesia on left ventricular function and distribution of cardiac output in dogs, with particular reference to the mechansim of tachycardia. Circ Res 1976; 39:512–577.

Nguyen-Duc-Kien. Testicular blood flow and testosterone secretion rates in rabbit and rat. PhD Dissertation. Ohio State University, mentioned by Free MF. Blood supply of the testes and its role in local exchange and transport of Hormones. In: Johnson AD, Gomes WR, eds. The testes. Vol. IV. New York: Academic Press, 1977; 39–90.

Pirke KM, Bofilias I, Sintermann R, Langhammer H, Wolf I, Pabst HW. Relative capillary blood flow and Leydig cell function in old rats. Endocrinology 1979; 105:842–845.

Pirke KM, Bofilias I, Spyra B, Langhammer H, Pabst HW. Capillary blood flow in the testes and testosterone secretion in the starved rat. Experientia 1982; 38:516–517.

Pirke KM, Vogt HJ, Geiss M. In vitro and in vivo studies on Leydig cell function in old rats. Acta Endocrinol 1978; 89:393–403.

Rudolph AM, Heyman MA. The circulation of the fetus in utero: methods for studying distribution of blood flow, cardiac output and organ blood flow. Circ Res 1967; 21: 163–184.

Setchell BP. The mammalian testis. London, Ithaca: Elek and Cornell University Press, 1978.

Setchell BP, Sharpe M. Effect of injected human chorionic gonadotrophins on capillary permeability, extracellular fluid volume and the flow of lymph and blood in the testes of the rat. J Endocrinol 1981; 91:245–254.

Vermeulen A. Determination of androgens in plasma. In: The endocrine function of human testis. Vol. I. New York: Academic Press, 1973; 91–104.

Waites GMH, Setchell BP. Changes in blood flow and vascular permeability in the testis, epididymis and accessory reproductive organs of the rat after the administration of Cadmium chloride. J Endocrinol 1966; 34:329–342.

Wong CC, Döhler KD, Atkinson MJ, Geerlings H, Hesch RD, Von zur Mühlen A. Circannual variation in serum concentration of pituitary, thyroid, parathyroid gonadal and adrenal hormones in male laboratory rats. J Endocrinol 1983; 97:179–185.