Capillary blood flow in the testes and testosterone secretion in the starved rat

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Summary. The average capillary blood flow in the testes was found to be 181 µl/min/g testis tissue (n = 19) in rats starved for 5 days and 273 µl/min/g (n = 18, $p < 0.01$) in the control group. Plasma testosterone was significantly decreased in the starved animals (1.00 ± 0.06 ng/ml vs 5.43 ± 0.63 ng/ml). When starved and control rats were stimulated with human chorion gonadotropin, testosterone values in plasma were greatly increased in both groups. The capillary blood flow was not altered. The data indicate that human chorion gonadotropin can stimulate testosterone production in the starved rat without influencing the reduced capillary blood flow.

In male and female patients with anorexia nervosa an impairment of gonadal function occurs rather early in the course of the disease[1]. Since this endocrine dysfunction is a consequence of starvation[2], we studied the male Wistar rat as an animal model, in order to evaluate the underlying pathophysiological mechanisms. In this species starvation causes a decreased gonadal hormone secretion[3].

Eik-Nes[4] described a relationship between testicular blood flow and testosterone secretion. We report here studies on testicular capillary blood flow and on plasma testosterone in the starved rat, which indicate that an impairment of blood flow may contribute to the decrease of testosterone secretion during starvation.

Material and methods. Male Wistar rats (200–220 g b.wt) were kept in groups under a 10 h dark and 14 h light schedule. Water and Purina rat chow® were available ad libitum for the control groups and water only for the starved rats.

Experiment 1. 24 rats were starved for 5 days, while the same number of animals served as controls. On the last 3 days, 10 animals from each group were injected with 10 IU of human chorion gonadotropin (HCG) per day, while the rest of each group was injected with saline. The animals were decapitated between 9.00 and 12.00 h. Trunk blood was collected. Testosterone was measured by radioimmunoassay[5]. The interassay variability was 7.1%, at an average concentration of 3.25 ng/ml.

Experiment 2. Testicular capillary blood flow was measured by means of the inert gas clearance technique[6]. The technique was described in detail by our group earlier and will only be outlined here in general.

Table 1. Body weight and testosterone in plasma of starved and control rats. Groups 3 and 4 were stimulated with 10 IU of HCG for 3 days

		Group 1 control	Group 2 starved	Group 3 control +HCG	Group 4 starved +HCG
	n	14	14	10	10
Final body weight g	$\bar{x}$	243.9	160.7	228.0	148.5
	SEM	2.7	1.6	3.5	1.3
Testosterone ng/ml	$\bar{x}$	5.43	1.00	26.58	68.38
	SEM	0.63	0.06	1.77	4.78

Table 2. Testicular capillary blood flow (TCBF) in starved and control rats with and without HCG-stimulation

		Control	Starved	Control +HCG	Starved +HCG
	n	8	8	10	10
Final body weight g	$\bar{x}$	208.8	173.8	215.5	175.0
	SEM	4.0	2.1	2.5	2.9
TCBF µl/min^{-1}/g^{-1}	$\bar{x}$	273	182	271	181
	SEM	38	17	27	15

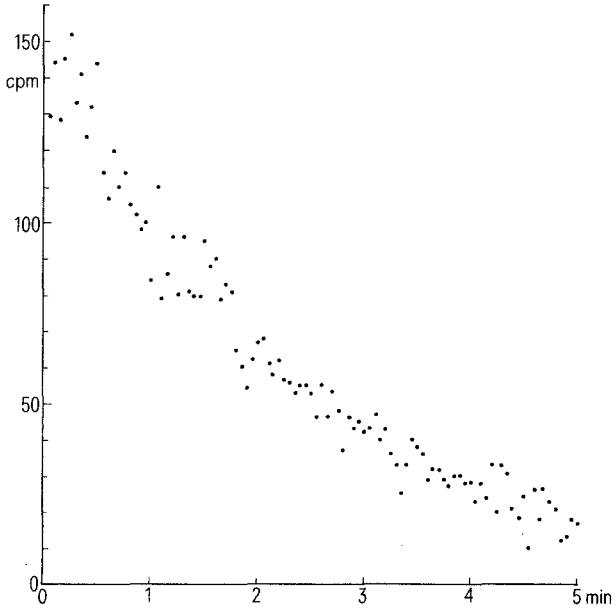
8 control rats and 8 rats starved for 5 days were studied without stimulation, while 10 control rats and 11 starved rats received a daily s.c. injection of 10 IU HCG for 3 days prior to blood flow measurement. The blood flow was measured between 17.00 and 21.00 h on the day of the last HCG injection. Rats were anesthetized by i.p. injection of Ketanest® (100 mg/kg b.wt). For each measurement 6 rats were placed on their backs directly on the collimator. 50 µl 0.9% NaCl in which 50 µCi ^{133}Xenon were dissolved were directly injected into the testes. The disappearance of radioactivity from the site of injection was recorded according to the region of interest method. The activity over the region of interest (site of injection) was recorded in 3 sec intervals over a period of 5 min. An example is given in the figure 1.

The half-time (t/2) of the first rapid component of the disappearance rate was determined as described earlier. The capillary blood flow was then calculated according to the formula of Kety[8]:

$$F = \frac{\ln 2 \cdot \lambda}{t/2 \cdot \delta} \quad \text{(ml/g tissue/min)}$$

F is the testicular blood flow, λ is the partition coefficient of Xenon between testicular tissue and blood (0.82)[6] and δ is the specific weight of the testes (1.05 g/ml). Statistical comparisons between groups were made by the Wilcoxon test.

Results and discussion. The effect of HCG stimulation on Leydig cell function is illustrated in table 1. The basal



Disappearance of radioactivity from rat testes after injection of 50 µCi ^{133}Xenon dissolved in saline.

testosterone concentrations in the plasma of the starved rats are significantly ($p < 0.01$) decreased. Under the maximal and unphysiological stimulation with HCG, both starved and control rats have greatly increased testosterone levels in plasma (table 1). This finding indicates that low plasma testosterone in starved rats is not primarily a consequence of impaired ability of the Leydig cells to secrete testosterone, but of the decreased stimulation by LH, the secretion of which is rather low during starvation[3,9,10].

It is surprising that the increase of testosterone was relatively and absolutely greater in the starved rats (table 1). Although we cannot explain this phenomenon at the moment, 2 factors may be responsible for the higher testosterone values in the plasma of the stimulated starved rats. First, we have observed a longer half-life of tritiated testosterone in the plasma of starved rats[11]. This finding means that the metabolic clearance rate is decreased and, as a consequence, the same production rate of testosterone would result in higher plasma levels. Second, Leydig cells may be more sensitive to stimulation with HCG. Since it is now well documented[12] that gonadotropins regulate the capacity and sensitivity of their gonadal receptors in the way that a higher LH secretion reduces receptor capacity and decreases the activity of the adenylate cyclase connected to this receptor, the low LH secretion in the starved rats may cause an increased sensitivity of Leydig cells to HCG.

The results of the measurement of capillary blood flow are listed in table 1. The data obtained in untreated control animals are in good agreement with the data of Joffre and Joffre[6], who observed an average of 240 µl/min/g, as well as with the results reported by other authors[13,14]. We observed a decrease in capillary blood flow in the starved rats by 33.5%. Stimulation with HCG did not reverse the effect of starvation. A similar observation was made by Setchel et al.[15], who studied the effect of chronic malnutrition on testicular function in the ram. Testosterone output, testicular weight and testicular blood flow were reduced. Oxygen and glucose uptake of testicular tissue was impaired. The explanation for this finding may be that the HCG acts rather specifically on the Leydig cells which occupy only about 4% of the total testes volume in this strain of rat[16]. According to Hardy and Scott[17], the capillary blood flow and the activity of cellular metabolism are closely related. We observed an increase in testosterone production, but not in testicular blood flow after HCG stimulation.

The secondary metabolic effects of testosterone on other testicular tissues, as for instance on spermatogenesis[18], may not become relevant after only a short stimulation with HCG as applied here.

In conclusion: incretory testicular function in starved rats is impaired primarily due to lack of stimulation by gonadotropins. A reduced capillary blood flow in the testes of the starved rats occurs as a consequence of malnutrition and may be responsible for a further impairment of testicular function.

1 M.P. Warren and R.L. Van de Wiele, Am. J. Obstet. Gynec. *117*, 435 (1973).
2 K.M. Pirke, M.-M. Fichter, R. Lund and P. Doerr, Acta endocr., Copenh. *92*, 193 (1979).
3 K.M. Pirke and B. Spyra, Acta endocr., Copenh. *96*, 413 (1981).
4 K.B. Eik-Nes, Can. J. Physiol. Pharmac. *42*, 671 (1964).
5 K.M. Pirke, Acta endocr., Copenh. *74*, 168 (1973).
6 M.M. Joffre and J. Joffre, C.r. hebd. Séanc. Acad. Sci. Paris *273*, 486 (1971).
7 K.M. Pirke, I. Bofilias, R. Sintermann, H. Langhammer, I. Wolf and H. Pabst, Endocrinology *105*, 842 (1979).
8 S.S. Kety, Pharmac. Rev. *3*, 1 (1951).
9 H.H. Srebnik, Biol. Reprod. *3*, 96 (1970).
10 G.A. Campbell, M. Kurcz, S. Marshall and J. Meites, Endocrinology *100*, 580 (1977).
11 K.M. Pirke, J.L. Baranao, R. Calandra, I. Lüthy and B. Spyra, J. Steroid Biochem, in press (1981).
12 K.J. Catt and M.L. Dufau, Adv. exp. Biol. Med. *36*, 379 (1973).
13 N. Einer-Jensen and G. Soofi, Prostaglandins *7*, 377 (1974).
14 J.M. Free, in: The testes, p.39. Eds A.D. Johnson and W.R. Gomes. Academic Press, New York 1977.
15 B.P. Setchel, G.M. Waites and H.R. Lindner, J. Reprod. Fert. *9*, 149 (1965).
16 K.M. Pirke, M. Geiss and R. Sintermann, Acta endocr., Copenh. *89*, 789 (1978).
17 F. Hardy and J.B. Scott, Physiol. Rev. *48*, 663 (1968).
18 E. Steinberger, Physiol. Rev. *51*, 1 (1971).

PRO EXPERIMENTIS

Unsuitability of urethane anesthetized rats for testing potential β-adrenoreceptor blockers

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Summary. Isoprenaline induced tachycardia in urethane, but not sodium barbital anesthetized rats depends upon resting heart rate values. This makes urethane anesthesia unsuitable for testing β-blockers.

Inhibition of isoprenaline-induced tachycardia (IIT) in anesthetized animals is often used to assess potential β_1-adrenoreceptor blockers. Preliminary experiments in our laboratory indicated that urethane-anesthetized rats had lower resting heart rate (RHR) and IIT than sodium barbital-anesthetized rats. In view of the above it appeared worthwhile to determine the influence of these 2 anesthetics on the relationship between RHR and IIT and to develop, if possible, a simple and suitable procedure for screening potential β-adrenoreceptor blockers in the rat.

Methods. Male albino rats, Wistar-Morini strain, weighing 350 g were anesthetized with urethane (1.2 g/kg) or sodium barbital (200 mg/kg) given i.p. In some experiments rats were pretreated with i.p. reserpine (5 mg/kg), 48 h before induction of surgical anesthesia. The right carotid artery and left jugular vein were cannulated for blood pressure recording and isoprenaline injection respectively. Heart rate was measured by coupling a DC Counter MARB 80Cl to a 8805B Hewlett Packard carrier preamplifier connected with the pressure transducer. After a 10-min stabilization period, heart rate was recorded before and after i.v. isoprenaline (0.15 µg/kg). When testing the effects of β-adrenoreceptor blockers, (administered orally 1 h before isoprenaline challenge), the isoprenaline-induced fall in diastolic blood pressure (IFDP) was recorded as a measure of β_2-stimulating properties.

IIT-values were plotted against those relative to RHR and the linear regression calculated according to the method of