TRANSPORT OF 5 α -DIHYDROTESTOSTERONE BY TESTICULAR LYMPH IN THE DOG

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Summary. When [^3H]testosterone was infused through the spermatic artery of anaesthetized dogs, [^3H]testosterone and [^3H]5 α -dihydrotestosterone could be found both in the lymph and in the spermatic venous blood from the infused gonad. The concentration of radioactive testosterone and dihydrotestosterone per ml of testicular lymph was comparable to that per ml of spermatic venous blood. Because of the marked difference in flow rate, most of the androgens were, however, delivered from the testis through the spermatic vein.

INTRODUCTION

Testicular lymph of rams contains the androgens, testosterone and androstenedione (Lindner, 1963). Moreover, both progesterone and its 20 α -reduced metabolite can be measured in the ovarian lymph of the ewe obtained during pregnancy or the luteal phase of the oestrous cycle (Lindner, Sass & Morris, 1964). It was recently shown in our laboratory that testes of anaesthetized dogs produce and secrete 5 α -dihydrotestosterone (Folman, Haltmeyer & Eik-Nes, 1972). Since 5 α -dihydrotestosterone (DHT) is a potent androgen, it was deemed important to determine the concentration of DHT in the testicular lymph.

MATERIALS AND METHODS

Healthy, mature mongrel dogs, weighing between 18 and 28 kg, were anaesthetized with intravenous sodium pentobarbital (35 mg/kg). A polyethylene cannula was inserted into the left femoral artery and the left spermatic vessels were exposed through an incision in the flank and prepared for cannulation (Eik-Nes, 1971). A polyethylene cannula (inside diameter, 0.011 in., outside diameter, 0.024 in.) was then placed in the major lymphatic vessel cranial to the plexus pampiniformis and the animal was given 10,000 i.u. heparin intravenously. The left spermatic artery was cannulated using a polyethylene cannula (inside diameter, 0.023 in. or 0.038 in.; outside diameter, 0.030 in. or 0.048 in.). This cannula had a blunt tip. The spermatic artery and veins were then severed and the testis transferred to a metabolic chamber at 37.5° C. The cannula in the femoral artery was connected to a Harvard constant rate

withdrawal–infusion pump and the femoral arterial blood was delivered to an oxygenator placed inside the metabolic chamber. The blood was oxygenated with 95% O₂:5% CO₂. The oxygenated arterial blood was withdrawn from the bottom of the oxygenator by a Harvard constant rate withdrawal–infusion pump placed in the metabolic chamber. This pump delivered the arterial blood to the testis through the cannula in the spermatic artery at a constant rate of 3.76 ml/min. This animal preparation (animal preparation II) has been described in detail elsewhere (Eik-Nes, 1971). When testes thus prepared were infused with oxygenated arterial blood, it was observed that the flow of fluid in the lymphatic cannula was extremely low. It was, therefore, decided to employ testis preparation I (Eik-Nes, 1971) in these experiments. Following cannulations of the left femoral artery and left testicular lymphatic

Table 1. Mean concentrations of [³H]testosterone and [³H]dihydrotestosterone in spermatic venous blood plasma and lymph of four dog testes infused with 7.5 µCi [³H]-testosterone the through spermatic artery

Time of infusion (min)	Spermatic venous blood plasma			Testicular lymph		
	ml*	[³ H] (d/min/ml)		ml*	[³ H] (d/min/ml)	
		Testosterone	DHT		Testosterone	DHT
0 to 30	43.6	26420	390	2.5	5310	10
30 to 60	41.9	42440	760	2.2	22680	190
60 to 90	39.9	45405	1060	1.9	23210	380
90 to 120	41.8	44400	1260	2.0	24150	530

* Mean volumes obtained of either fluid. The average concentrations of [³H]testosterone and [³H]DHT (d/min/g testis weight) at the end of the infusion were 13,345 and 1390, respectively.

vessel as described above, the right testis was removed. The dog was heparinized and the left spermatic vein was cannulated using a polyethylene cannula with an inside diameter of 0.055 in. to 0.066 in. The left spermatic artery was then cannulated and connected to the outlet channel of the Harvard constant rate withdrawal–infusion pump through a 3.5-m coiled polyethylene cannula placed in a water bath maintained at 37.5° C. A temperature probe was placed under the left testis through an opening in the scrotum. This probe was attached to an automatic temperature recorder and changes in testicular temperature could be controlled by changing the temperature in the water bath containing the spermatic arterial cannula. The spermatic artery was infused with arterial blood at a rate of 3.76 ml/min. Considerable variability was, however, observed in the flow of lymph from testes infused with arterial blood through the spermatic artery (Table 1). These variations may be due to variable lymphatic drainage between individual testes (weight: 16 to 21 g). In an attempt to reduce this variation, small lymph vessels in the spermatic cord were tied off if they did not appear to communicate with the cannulated lymphatic vessel. The irregularity and the relative complexity of this ‘artificial’ drainage system were also borne out by data from intravital injection of Indian ink through the spermatic artery at the termination of the experiments.

In all experiments, testes were infused for a preliminary 30-min period at 37° C (Eik-Nes, 1971), then 1 α ,2 α [³H]testosterone (sp. act. 45 Ci/mmol, obtained from New England Nuclear Corporation, 575 Albany Street, Boston, Mass., U.S.A.) dissolved in 0.9% NaCl solution, was added to the arterial blood in the spermatic artery at a rate of 0.36 ml/min. The [³H]testosterone was purified by thin-layer chromatography before being infused. Spermatic venous blood samples of 30-min duration were collected by way of the cannula in the spermatic vein and 30-min samples of spermatic lymph were collected by way of the cannula in the major lymphatic vessel. The venous blood was centrifuged and the plasma removed (Eik-Nes, 1971). The testes were homogenized in 0.9% NaCl solution at the end of infusion. All samples were then frozen and kept at -10° C until analysed by gas-liquid chromatography for testosterone by the method of Brownie, van der Molen, Nishizawa & Eik-Nes (1964) and for DHT by the method of Haltmeyer & Eik-Nes (1972a). The radiochemical purity of testosterone (Ewing & Eik-Nes, 1966) and of DHT (Haltmeyer & Eik-Nes, 1972b), isolated by these methods from biological samples, has been published.

RESULTS

When [³H]testosterone was infused through the spermatic artery, [³H]-testosterone and [³H]DHT were found in spermatic venous plasma and in spermatic lymph (Table 1). The concentration of [³H]testosterone per ml of

Table 2. Mean ratios of [³H]testosterone and [³H]dihydrotestosterone/ml spermatic venous plasma and lymph of testes infused with a total of 7.5 μ Ci [³H]testosterone through the spermatic artery*

<i>Time of infusion (min)</i>	<i>[³H]testosterone ratios (spermatic venous plasma/lymph)</i>	<i>[³H]DHT ratios (spermatic venous plasma/lymph)</i>
0 to 30	5.0	34.8
30 to 60	1.9	3.9
60 to 90	1.9	2.8
90 to 120	1.8	2.4

* Data used for calculation are presented in Table 1.

spermatic venous plasma or testicular lymph remained relatively constant during 30 to 120 min of infusion, while that of [³H]DHT increased in the spermatic venous plasma ($P < 0.01$) and lymph ($P < 0.01$) throughout the experiment (data processed by analysis of variance with repeated measures; Winer, 1962). The ratio (per ml) of [³H]testosterone between spermatic venous plasma and lymph remained steady for 30 to 120 min of the experiment while this ratio of [³H]DHT declined throughout the infusion (Table 2). By the termination of the infusion, the concentration of radioactive testosterone and DHT per ml of spermatic venous plasma was 1.8 and 2.4 times greater, respectively, than that of spermatic lymph (Table 1).

In three dogs infused with [³H]testosterone through the spermatic artery, non-radioactive testosterone and DHT were also measured in spermatic

Table 3. True specific radioactivity of testosterone and dihydrotestosterone in venous blood plasma and tissue of testes infused with [^3H]testosterone through the spermatic artery

Dog no.	Plasma testosterone during infusion (min)				Testis testosterone (at the end)	Plasma DHT during infusion (min)				Testis DHT (at the end)
	0 to 30	30 to 60	60 to 90	90 to 120		0 to 30	30 to 60	60 to 90	90 to 120	
I	7415	7600	7840	N.D.	N.D.	960	4990	3800	N.D.	N.D.
II	810	920	1035	1360	130	255	620	540	430	95
III	3380	2030	1050	1140	170	1030	1210	520	780	145

Details of experiments are state in 'Materials and Methods'. Specific radioactivity values expressed as d/min/ng.
N.D. = not determined.

venous blood plasma. The true specific radioactivity of secreted testosterone was higher than that of secreted DHT (Table 3), a finding which repeats that of work already published from our laboratory (Folman *et al.*, 1972). Non-radioactive testosterone could also be determined in 2-ml samples of spermatic lymph, but the concentrations of non-radioactive DHT in such volumes of spermatic lymph were too low to be determined with adequate precision (Haltmeyer & Eik-Nes, 1972a). Non-radioactive testosterone and DHT were present in all testes investigated at the end of infusion (Table 3).

DISCUSSION

Some of the DHT produced from testosterone in the testis of the dog (Folman *et al.*, 1972) leaves this gland through the spermatic lymph (Table 1). Since the flow of lymph from the dog testis (Table 1) is much lower than that of spermatic venous blood (Eik-Nes, 1962), the quantitative significance of lymphatic transport of testosterone or DHT from the testis of the dog is minute. The major portion of testicular androgens, including DHT, is delivered to the body by way of the spermatic venous blood (Table 1). This was also the conclusion of Lindner (1963) working with testosterone and androstenedione in testicular venous blood and in testicular lymph of the ram.

In our work, considerable variation in the flow of testicular lymph was seen from animal to animal. The condition of anaesthesia will decrease the flow of testicular lymph (Lindner, 1969), and we observed a dramatic decrement in such flow following denervation of the testis which is the main difference between animal preparations I and II (Eik-Nes, 1971). Thus, intact function of testicular nerves could be important for proper flow of testicular lymph.

The rôle played by the lymphatic system for intratesticular transport of androgens has yet to be determined. It is clear that the extracellular space of the Leydig cells has lymphatic drainage (Waites & Setchell, 1969; Morris & McIntosh, 1972). These cells produce the major portion of testicular testosterone (Christensen & Mason, 1965) but compared to the seminiferous tubules, they show little ability to convert [^3H]testosterone to [^3H]DHT, at least in the mature rat in experiments *in vitro* (Folman, Ahmad, Sowell & Eik-Nes, 1973). If the Leydig cells had lymphatic drainage (Waites & Setchell, 1969), one would expect that the DHT concentration of the testicular lymph would be extremely low. In testes secreting both testosterone and DHT (Table 3) in measurable quantities, we had difficulty in determining DHT in 2-ml portions of spermatic lymph though testosterone could be determined in such samples. The fact, however, that, under steady state conditions for testicular metabolism of infused [^3H]testosterone (60- to 120-min samples, Tables 1 and 3), the concentration of [^3H]testosterone and [^3H]DHT per ml spermatic venous blood plasma was only about twice that of spermatic lymph (Table 1) may suggest that some of the [^3H]DHT in spermatic lymph could have been produced outside the Leydig cells. These data are highly indicative of the fact that both testosterone and DHT will readily diffuse through the tissues separating testicular venous blood from testicular lymph. Estimation of DHT in rete testis fluid is therefore called for since it is commonly held that this fluid arises within the seminiferous

tubules and is quite distinct from testicular lymph which originates from the interstitial tissue (Waites & Setchell, 1969). The concentration of testosterone in rete testis fluid is high (Voglmayr, Waites & Setchell, 1966; White & Hudson, 1968).

The presence of DHT in the lymph and venous blood of testes (Table 1) implies that the testicular tubules could be exposed to its action, which will promote spermatogenesis in testes of mature hypophysectomized rats (Ahmad, Haltmeyer & Eik-Nes, 1973). The dose discrepancy between ICSH and testosterone with regard to restoration of tubular function in the testes of hypophysectomized rats has been known for some time (Woods & Simpson, 1961). Thus, in addition to the biological significance of DHT formation and secretion by the canine testis (Folman *et al.*, 1972; Tremblay, Forest, Shale, Martel, Kowarski & Migeon, 1972), transport of DHT in the testicular lymph could also be of importance for spermatogenesis in sexually mature dogs.

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