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DIFFERENT MECHANISMS ARE INVOLVED IN ^{3}H-ANDROGEN UPTAKE BY THE RAT SEMINIFEROUS AND EPIDIDYMAL TUBULES IN VIVO

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ABSTRACT

Proluminal movement of ^{3}H-androgen from peritubular to intratubular fluids of the adult rat testis and epididymis was investigated by using in vivo microperifusion and subsequent micropuncture of seminiferous tubules and caput, corpus, and cauda epididymal tubules. Tubules were perifused with Minimum Essential Medium containing ^{3}H-testosterone. ^{14}C-polyethyleneglycol was included in the perifusion fluid as a marker for contamination of intraluminal fluid by peritubular fluid. Radioactivity of isotopes in interstitial and intraluminal fluids was determined at 1 and 2 hours after perifusion and the percentage of peritubular isotopes appearing in the intraluminal fluid was determined. Proluminal movement of ^{3}H-androgen across the seminiferous epithelium was significantly restricted. In contrast, intraluminal ^{3}H-androgen concentrations in the caput epididymal fluid were 200−300% of those in the peritubular fluid at both 1 and 2 hours after perifusion. Similar results were found in the corpus epididymis. ^{3}H-androgen concentrations in the cauda epididymal fluid were approximately 125% of the peritubular isotope concentrations. The exact mechanism underlying this uphill transepithelial movement of ^{3}H-androgen in the rat epididymis continues to be elucidated.

Key Words: Testosterone, Testis, Epididymis, Micropuncture, Microperifusion

INTRODUCTION

Although both the testis and epididymis are androgen target tissues,[1] very little is known about local mechanisms that regulate movement of androgen across the seminiferous or epididymal epithelium. It is important to develop an understanding of these mechanisms because seminiferous and epididymal tubules provide an important specialized microenvironment for spermatogenesis, sperm maturation processes and sperm storage.[2,3,4]

To investigate the proluminal androgen movement into seminiferous tubules, Parvinen et al. dissected rat seminiferous tubules after intravenous injection of ^{3}H-testosterone and determined uptake of radioactivity by tubules.[5] They reported a restricted movement of ^{3}H-androgen into the tubules. Setchell and Main studied the proluminal movement of ^{3}H-androgen into the seminiferous tubule lumen after intravenous infusion of ^{3}H-testosterone.[6] They showed that net entry of ^{3}H-testosterone into the tubules does not exceed 30−40% of ^{3}H-testosterone concentrations in the blood.

Many previous studies on the movement of ^{3}H-androgen across the epididymal epithelium have been restricted to the cauda epididymis.[7,8,9] They have demonstrated that proluminal

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movement of androgens from the blood into the cauda epididymal lumen is generally low. They have speculated that the binding capacity of intraluminal fluids is an important factor in steroid entry into the epididymal lumen.

For a more specific view of transepithelial androgen movement, we have established a new system of in vivo microperifusion and subsequent in vivo micropuncture that allow direct examination of androgen movement specifically from the interstitial space into the seminiferous tubule or the caput, corpus or cauda tubule lumen and the elimination of vascular factor in proluminal molecular movement. To determine whether proluminal androgen movement is affected by testosterone secreted into testicular and epididymal interstitial fluids we attempted to measure the testosterone concentration in interstitial fluids after 1-h perifusion.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (425–620g) were housed in a constant temperature (22°C), constant humidity (50%) vivarium on a 12 hr:12 hr, light:dark cycle. They had free access to rat chow and water. They were allowed to acclimate after shipment for at least one week before experimental use.

Isotopes

The following isotopes were purchased from New England Nuclear (Boston, Massachusetts): ^{3}H-Testosterone (specific activity: 55.2 Ci/mmol, M.W. 288.4) and ^{14}C-polyethyleneglycol (^{14}C-PEG; specific activity: 15.0 mCi/g, M.W. 4000). All compunds were used as provided by the company.

Preparation of micropipettes for perifusion and micropuncture

Micropipettes for perifusion and puncture were drawn on a vertical pipette puller (David Kopf Instruments, La grange, IL) from constant-bore flint glass tubing with an outside diameter of 0.9 mm and an inside diameter of 0.6 mm. In order to facilitate penetration of the tubule wall, the pipette tips were sharpened on a rotating wet stone grinder (Bausch & Lomb Incorporated, Rochester, NY) to a diameter of 50 to 100 μm, depending on the section of tubule to be punctured. Suitable tip size for perifusion pipette was approximately 100 μm while micropuncture tip size was 50 μm for seminiferous tubule, 75 μm for caput or corpus epididymis, and 100 μm for cauda epididymis. After the pipettes had been ground, they were cleaned with acetone and distilled water and rendered hydrophobic by rinsing with 1% aqueous solution of Siliclad (Clay Adams, Parsippany, NJ) and dried for 15 min in an oven at 110°C.

Volumetric pipettes

The pipettes were placed in a microforge (Stoeling Co., Chicago, IL) and heat was applied focally to the pipette wall. A constriction was made in the pipette lumen by melting the glass wall. These constriction pipettes were calibrated with the standards of known radioactivity volume. They were used for transferring constant sample volumes from collecting pipette to scintillation vial. Constriction pipettes of 50 nl volume were used in this experiment.

In vivo perifusion of reproductive tract tubules and subsequent micropuncture

The animals were anesthetized with intraperitoneal injections of Inactin (sodium 5-ethyl-5-(1-methylpropyl)-2-thiobarbiturate, Byk Guilden Konstanz, Hamburg, Germany) in a dose of 100 mg/kg body weight and prepared for micropuncture. Conventional tracheostomy was performed when necessary to ensure the animal had an unobstructed air passage. Body temperature was maintained at 37.5 ± 0.5°C.

The testis and epididymis were exposed through a scrotal incision and placed in a 35°C

testicle warmer specially fabricated for this purpose. The exposed thestis or epididymis was immobilized by surrounding it in 2% agar. A small area of the immobilized testis or epididymis was uncovered with agar and was moistened with water-equilibrated mineral oil to prevent surface dehydration and to improve temperature regulation.

A 100 μm tip micropipette was placed in a micromanipulator (Leitz, Hamburg, Germany) for well-controlled movement and connected to a 3 ml glass syringe with PE-60 tubing. The pipette, tubing, and syringe were filled with perifusion fluid. The perifusion fluid consisted of 0.3% lissamine-green-dyed solution including Minimum Essential Medium (MEM; pH 7.0–7.4; Gibco Laboratories, Grand Island, NY). Three ml of this fluid contained 80 μCi ^{3}H-testosterone and 4.0 μCi ^{14}C-PEG. The filled syringe was attached to a perifusion pump (model 341B, Sage Instruments, Cambridge, MA).

The perifusion micropipette was used to puncture directly through the transparent testicular or epididymal tunica and the pipette tip was left in the interstitial space. Figure 1 shows the exact location of the tip of the pipette in the epididymal interstitial space. The perifusion pump was set at a priming rate of 36 μl/min in the testis and 6 μl/min in the epididymis for 15 min.

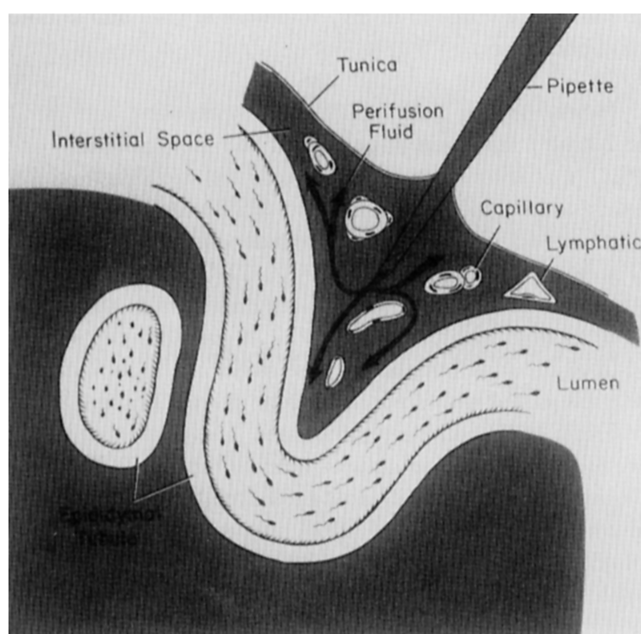

Fig. 1. Schematic description of microperifusion around the epididymal tubules. The tip of the pipette should be located beneath the tunica but outside the tubule. Perifusion fluid (arrows) diffuses in the interstitial space.

Subsequently, a sustaining perifusion rate was set at 6 μl/min in the testis, 2 μl/min in the caput and corpus epididymis, and 3 μl/min in the cauda epididymis, respectively, for the remainder of the experiment. These pump rates have been determined empirically (1) to allow relatively rapid diffusion of solution into the interstitial space, and (2) to allow consistent interstitial concentra-

tions of radioactive material over the complete time course of the experiment.

The perifusions continued for 1 or 2 h. Preliminary experiments have indicated that values for transepithelial ^{3}H-androgen movement in the testis and epididymis reached a plateau by 1-h perifusion. Therefore, subsequent data were collected at 1 and 2 h after sustaining perifusion. During perifusion, the tip of the pipette was always kept in sight to ensure that the testicular or epididymal tubule was not cut or punctured (Fig. 1). Samples of fluid from the testicular or epididymal interstitial space and intraluminal fluids of seminiferous or epididymal tubules were collected by in vivo micropuncture methods at 1 and 2 h after initiation of the sustaining perifusion.

Centrifugation and aliquanting of samples

After the samples were collected, the tip of the pipette was cut off leaving the sample sandwiched between columns of mineral oil in the collection pipette. One end of the pipette was sealed with Critoseal (Monoject Scientific, St. Louis, MO) and the pipette was inserted into a standard capillary hematocrit tube. This tube was inserted into a special plexiglass adapter which was inserted into a rotor head on an IEC model B-20 refrigerator centrifuge. The sample was centrifuged at 10,000 rpm for 20 min at 0°C. The centrifuged sample in the original micropipette was placed into a vertical transfer apparatus (Bunton Instruments Co., Rockville, MD). Aliquots of cell-free fluid were transferred into scintillation vials and counted for radioactivity by scintillation spectrophotometry.

Calculation

Radioactivity of ^{3}H-testosterone and ^{14}C-PEG in peritubular and intraluminal fluids was determined at 1 and 2 h after initiation of perifusion and the percentage of peritubular ^{3}H-testosterone and ^{14}C-PEG appearing in the intraluminal fluid was determined at each time point. Since ^{14}C-PEG is essentially excluded by the blood-testis and blood epididyml barriers, it was included in the perifusion fluid as a marker for contamination of intraluminal fluid by blood or extratubular fluid. The proportion of extratubular ^{14}C-PEG appearing in the intraluminal fluid was used to correct the ^{3}H-testosterone data by subtraction. When net entry of peritubular ^{14}C-PEG into the intraluminal fluid was greater than 7%, it was deemed to be substantial contamination and excluded from the experiments. Because radioactivity subsequently appearing in the various fluids becomes associated with other androgens as well, primarily 5 alpha-dihydrotestosterone, this report will refer to ^{3}H-androgen rather than ^{3}H-testosterone per se.

Assay for testosterone in testicular and epididymal interstitial fluids

One microliter aliquots of testicular and caput epididymal interstitial fluid after 1-h perifusion with lissamine-green-dyed solution including MEM only were applied to measurement of testosterone. Briefly, the fluids were collected into capillary tubes, stored frozen, thawed, flushed from the capillary tubes with 50 μl saline, and ^{3}H-testosterone was added to the saline and extracted with 4 ml diethyl ether twice. The evaporated ether extracts were subjected to HPLC as described proviously.[10] Briefly, testosterone was separated on a Partisil PXS 10/25 OP53 column (Watman, Inc., Clifton, NJ) at 3 ml/min with acetonitrile-H_2O (50:50, vol/vol). Testosterone in eluates of the HPLC were quantified by RIA as described previously.[10]

Data analysis

Chauvenet's criterion was applied to all the data.[11] They were presented as mean and SEM. Data were analyzed by Student's t test or analysis of variance followed by Duncan's multiple range test.[12] All significant differences were set at $p < 0.05$.

RESULTS

In vivo perifusion and androgen movement

Isotope concentrations remaining in interstitial fluids were geneally stable between 1 and 2 h in all tissues (Table 1). At both 1 and 2 h after initiation of the sustaining perifusion in the testis ^{14}C-PEG concentrations were approximately 60% of those in the original perifusion fluid, and ^{3}H-androgen concentrations were stable at approximately 9% of those in the original perifusion fluid (Table 1) and significantly lower than those found in the epididymis at both 1 and 2 h after perifusion ($p < 0.05$). In the caput and cauda epididymis, concentrations of ^{14}C-PEG remaining around the tubules were not statistically different from those found in the testis, but in the corpus epididymis concentrations of ^{14}C-PEG remaining around the tubules at 1 h only were significantly lower than those seen in the testis or other epididymal tubules at the same time point ($p < 0.05$).

Additionally, ^{3}H-androgen remaining around the corpus epididymal tubules at 2 h was significantly higher than that at 1 h ($p < 0.05$). Concentrations of ^{14}C-PEG remaining in the interstitial space of the cauda epididymis were highest both at 1 and 2 h, but were not statistically different (Table 1).

Table 1. Relative Concentration of Radiolabeled Compounds in Peritubular Fluid Containing ^{3}H-testosterone and ^{14}C-PEG after 1- and 2-h Perifusion.

Perifusion site		%^{14}C-PEG remaining*		%H-androgen remaining**	
		1 h	2 h	1 h	2 h
Testis	(11)	$64.1 \pm 3.1^{a,1}$	$62.2 \pm 2.1^{a,1}$	$9.1 \pm 1.6^{b,1}$	$8.6 \pm 0.3^{b,1}$
Caput Ep.	(9)	$78.3 \pm 5.6^{a,1}$	$77.3 \pm 7.0^{a,1}$	$23.8 \pm 3.5^{a,1}$	$25.1 \pm 5.1^{a,1}$
Corpus Ep.	(5)	$51.1 \pm 0.5^{b,1}$	$54.2 \pm 2.7^{a,1}$	$13.4 \pm 5.8^{a,1}$	$20.7 \pm 4.8^{a,2}$
Cauda Ep.	(7)	$80.8 \pm 8.5^{a,1}$	$80.2 \pm 9.7^{a,1}$	$16.2 \pm 2.9^{a,1}$	$18.5 \pm 4.5^{a,1}$

*Percentage of original perifusion fluid ^{14}C-polyethyleneglycol (^{14}C-PEG) concentration remaining in the fluid from the peritubular space at either 1 or 2 h after perifusion.

**Percentage of original perifusion fluid ^{3}H-androgen concentration remaining in the fluid from the peritubular space at either 1 or 2 h after perifusion.

Numbers in parentheses indicate the number of animals.

^{1,2}Within isotopes and tissues, but between times, means ± SEs not sharing the same numerial superscript are significantly different ($p < 0.05$).

^{a,b}Within isotopes and times, but between tissues, means ± SEs not sharing the same letter superscript are significantly different ($p < 0.05$).

After 1- and 2-h perifusion of the seminiferous tubules, approximately 10% and 5% of peritubular isotope concentrations, respectively, appeared in the intraluminal fluid (Fig. 2). Intraluminal ^{3}H-androgen concentrations in the caput epididymal fluid were 200–300% of those in the peritubular fluid at both 1 and 2 h after initiation of sustained perifusion (Fig. 2). Similar results were obtained from the corpus epididymis, but the 2-h value was significantly lower than the 1-h value ($p < 0.05$). ^{3}H-androgen concentrations in the cauda epididymal fluid were approximately 125% of the peritubular isotope concentrations (Fig. 2). These data demonstrate that the net transepithelial movement of ^{3}H-androgen in the reproductive tubule is in the order

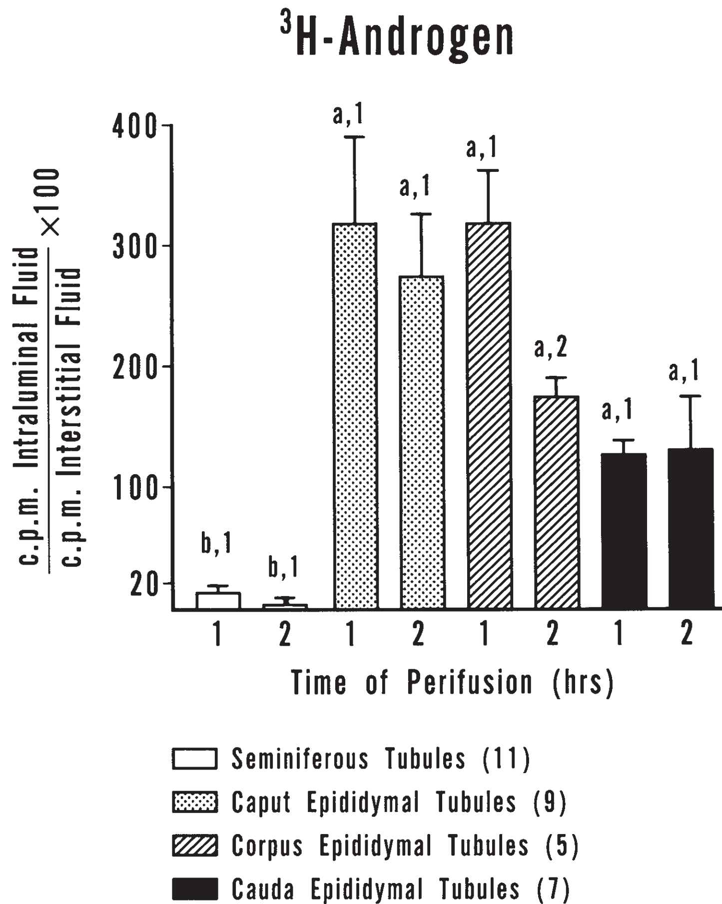

Fig. 2. Proluminal movement of isotope perifused into testicular and epididymal interstitial space as ^{3}H-testosterone. ^{3}H-androgen concentrations in the seminiferous tubules never rose above approximately 10% of ^{3}H-androgen concentrations in the testicular interstitial space. Intraluminal ^{3}H-androgen concentrations in the caput lumen exceed 200% of the epididymal peritubular isotope concentrations and in the cauda tubules exceed 150% of the peritubular isotope concentrations.

of caput epithelium, corpus epithelium > cauda epithelium > seminiferous epithelium.

Testosterone concentrations in testicular and epididymal interstitial fluids

Testosterone concentrations in serum, testicular and epididymal interstitial fluid after 1-h perifusion were 1.3, 13 and 3.1 ng/ml, repsectively (Table 2). These values were much lower than the ^{3}H-testosterone concentration in the original perifusion fluid (approximately 138 ng/ml).

Table 2. Concentrations (mean ± SE) of testosterone in sera, testicular and epididymal interstitial fluids.

Fluids	Testosterone (ng/ml)	n[a]
CBS	1.25±0.2	7
TIF	13.3±2.6	3
EIF	3.05±0.43	3

CBS: Cardiac blood serum, TIF: Testicular interstitial fluid, EIF: Epididymal interstitial fluid. [a]Number of samples analyzed.

DISCUSSION

Proluminal movement of ^{3}H-androgen into seminiferous tubules

The present study demonstrated that movement of ^{3}H-androgen across the seminiferous tubules is restricted. ^{3}H-androgen concentrations remaining around the seminiferous tubules were significantly lower than those seen in the epididymis. Nevertheless, proluminal movement of ^{3}H-testosterone in the seminiferous tubules was restricted. As a partial explanation of this discrepancy, it can be considered that some portion of total androgen in peritubular fluid is drained into the lymphatic or venous vessels in testicular interstitial tissues. Another possible mechanism of this restriction of androgen movement into the seminiferous tubule lumen is the binding of androgen in the androgen target cells of the seminiferous epithelium, thus removing some portion of the total androgen available for diffusion into the tubule lumen.[13] Testosterone concentration in the testicular interstitial fluid after sustained perifusion was significantly lower than the ^{3}H-testosterone concentration in the original perifusion fluid (Table 2). Therefore, testosterone secreted into the peritubular space does not seem to affect the proluminal androgen movement.

That androgens indeed have low access to the seminiferous tubule lumen is demonstrated by the present results and by different in vivo techniques in hamsters.[9] Also consistent with these results are the findings of Comhaire and Vermeulen[14] and Turner et al.[15] that testosterone concentrations in rat seminiferous tubule fluid are significantly lower than in testicular interstitial fluid. Turner et al. demonstrated that testicular intraluminal androgens exist in lwoer concentrations than in extratubular fluids and are in excess of intraluminal androgen binding protein (ABP); therefore, it is not necessary to call upon ABP as a retainer of testosterone in the lumen of the seminiferous tubule. The effect on proluminal androgen movement of androgen binding to Sertoli cell's androgen receptors or to peritubular androgen binding molecules is uncertain, but is potentially different than in the caput epididymis where proluminal androgen movement is very high.

Proluminal movement of ^{3}H-androgen into epididymal tubules

Androgens reach the epididymis by way of the rete testis and the blood stream. Testicular lymphatic drainage has been suggested as an additional route, but the extent of this contribution is not known. Androgens arriving from either route may be modified by epididymal metabolism before entering the lumen more distally. Since sperm maturation and storage are unaffected by ligation of the efferent ducts, and injected steroids can maintain these functions in the orchiectomized male, circulating androgens have been credited with having the major influence on epididymal function.[7]

Despite the lower concentration of androgens in arterial blood compared with rete testis fluid, more androgens reach the epididymis from the blood. The greater blood flow to the caput epididymis[16] with possible vascular transfer of androgens from spermatic veins to the epididymal artery in the pampiniform plexus would further enrich androgen supply to this region. However, androgen movement from blood to epididymal lumen is at a rate that does not allow androgens to reach equilibrium with blood concentrations of androgens.[13] This low availability of vascular androgens to caput lumen fluid is influenced by capillary endothelium.[13] Movement of ^{3}H-androgen from the blood to the epididymal lumen was relatively slow; however, movement from the epididymal interstitial space to the lumen was extremely rapid and against a concentration gradient. This is very different from the case in the testis where proluminal androgen movement was similar, with or without the presence of the capillaries, between ^{3}H-androgen and the tubule lumen.[13] Intraluminal ^{3}H-androgen concentrations in the caput and corpus epididymis exceeded peritubular ^{3}H-androgen concentrations by 200—300% after 1 h of perifusion. A similar but smaller result was obtained in the cauda epididymis. ^{3}H-androgen remaining around the tubules of the corpus epididymis at 2 h was significantly higher than that at 1 h. This result is consistent with the significant decrease in the value of the proluminal movement of ^{3}H-androgen at 2 h. Testosterone concentration in the epididymal interstitial fluid after sustained perifusion containing MEM only was significantly lower than ^{3}H-testosterone concentration in the original perifusion fluid (Table 2). Therefore, testosterone secreted into the peritubular space does not seem to have a competitive effect on the proluminal androgen movement.

Little is known about the process by which androgens enter the epididymal tubules from the interstitial space. Cooper and Waites[7] and Cooper[8] have investigated factors affecting the proluminal movement of androgens in the cauda epididymal tubules of rats and have shown that the presence and nature of proteins in the cauda lumen may influence the partition of infused steroids between blood, epithelium, and lumen. No studies have been reported which examine further the mechanism of movement across the epididymal epithelia. The present study has demonstrated that androgen movement across the epididymal epithelium, in contrast to the seminiferous epithelium, is against a concentration gradient. This implies the presence of a cell membrane androgen receptor in the epididymal epithelial cells or the presence of an androgen receptor in the lumen of the epididymis (e.g., androgen binding protein, spermatozoa).

In conclusion, the net proluminal movement of ^{3}H-androgens into seminiferous tubules is restricted. However, transepithelial movement of ^{3}H-androgens in epididymal tubules occurred against a concentration gradient. Further studies will determine whether or not this proluminal, antigrade ^{3}H-androgen movement is receptor-mediated, concentration-dependent, or energy-depenedent. Transepithelial movement of androgens is a major factor that affects the endocrine microenvironment in which sperm cells develop and mature and to which the tubule epithelia are exposed. Little is understood about this movement and the factors controlling it. Studies that detial the effect of movement on the endocrine microenvironment inside the male tract tubules and that investigate the mechanism by which these factors operate will be needed.

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