Nagoya J. Med. Sci. 57. 77 ~ 83, 1994

INTRALUMINAL CONTENT IS REQUIRED FOR THE MAINTENANCE OF ANTIGRADE PROLUMINAL MOVEMENT OF ^{3}H-ANDROGENS INTO RAT CAPUT EPIDIDYMAL TUBULES

MASANORI YAMAMOTO, HATSUKI HIBI, YOSHIKAZU TSUJI and KOJI MIYAKE

Department of Urology, Nagoya University School of Medicine, Nagoya, Japan

ABSTRACT

The present study was undertaken to determine whether or not antigrade, proluminal ^{3}H-androgen movement in the caput epididymis occurs in the absence of native lumen content. A single tubule was perfused with artificial caput fluid containing no androgen-binding protein for 30 min and subsequently tubules were perifused with Minimum Essential Medium perifusion fluid containing 26.7 μCi/ml ^{3}H-testosterone and 1.3 μCi/ml ^{14}C-polyethyleneglycol for 1 h. Radioactivity of isotopes in perifusion and intraluminal fluids was determined at 1 h after sustaining perifusion, and the percentage of peritubular isotopes appearing in the intraluminal fluid was determined. Net entry of ^{3}H-androgen into the epididymal tubules in the presence of native intraluminal content was approximately 323%. In contrast, intraluminal ^{3}H-androgen concentrations in the epididymal fluid in the absence of native lumen content were significantly reduced, to 100% of those in the peritubular fluid. Antigrade, proluminal movement of ^{3}H-androgen in the caput epididymis does not occur in the absence of native lumen content. Androgen-binding protein in the epididymal lumen may be required to maintain uphill proluminal movement of ^{3}H-androgen into the tubules.

Key Words: Androgen-binding protein, Androgen uptake, Epididymis.

INTRODUCTION

The epididymis is an androgen target tissue and its metabolism and epithelial secretion depends on the presence of androgens.[1] Consequently, epididymal sperm maturation does not occur in the absence of androgens.[2] Androgen concentrations in epididymal tissue extracts are high, especially in the caput epididymis of several species,[3,4] and caput epididymal fluid androgen concentrations are higher still.[5] It has been a primary goal of our experiment to understand the mechanism by which this intraluminal microenvironment of concentrated androgens is maintained and, secondly, to understand why such high intraluminal androgen concentrations are necessary for normal epithelial functions.

Previous reports have demonstrated that transepithelial movement of ^{3}H-androgens in the epididymis occurs against a concentration gradient and is subject to competitive inhibition.[6–8] It has also been shown that hypophysectomy decreases the secretion of androgen-binding protein from Sertoli cells, and proluminal ^{3}H-androgen movement is significantly reduced from the control levels. Follicle-stimulating hormone or luteinizing hormone replacement returns proluminal ^{3}H-androgen movement to normal.[9,10] Therefore, our results and others' have brought attention

Correspondence: Dr. Masanori Yamamoto, Department of Urology, Nagoya University School of Medicine, Tsurumaicho, Showa-ku, Nagoya 466, Japan
Accepted for Publication in November 29, 1993

to androgen-binding protein as the putative regulator of the epididymal androgen uptake.

Recently, we have reported that metabolic inhibitors completely eliminated this antigrade or uphill aspect of proluminal androgen movement and suggested that an energy dependent mechanism may be involved in the epididymal androgen uptake.[11] However, we are very hesitant to accept the concept that an active transport process of steroids exists in the epididymis, because a vast amount of information reflects that steroids should diffuse across the cell membranes.[12,13]

The next experiment that we should do is to determine whether or not antigrade proluminal movement of ^{3}H-androgen in the caput epididymis is still maintained when intraluminal native fluids are replaced by artificial perfusion fluid not containing androgen-binding protein. If androgen-binding protein is important in androgen movement, and if native luminal content is replaced by an artificial caput fluid, uphill movement of ^{3}H-androgen into the intraluminal compartment should be eliminated. We here report the first direct evidence for the need for the presence of intraluminal content to maintain the uphill movement.

MATREIALS AND METHODS

In Vivo Microperfusion

Adult, male rats (520-680 gm) were anesthetized with intraperitoneal injections of inactin (sodium 5-ethyl-5-(1-methyipropyl)-2-thiobarbiturate, Byk Guilden Konstanz, Hamburg, Germany; 100 mg/kg body weight) and prepared for micropuncture as previously described.[6,7] In the present procedure, the caput epididymis was immobilized by surrounding it with 2% agar. An exposed portion of the tunica albuginea was incised with microscissors to present the underlying epididymal tubules. One tubule was selected for perfusion and micropunctured with a sharpened glass micropipette (75-μm tip) attached to a micromanipulator. The micropipette was attached to a 100-μl glass syringe by a length of polyethylene cannula. The perfusion system was filled with artificial caput fluid (Table 1) stained with 0.3% lissamine-green. Composition of the fluids was based on available analytical results describing epididymal fluids.[14] Glycerylphosphorylcholine was not included because of the difficulty in rendering it cadmium-free. The perfusion system was driven by a multispeed infusion pump (Model 341B, Sage Instruments, Cambridge, MA) at a rate of 225 nl/min. This was done to allow sufficient tubule perfusion in a reasonable amount of time. This perfusion rate did not cause tubule distention observable under the dissecting microscope. Perfusion of the tubule continued for 30 min.

Table 1. Concentrations of Compounds Used in Perfusion Fluids for the Caput Epididymis.

Artificial Caput Epididymal Fluid

Compound	Concentration (mM)
NaCl	100
$KHCO_3$	25
Mannitol	80
Bovine Serum Albumin (mg/ml)	30
PIPES buffer	30

[mOsm] = 360, pH = 6.5

In Vivo Microperifusion

Thirty minutes after beginning the sustaining tubule perfusion, the intraluminal perfusion was halted and the animal began receiving the priming perifusion around the caput epididymal tubules. The perfused tubule was maintained in this "stop flow" condition during perifusion. A 150-μm tip micropipette was used to puncture directly through the epididymal tunica, and the pipette tip was left in the interstitial space. The perifusion fluid consisted of 0.3% lissamine-green-dyed solution including Minimum Essential Medium (pH 7.0 to 7.4; Gibco Laboratories, Grand Island, NY). Three ml of this fluid contained 80 μCi ^{3}H-testosterone and 4.0 μCi ^{14}C-polyethyleneglycol (PEG). The perifusion pump was set for a priming infusion at a rate of 6 μl/min for 15 min. Subsequently, a sustaining perifusion rate was set at 2 μl/min for the remainder of the experiment. The perifusion continued for one hour.

A specific order must be followed at fluid collection. First, the microperfusion pipette must be withdrawn. The small tip of the pipette makes such a small hole in the tunica and the tubule that they reseal, and no detectable leakage occurs. The microperfusion micromanipulator then becomes the micropuncture micromanipulator, and 75-μm-tip micropipettes are used to micropuncture separately, first, the interstitial compartment, then, the lumen of the perfused tubule. Radioactivity of ^{3}H-androgen and ^{14}C-PEG in the perifusion and intraluminal fluids is determined, and the percentage of peritubular ^{3}H-androgen and ^{14}C-PEG appearing in the intraluminal fluid is calculated.

The perfused lumen fluid had the same exposure to the outside ^{3}H-androgen as did the normal perifusion system, which has been previously described.[6,7] These perfusion/perifusion experiments established values for net entry of ^{3}H-androgen into epididymal tubules that have been perfused with a defined fluid not containing androgen-binding protein. Since ^{14}C-PEG is essentially excluded by the blood-testis barrier,[6,7] it was included in the perifusion fluid as a dilution marker for the perifusion fluid as well as a marker for contamination of intraluminal fluids by the peritubular fluid. All intraluminal ^{3}H-testosterone data were corrected for contamination following ^{14}C-PEG.[9,10]

Data Analysis

Chauvenet's criterion was applied to all the data.[15] They are presented as mean and SEM. The significance of differences between perifusion alone and perfusion followed by perifusion experiments was assessed by the Wilcoxon rank sum test ($p < 0.05$) because data were not normally distributed.

RESULTS

Although ^{3}H-testosterone was the steroid perifused around the tubules, it is known that the radioactivity subsequently detected in the various fluids becomes associated with other androgens as well[10,13]; thus, the present report will refer to ^{3}H-androgen(s) in the various fluids rather than to ^{3}H-testosterone itself.

Isotopes remaining in the peritubular fluids were generally stable between the two experimental groups (Figs. 1 and 2). In the perifusion-alone experiment, concentrations of ^{14}C-PEG and ^{3}H-androgen remaining around the tubules were 74.6 ± 5.9% and 22.5 ± 3.7%, respectively (Figs. 1 and 2). In the perfusion followed by perifusion experiment, concentrations of ^{14}C-PEG and ^{3}H-androgen remaining around the tubules were 73.2 ± 2.7% and 21.5 ± 1.0%, respectively (Figs. 1 and 2).

After 1-h perifusion of the epididymal tubules of the control rats, 323.4 ± 73.2% of peritu-

bular ^{3}H-androgen concentrations appeared in the intraluminal fluid (Fig. 3). In contrast, ^{3}H-androgen concentrations entering the lumen of the epididymal tubules after completion of 30-min perfusion followed by 1-h perifusion were 100.2 ± 5.0% (Fig. 3). These values were significantly lower than those in the perifusion-alone group ($p < 0.05$). When intraluminal native fluid of the caput epididymis was replaced with an artificial fluid containing no androgen-binding protein, antigrade, proluminal androgen movement was completely eliminated.

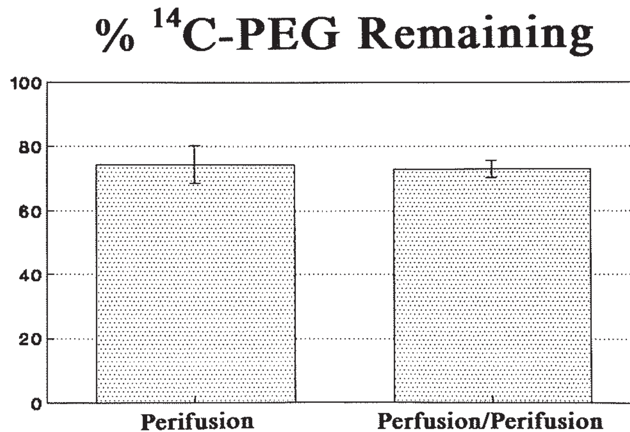

Fig. 1. Percentage of original perifusion fluid ^{14}C-polyethyleneglycol (^{14}C-PEG) concentration remaining in the fluid from the interstitial space in the 1-h perifusion-alone experiment (Perifusion) and 30 min perfusion with artificial caput fluid containing no androgen-binding protein followed by 1-h perifusion experiment (Perfusion/Perifusion).

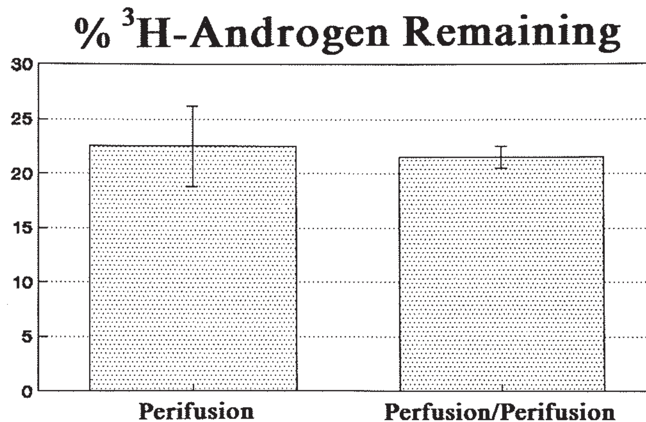

Fig. 2. Percentage of original perifusion fluid ^{3}H-androgen concentration remaining in the fluid from the interstitial space in the 1-h perifusion-alone experiment (Perifusion) and 30-min perfusion with artificial caput fluid containing no androgen-binding protein followed by 1-h perifusion experiment (Perfusion/Perifusion).

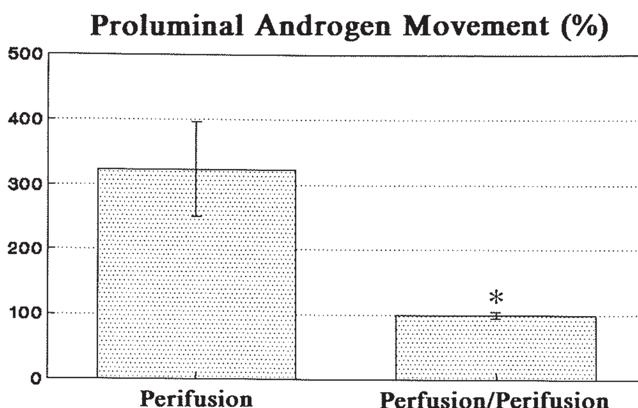

Fig. 3. Proluminal movement of ^{3}H-androgens perifused in the caput epididymal interstitial space in the 1-h perifusion-alone experiment (Perifusion) and 30-min perfusion with artificial caput fluid containing no androgen-binding protein followed by 1-h perifusion experiment (Perfusion/Perifusion). Radioactivity in the tubule lumina was expressed as a percentage of radioactivity appearing in the same volume of interstitial fluid surrounding the same tubule at the same time period (intraluminal fluid counts per minute [CPM] ÷ interstitial fluid CPM X 100). Antigrade, uphill proluminal movement of ^{3}H-androgens in the perifusion-alone experiment is completely eliminated by removing native luminal androgen-binding protein.

DISCUSSION

It has been determined that ^{3}H-androgens appear in caput epididymal fluid in concentrations that are 250 to 300 percent of the concentrations present in interstitial fluid.[6,7] This antigrade, proluminal ^{3}H-androgen movement is subject to inhibition with unlabeled testosterone.[8] Additionally, antigrade, proluminal androgen movement is eliminated by hypophysectomy and returned to normal by follicle-stimulating hormone or luteinizing hormone replacement.[9,10] We have recently demonstrated that adenosine triphosphate (ATP) concentrations in caput epididymal tissues after 0.1 mM dinitrophenol (DNP) or potassium cyanide treatment were significantly decreased and that these metabolic inhibitors at the same concentration then significantly reduced proluminal androgen movement in the caput epididymis and removed the antigrade or uphill aspect of proluminal androgen movement.[11] We seem to be getting two differing and confusing lines of results, which we have not completely resolved. One is the previously accepted hypothesis that ^{3}H-androgens diffuse across the epididymal epithelia, but bind to intraluminal androgen-binding protein in the epididymis (concentration: approx. 270 nM)[16], thus accounting for the antigrade movement of ^{3}H-androgens and the maintenance of high androgen concentrations in the caput lumen.[5–8] The other is that an energy-requiring mechanism may be involved in the epididymal androgen uptake.[11]

If this unique uphill proluminal androgen movement is accurate active transport, and if intraluminal native fluids are replaced with an artificial fluid containing no androgen-binding protein, antigrade proluminal androgen movement should still be maintained because androgen-binding protein in that case should not be important. Active transport should cause uphill movement into the intraluminal compartment even if intraluminal fluids are replaced with an artificial caput fluid. Therefore, we did the experiment in which a single epididymal tubule was perfused with an artificial fluid not containing androgen-binding protein, for 30 min, and then the normal perifusion system was started and continued for 1 h before taking the micropunctured sample

back again. The present results indicate that antigrade proluminal movement of ^{3}H-androgen in the caput epididymis does not occur in the absence of luminal androgen-binding protein.

Gerard et al.[17] have recently given evidence that the androgen-binding protein internalized by receptor-mediated endocytosis still retains its androgen. This was the first direct evidence that androgen-binding protein and androgens are internalized together in the caput epididymal epithelium. Gerard[17] gave evidence that androgen-binding protein brings androgen into the epithelial cell, and Tindall et al.[18] earlier found that the epididymal cytosolic androgen receptor exhibited increased androgen binding in the presence of androgen-binding protein, but the meaning of this relative to epithelial cell function for androgen uptake remains unclear. More recently, Turner et al.[19] have shown that caput tubule uptake of ^{3}H-androgens from the peritubular incubation fluid was significantly reduced by removal of native lumen content and significantly increased toward normal by perfusion with a fluid containing androgen-binding protein. This finding of Turner et al. that intraluminal androgen-binding protein enhances net androgen uptake by caput epididymal tubules from their surrounding medium in vitro is certainly consistent with our in vivo data. Our ^{3}H-androgen transport data is generally, though not inevitably, consistent with the concept that ^{3}H-androgens move across the epididymal epithelium where they bind to intraluminal androgen-binding protein, are removed from the diffusible ^{3}H-androgen pool in the interstitial space, and thus concentrate on the luminal side of the epididymal epithelium. Nevertheless, one aspect of antigrade, proluminal androgen movement is not fully explained by the hypothesis that all free androgen movement across the epithelium is due to simple diffusion.

In conclusion, removal of caput epididymal lumen content significantly reduced antigrade proluminal movement of ^{3}H-androgens into the caput epididymal lumen. This uphill proluminal androgen movement against concentration gradient occurs in the presence of intraluminal content, yet an energy-dependent process is also important in this phenomenon. The relationship between androgen-binding protein and energy-requiring mechanism remains undefined. Further experiments are needed to clarify the mechanism of androgen movement specifically across the caput epithelium.

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