Nagoya J. Med. Sci. 56. 81 ~ 87, 1993

TRANSEPITHELIAL MOVEMENT OF ^{3}H-ANDROGEN IN RAT SEMINIFEROUS AND CAPUT EPIDIDYMAL TUBULES: SATURABILITY AND EFFECT OF COMPETITION WITH ESTRADIOL

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ABSTRACT

Proluminal movement of ^{3}H-testosterone from peritubular space to intratubular fluids of the adult rat testis and epididymis was studied by using in vivo microperifusion and subsequent micropuncture of seminiferous tubules and caput epididymal tubules. Tubules were perifused with Minimum Essential Medium containing ^{3}H-testosterone. To determine if androgen transport is saturable, 40, 80, 160, and 320 μCi ^{3}H-testosterone were included in the perifusion fluid. Radioactivity of ^{3}H-androgens in the intraluminal fluids was determined at 1 hour after perifusion. Transepithelial ^{3}H-androgen movement in the testis was linear ($r=0.735$, $p<0.001$, $y=0.17x+5.6$). The movement of ^{3}H-androgen across the epididymal epithelium was saturable (V_{max} of 326.5 nM/hr and K_m of 77 nM). To determine the effect of estradiol on proluminal androgen movement, estradiol at 10X the cocentration of ^{3}H-testosterone was incorporated in the perifusion fluid. Proluminal ^{3}H-androgen movement into the seminiferous and epididymal tubules was not affected by addition of estradiol to the perifusion fluid. These findings support our previous observations that proluminal transepithelial movement of ^{3}H-androgens could be mediated by its binding to a specific intraluminal androgen binding protein.

Key Words: Androgen movement; epididymis; estradiol; microperifusion; micropuncture.

INTRODUCTION

The mammalian testis and epididymis provide an important environment for spermatogenesis and maturation of spermatozoa.[1] Androgens are important components of this environment and play an essential role in the regulation of spermatogenesis and the control of epididymal metabolism and epididymal epithelian secretion.[1,2,3] Nevertheless, little is known about local mechanisms that regulate androgen movement across the seminiferous or epididymal epithelium.

It has been recently demonstrated that the transepithelial movement of ^{3}H-androgen in the epididymal tubules occurs against a concentration gradient[4,5] and is subject to competitive inhibition.[6] It has also been shown that ^{3}H-androgen movement across the caput epididymal epithelium is reduced by hypophysectomy and completely returned to normal in the hypophysectomized rat supplemented with FSH or LH.[7] All of these findings are consistent with a hypothesis that androgens diffuse across the epididymal epithelium and exist there in high concentrations due to their binding to androgen binding protein (ABP). The objective of the present study was to determine whether or not proluminal androgen movement across the seminiferous

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Accepted for Publication in January 12, 1993

tubules and epididymal tubules is altered by various concentrations of ^{3}H-testosterone in the intertubular compartment and to determine if proluminal, antigrade movement of ^{3}H-androgen into these tubule lumina is affected by competition with estradiol in the perifusion fluid.

MATERIALS AND METHODS

Rats

Adult male Sprague-Dawley rats (440–670 g) were maintained in a constant temperature (22°C), constant humidity (50%) vivarium on a 12 hr:12 hr light:dark cycle. Food and water were available ad libitum. 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).

In vivo microperifusion and micropuncture

The animals were anesthetized with Inactin (sodium 5-ethyl-5-(1-methylprophyl)-2-thiobarbiturate, Byk Guilden Konstanz, Hamburg, Germany; 100 mg/kg body weight) and prepatred for in vivo microperifusion and micropuncture of reproductive tract tubules as previously described.[4)] Briefly, a testis and epididymis were exteriorized in a 35°C testicle holder and stabilized in 2% agar. A small area of the immobilized testis or epididymis was exposed and covered with mineral oil. The tubules were perifused in vivo through a 100 μm tip micropipette inserted through the tunica albuginea. The perifusion fluids were lissamine-green dyed Minimum Essential Medium (MEM; pH 7.0–7.4; Gibco Laboratories, Grand Island, NY) containing 13.3, 26.7, 53.3, or 106.7 μCi ^{3}H-testosterone/ml (240, 480, 960, or 1920 nM testosterone, respectively) and 1.3 μCi ^{14}C-PEG/ml. Data from these experiments were used for the estimation of K_m and V_{max}. Priming and sustaining perifusion rates for the seminiferous and caput epididymal tubules were as previously described.[4)].

In other experiments MEM containing 26.7 μCi ^{3}H-testosterone/ml and 1.3 μCi ^{14}C-PEG/ml was supplemented with 1% (v/v) ethanol and 4.8 μM estradiol (10X the concentration of ^{3}H-testosterone) or with 1% ethanol alone prior to perifusion around seminiferous and caput epididymal tubules.

Intraluminal fluids and adjacent interstitial fluids (perifusion fluid remaining in the peritubular space) were collected by micropuncture at 1 hour after initiation of the sustaining perifusion in the experiments using as perifusion fluid MEM containing ^{14}C-PEG and increasing concentrations of ^{3}H-testosterone. Previous reports have indicated that values for transepithelial ^{3}H-androgen movement have reached plateau by 1 hour perifusion.[4,5)] In the experiments using perifusion fluid containing ^{14}C-PEG and ^{3}H-testosterone along with 1% ethanol or estradiol plus 1% ethanol, the perifusion continued for 1 or 2 hours and fluids were collected from the testicular and epididymal interstitial space and lumen at 1 and 2 hours after initiation of perifusion. Cell free fluids were obtained by centrifugation and aliquanted as previously described.[8)] Each fluid sample was analyzed in triplicate for ^{3}H and ^{14}C radioactivity.

Analysis of data

Net proluminal movement of isotope into the seminiferous and caput epididymal tubule fluid

has been previously described.[4] Briefly, using ^{14}C-PEG as a marker for inadvertent contamination of intraluminal fluid, the concentration of ^{3}H-testosterone in intraluminal fluid is reported as a percentage of the ^{3}H-testosterone concentration existing in peritubular fluid at that same time period.

Chauvenet's criterion[9] was applied to all the data. Statistical comparison for the intraluminal ^{3}H-androgen concentrations of the seminiferous tubules and the caput epididymal tubules at each peritubular ^{3}H-androgen concentration was done by Student's t-test. Multiple comparisons were made by the Kruscal-Wallis test followed by the Wilcoxon Rank-Sum test. A p value of 0.05 or less was considered significant.

RESULTS

While ^{3}H-testosterone was the steroid perifused around the tubules, it is known that ^{3}H-testosterone is partially converted to other ^{3}H-androgens[4,8]; thus we subsequently refer to ^{3}H-androgen(s) in the various fluids rather than ^{3}H-testosterone, per se.

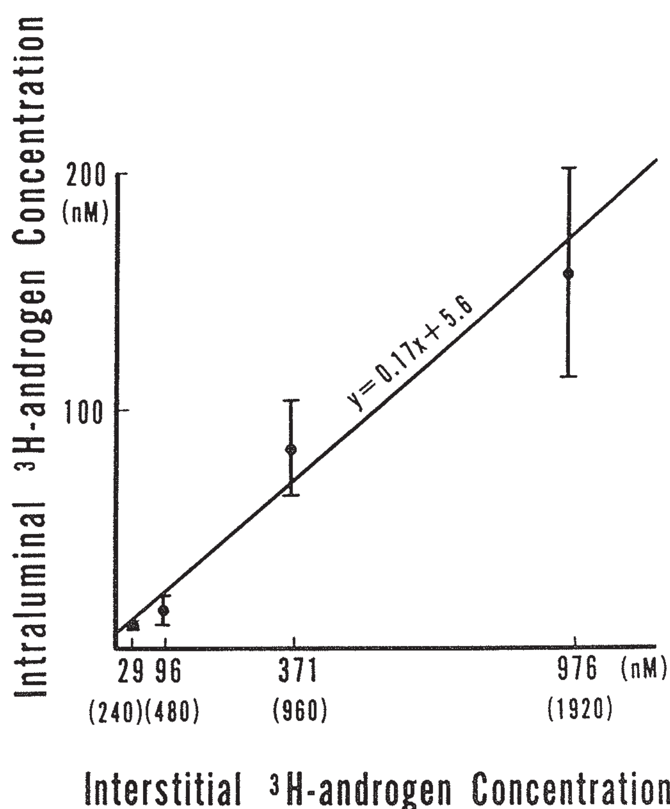

Fig. 1. Intraluminal ^{3}H-androgen concentrations in the seminiferous tubule lumen in different concentrations of interstitial ^{3}H-androgen at 1 hour after sustaining perifusion. Abscissa is the mean interstitial ^{3}H-androgen concentration after 1 hour perifusion. ( ), ^{3}H-testosterone concentration in original perifusion fluid. Each value of intraluminal ^{3}H-androgen concentration is the mean ± SEM of n=7–8 animals. These values increased in a linear fashion ($r=0.735$, $y=0.17x+5.6$, $p<0.001$).

Intraluminal ^{3}H-androgen concentrations in the seminiferous tubules increased linearly with increasing interstitial concentrations ($r=0.735$, $p<0.001$; Fig. 1). Intraluminal ^{3}H-androgen concentrations reached approximately 157 nM at highest concentration of interstitial ^{3}H-androgen, and no plateau concentration was reached (Fig. 1). In the caput epididymal tubules proluminal androgen movement was shown to be saturable (Fig. 2). Additionally, caput intraluminal ^{3}H-androgen concentrations were significantly higher than those in the siminiferous tubules at each peritubular ^{3}H-androgen concentration (t-test; $p<0.05$). The apparent K_m and V_{max} values were approximately 77 nM and 326.5 nM/hr (Fig. 2).

After 1 and 2 h perifusion of seminiferous tubules, intraluminal isotope concentrations averaged 20% of peritubular isotope concentrations or less (Fig. 3A). In the caput epididymal tubules at both 1 and 2 hours after the initiation of perifusion intraluminal ^{3}H-androgen

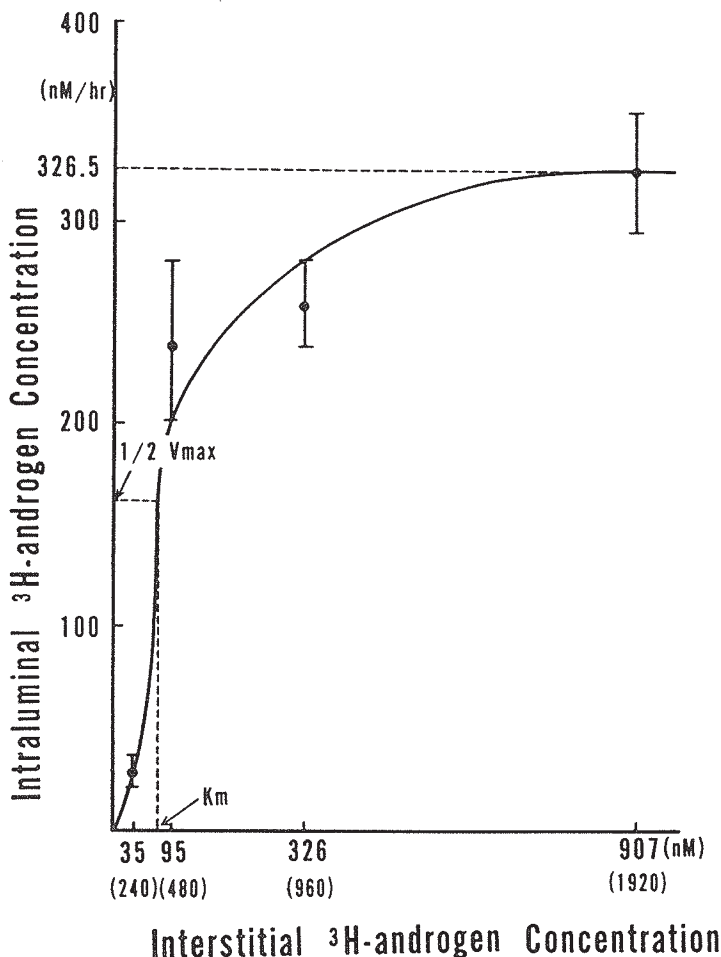

Fig. 2. Intraluminal ^{3}H-androgen concetrations in the caput epididymal lumen with different concentrations of interstitial ^{3}H-androgen at 1 hour after sustaining perifusion. Abscissa is the mean interstitial ^{3}H-androgen concentration after 1 hour perifusion. ( ), ^{3}H-testosterone concentration in original perifusion fluid. Each value of intraluminal ^{3}H-androgen concentration is the mean ± SEM of n=5–9 animals. The apparent V_{max} and K_m were calculated to be 326.5 nM/hr and 77 nM, respectively.

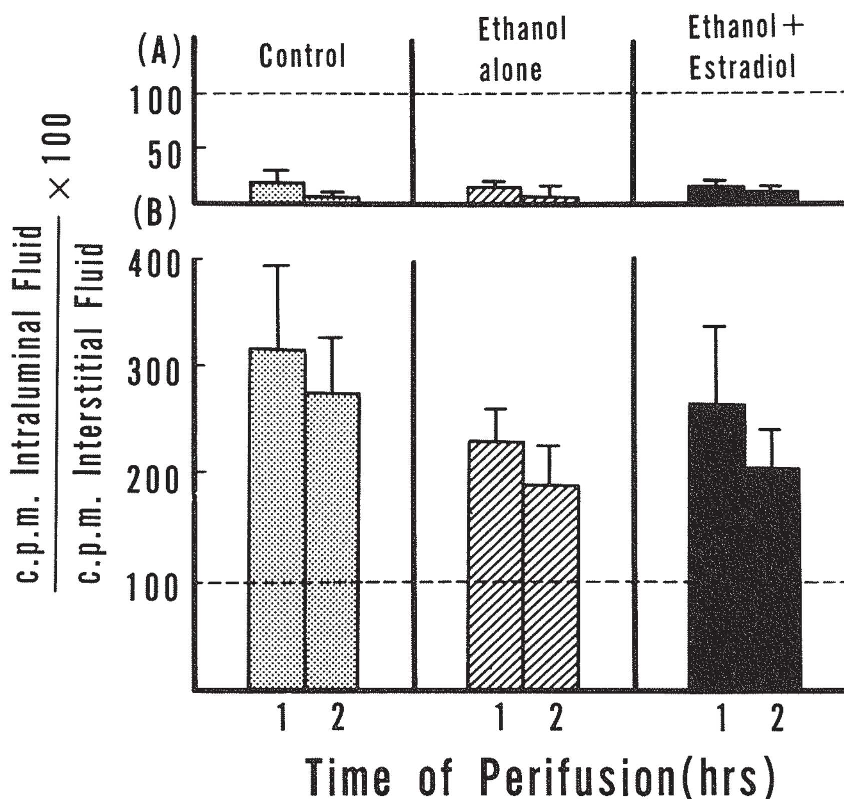

Fig. 3. (A) Net proluminal movement of ^{3}H-androgens into the seminiferous tubules at 1 and 2 h after exposure to ^{3}H-testosterone-containing perifusion fluid (PF), PF with 1% etanol, or PF with 4.8 μM estradiol and 1% ethanol. The dotted line indicates the value for equilibrium between interstitial and intraluminal isotope concentrations. Each value is the mean ± SEM of n=4 animals.

(B) Net proluminal movement of ^{3}H-androgens into the caput epididymal tubules at 1 and 2 h after exposure to ^{3}H-testosterone-containing perifusion fluid (PF), or PF with 1% ethanol, or PF with 4.8 μM estradinol and 1% ethanol. The dotted line indicates the value for equilibrium between interstitial and intraluminal isotope concentrations. Each value is the mean ± SEM of n=4–9 animals.

concentrations reached approximately 300% of interstitial androgen concentrations (Fig. 3B). As demonstrated previously,[7] concentrations of ^{14}C-PEG and ^{3}H-androgen around the tubules were consistent at 1 and 2 h (data not shown). Proluminal movement of ^{3}H-androgen into the siminiferous and caput epididymal tubules was not significantly inhibited by addition of 1% (v/v) ethanol or 1% ethanol plus 4.8 μM estradiol to the perifusion fluid (Fig. 3A and 3B).

DISCUSSION

Transepithelial movement in seminiferous tubules

The present study has demonstrated that movement of ^{3}H-androgen across the seminiferous tubules is elevated in linear fashion when ^{3}H-testosterone concentrations in the perifusion fluid are increased. Nevertheless, intraluminal androgen concentration during the perifusion containing the highest interstitial ^{3}H-testosterone concentration (976 Nm) was 157 nM, a value

significantly lower than that in the caput epididymis when perifused with similar concetrations of ^{3}H-testosterone (Fig. 1 and 2). Percentage of peritubular fluid ^{3}H-androgen concentration appearing in the intraluminal fluid at 1 and 2 h after initiation of the sustaining perifusion averaged 20% or less (Fig. 3A) while similar values obtained from the caput epididymides generally averaged above 200% (Fig. 3B). Thus, there is significantly less net proluminal androgen movement across the seminiferous epithelium than the epididymal epithelium. It has been previously reported that proluminal ^{3}H-androgen movement across the seminiferous epithelium is restricted[4] and not inhibited by competition with unlabelled testosterone.[6] These previous results and the present result that net proluminal androgen movement into rat seminiferous tubules is no inhibited by competition with estradiol (Fig. 3A) are consistent with simple diffusion of free androgen across the siminiferous epithelium. The possibility that there is a specific androgen transport mechanism in the seminiferous tubules has been offered by others, but the evidence for this is sparse and problematical.[10]

While transepithelial androgen movement in the seminiferous tubule can be accounted for by simple diffusion, the reason for restricted net movement of androgens into the tubule lumen is unclear. That androgens indeed have low access to the seminiferous tubule lemen is demonstrated by the present results, by previous reports using the same techniques,[4,5] and by different in vivo techniques in both rats[4] and hamsters.[8] Also consistent with these results are the findings of Comhaire and Vermeulen[11] and Turner at al.[12] that testosterone concentrations in rat seminiferous tubule fluid are significantly lower than in testicular interstitial fluid (lymph). Recent unpublished data from our laboratory have confirmed that when rat testicular interstitial fluid and seminiferous tubule fluid are collected by in vivo micropuncture, the ratio of peritubular to intraluminal testosterone (as measured by R.I.A.) is appoximately 1:0.7.

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.

Transepithelial movement in caput epididymal tubules

The findings from the present study of transepithelial movement of ^{3}H-androgens in the caput epididymis indicate that intraluminal ^{3}H-androgen concentrations are saturable (Fig. 2). At 1 hour after initiation of the sustaining perifusion at 95 nM ^{3}H-androgen, intraluminal ^{3}H-androgen concentrations were approximately 240 nM. This was 15 times the ^{3}H-androgen concentration in seminiferous tubule lumen fluid surrounded by similar ^{3}H-androgen concentrations (Fig. 1 and 2). Proluminal androgen movement across the epididymal epithelium, contrast to the seminiferous epithelium, is against a concentration gradient (Fig. 3A and B).

When testosterone is taken up by epididymal epithelial cells, it is rapidly metabolized to dihydrotestosterone and other androgens. While there are many other studies of testosterone or dihydrotestosterone bindings to intracellular receptors or ABP in the epididymis in vitro,[3] we are not aware of previous studies of the kinetics of androgen movement across reproductive tract epithelia in vivo. The V_{max} estimate of 326.5 nM/hr is similar, through not identical, to the R.I.A. estimate of intraluminal ABP concentration in the rat caput epididymis (approx. 270 nM)[12] and is supportive of our hypothesis that androgen-ABP binding is important in the process of antigrade, proluminal androgen movement seen in the caput epididymis. The subsequent study of proluminal ^{3}H-androgen movement in competition with estradiol (Fig. 3) was performed with peritubular ^{3}H-androgen concentrations (approx. 95 nM) similar to the estimated K_m for transepithelial androgen movement (approx. 80 nM). As in the seminiferous tubules, net proluminal androgen movement was not significantly inhibited by competition with estradiol (Fig. 3B).

In conclusion, transepithalial ^{3}H-androgen movement in the testis is linear and restricted while proluminal movement of ^{3}H-androgen into the caput epididymal tubules is saturable and occurs against a concentration gradient. Proluminal movement of ^{3}H-androgen into the seminiferous tubule lumen is not inhibited by competition with unlabelled testosterone[6] or unlabelled estradiol in the perifusion fluid. However, antigrade, proluminal movement of ^{3}H-androgen into the caput epididymal tubule lumen has been shown to be subject to competitive inhibition,[6] but is not inhibited by competition with unlabelled estradiol in the perifusion fluid. These data are consistent with our hypothesis that androgens diffuse across the epididymal epithelium and bind to ABP in the epididymal lumen, thus accounting for the accumulation of ^{3}H-androgen in the epididymal lumen, and the estimated V_{max} for ^{3}H-androgen movement across the caput epithelium is also consistent with this hypothesis.

Such a mechanism would not require active transport across the epithelium as an explanation of the uphill ^{3}H-androgen movement, but the effect of metabolic inhibitors on proluminal ^{3}H-androgen movement has not yet been tested. It is also possible that differential androgen binding to molecules in the testicular and epididymal interstitial space may effect compartmentalization of androgens in the testis and epididymis. Experiments to study these phenomena are currently underway in our laboratory.

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