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EVIDENCE FOR ACTIVE TRANSPORT OF ^{3}H-ANDROGENS ACROSS THE EPIDIDYMAL EPITHELIUM IN THE RAT

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ABSTRACT

The effect of metabolic inhibitors on transepithelial movement of ^{3}H-androgens was investigated by in vivo perifusion and subsequent micropuncture of caput and cauda epididymal tubules. Epididymal tissue (adenosine triphosphate (ATP) concentrations were determined at 1 h after exposure of perifusion fluid with metabolic inhibitor. To determine whether or not metabolic inhibitor alters intraluminal androgen-binding protein concentration or androgen binding to interstitial proteins in the caput epididymis, ^{3}H-dihydrotestosterone (DHT) binding to interaluminal androgen-binding protein and bound vs free androgen ratio in the interstitial fluid after 1 h perifusion with fluid containing metabolic inhibitor around caput epididymal tubules were examined. Proluminal movement of ^{3}H-androgens and tissue ATP concentrations in the caput and cauda epididymis were significantly decreased by addition of dinitrophenol (DNP) or potassium cyanide to the perifusion fluid. Relative intraluminal androgen-binding protein concentration and bound vs free ^{3}H-androgen ratio in the interstitial fluid were not altered when DNP or potassium cyanide was added to the perifusion fluid. These results demonstrate for the first time that an energy-dependent mechanism may be involved in the epididymal androgen uptake.

Key Words: Active transport, Androgen, Epididymis

INTRODUCTION

In vivo epididymal microperifusion and tubule micropuncture have demonstrated that the proluminal movement of ^{3}H-androgens in the epididymis occurs against a concentration gradient.[1,2] This uphill, proluminal movement of ^{3}H-androgens is subject to competitive inhibition.[3] It has also been shown that ^{3}H-androgen movement across the caput epididymal epithelium is reduced by hypophysectomy[4] and is completely returned to normal in the hypophysectomized rat supplemented with FSH or LH.[5] All of these findings are consistent with a hypothesis that ^{3}H-androgens move across the epididymal epithelium by simple diffusion where the ^{3}H- androgen is then bound to intraluminal androgen-binding protein. Such antigrade, proluminal movement of ^{3}H-androgens into the tubule lumen can occur by active transport, which is energy dependent and also accounts for the competitive inhibition of ^{3}H-androgen movement. The objective of the present study was to determine if proluminal movement of ^{3}H-androgen into the epididymal tubule is subject to metabolic inhibition. We have reported here for the first time evidence for an active transport mechanism in the epididymal tubule uptake of ^{3}H-androgen.

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MATREIALS AND METHODS

In vivo Microperifusion and Micropuncture in the Caput and Cauda Epididymis

The animals were anesthetized with intraperitoneal injections of inactin (sodium 5-ethyl-5-(1-methyllpropyl)-2-thiobarbiturate, Byk Guilden Konstanz, Hamburg, Germany; 100 mg/kg body weight) and subjected to in vivo microperifusion and micropuncture as previously described.[1,2] Briefly, the epididymis was exteriorized through a scrotal incision, placed in a 35°C testicle warmer, and stabilized in 2% agar. The caput or cauda tubules were perifused in vivo through a 100 μm tip micropipette inserted through the tunica albuginea. The tip of the pipette was always kept in sight to ensure that the epididymal tubule was not cut or punctured. In the caput epididymis, the perifusion pump was set at a priming rate of 6 μl/min for 15 min, then at a sustaining rate of 2 μl/min. In the cauda pididymis, the priming and sustaining perifusion rates were 6 μl/min and 3 μl/min, respectively. The perifusion fluid was 0.3% lissamine- green dyed Minium Essential Medium (MEM) containing 26.7 μCi ^{3}H-testosterone/ml and 1.3 μCi ^{14}C-polyethyleneglycol (PEG)/ml, or the same fluid supplemented with 0.1 mM or 1 mM 2,4- dinitrophenol (DNP) or with 0.1 mM or 1 mM potassium cyanide. These five different fluids were used in different experiments on the caput and cauda epididymis. A fluid was perifused around the epididymal tubules, and interstitial (perifusion) fluid and adjacent intraluminal fluid were collected by micropuncture at 1 h after initiation of the sustaining perifusion. Radioactivity of ^{3}H-androgen and ^{14}C-PEG in the perifusion and intraluminal fluids was determined in all samples and the percentage of peritubular ^{3}H-androgen and ^{14}C-PEG appearing in the intraluminal fluid was determined. Since ^{14}C-PEG is essentially excluded by the blood epididymal barriers, it was included in the perifusion fluid as a marker for contamination of the intraluminal fluid by fluid from the extratubular compartments. The proportion of extratubular ^{14}C-PEG appearing in the intraluminal fluid was used to correct the ^{3}H-testosterone data by substraction.

Caput or Cauda Tissue ATP (adenosine triphosphate) Concentration

To prove deprivation of cell ATP energy by exposure of epididymal tubules to metabolic inhibitors, ATP concentrations were determined at 1 h after exposure to MEM perifusion fluid with or without metabolic inhibitor (0.1 mM, 1 mM DNP or potassium cyanide). Following 1 h perifusion around the proximal caput and distal cauda tubules (Fig. 1), perifused tissues were immediately dissected out, frozen in dry ice, weighed (usual weight 40–50 mg), and homogenized for 20 sec at setting 4 on a Polytron tissue homogenizer (Brinkman Instruments, Rexdale, Ontario, Canada) in 0.9 N perchloric acid.[4] After centrifugation, an aliquot of the supernatant was neutralized with one half volume of potassium carbonate. The precipitated potassium was removed bycentrifugation.[4] Aliquots of the supernatant were used for ATP analysis by the luciferin-luciferase reaction.[5] Luciferase in the presence of luciferin, oxygen, magnesium and ATP catalyses the formation of adenyl-luciferin. Adenyl-luciferin is oxidized by atmospheric oxygen to adenyl-oxyluciferin and this process is accompanied by emission of light. This light flash was counted by a Beckman liquid scintillation spectrophotometer (LS 7000, Beckman Instruments, Inc. Fullerton, CA).[5]

^{3}H-DHT Binding to Intraluminal Androgen-binding Protein in the Caput Epididymis

Metabolic inhibitors may somehow have reduced intraluminal androgen-binding protein, which has previously been shown to be important in the maintenance of antigrade, proluminal ^{3}H- androgen movement[3,6,7]; thus, the effect of peritubular metabolic inhibitors on intraluminal androgen-binding protein concentration was determined.

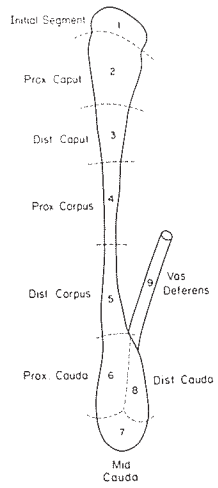

Fig. 1. Schematic representation of the epididymis. Dotted lines divide the epididymis into eight gross anatomical segments. 1: Initial segment; 2: proximal caput; 3: distal caput; 4: proximal corpus; 5: distal corpus; 6: proximal cauda; 7: mid cauda; 8: distal cauda.

One-microliter aliquots of cell-free caput epididymal fluid were obtained at 1 h after exposure to MEM perifusion fluid with or without metabolic inhibitor (0.1 mM DNP or potassium cyanide). These fluids were subjected to electrophoresis on 8% polyacrylamide mini-slab gels. The gels contained 10 μCi ^{3}H-DHT to allow steady-state binding of the labeled androgen to intraluminal androgen-binding protein.[8] ^{3}H-DHT was distributed uniformly throughout the length of the gel and was stationary in the electrophoretic field until it was bound to a protein moving through the gel. Labeled gels were sliced into 2- mm sections and radioactivity for each gel slice (CPM/2-mm gel slice) was determined by scientillation spectrophotometry.[9] Relative intraluminal androgen-binding protein concentrations were determined by comparing areas under the binding-profile curves of gels from each fluid type.

Measurement of Bound vs Free Androgen in the Interstitial Fluid in the Caput Epididymis

Metabolic inhibitors may have altered ^{3}H-androgen binding to interstitial and vascular proteins (secreted into the perifusion fluid) in the epididymis and affected proluminal movement of ^{3}H-androgen; thus, the effect of metabolic inhibitors in the perifusion fluid on bound ^{3}H-androgen in the interstitial fluid was determined.

Interstitial fluids in the caput epididymis were obtained at 1 h after exposure to MEM perifusion fluid containing ^{3}H- testosterone and ^{14}C-PEG with or without metabolic inhibitor (0.1 mM DNP or 0.1 mM potassium cyanide). Charcoal was dispersed in the fluid sample by vibration, then removed by centrifugation. Triplicate aliquots of each supernatant were assayed by scintillation spectrophotometry to determine protein-bound ^{3}H-androgen. Total radioactivity in a separate sample was determined.

Data Analysis

Chauvenet's criterion was applied to all the data.[10] They are presented as mean and SEM. All multiple comparisons were made by the Kruscal Wallis test[11] for nonparametric data followed by the Wilcoxon rank sum test ($p<0.05$).

RESULTS

Transepithelial ^{3}H-androgen Movement in the Caput and Cauda Epididymis

Isotope concentrations remaining in the perifusion fluid around the caput and cauda tubules after 1 h were not significantly different whether originating from ^{14}C-PEG or ^{3}H- testosterone (data not shown). This was true within the control and treated groups. These data demonstrate that the radiolabelled compounds available to the bubules were relatively similar from group to group.

After 1 h perifusion of the caput epididymal tubules of the control rats, $315.3 \pm 74.5\%$ of peritubular ^{3}H-androgen concentrations appeared in the intraluminal fluid (Fig. 2). These values were significantly decreased by exposure of the epididymal tubules to 0.1 mM DNP and 0.1 mM potassium cyanide ($142 \pm 23.8\%$ and $73.74 \pm 34\%$, respectively) (Fig. 2). Proluminal movement of ^{3}H-androgen into the caput epididymal tubules was also significantly inhibited by 1 mM DNP and 1 mM potassium cyanide ($149.4 \pm 29.3\%$ and $79.0 \pm 32.0\%$, respectively) (Fig. 3).

After 1 h perifusion of the cauda epididymal tubules of the control rats ^{3}H-androgen concentrations in the luminal fluid were $124.73 \pm 11.7\%$ (Fig. 4). Addition of 0.1 mM DNP and 0.1 mM potassium cyanide caused a significant decline in the proluminal movement of ^{3}H-androgen ($35.16 \pm 5.1\%$ and 42.82 ± 6.3) (Fig. 4). One mM DNP and 1 mM potassium cyanide significantly reduced net proluminal movement of ^{3}H-androgen ($33.7 \pm 4.8\%$ and $39.5 \pm 5.3\%$, respectively) (Fig. 5). Metabolic inhibitors completely eliminated the antigrade or uphill aspect of proluminal androgen movement.

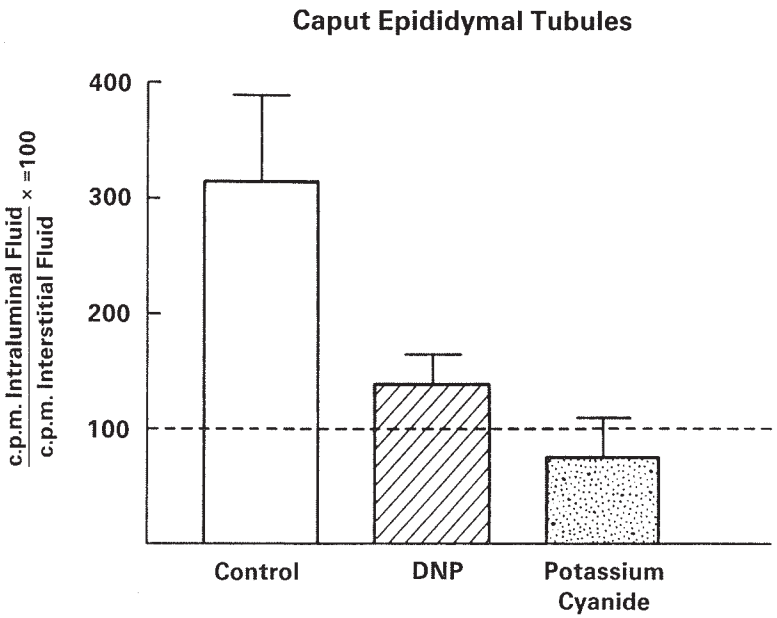

Fig. 2. Effect of 0l.1 mM DNP or potassium cyanide on proluminal movement of ^{3}H-androgen into caput epididymal tubules. Both metabolic inhibitors significantly eliminated ($p<0.05$) proluminal ^{3}H-androgen movement at levels greater than those expected from diffusion alone (dashed line).

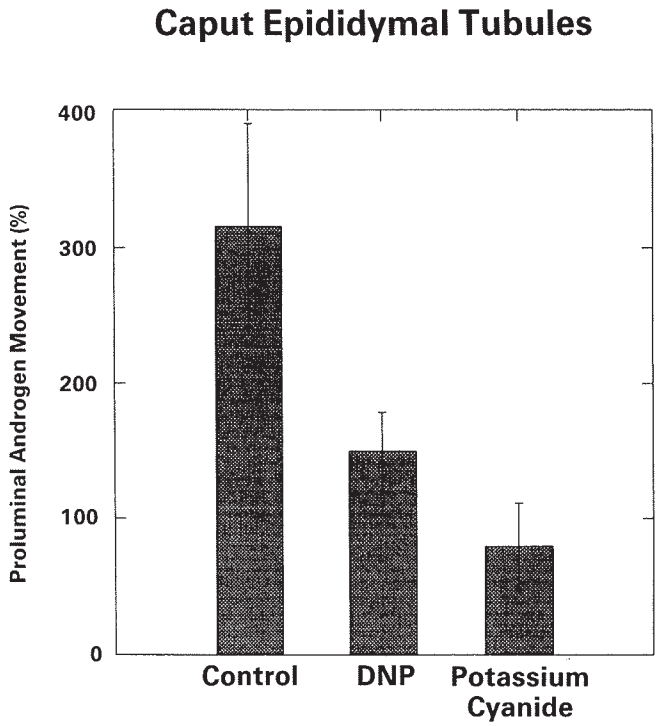

Fig. 3. Effect of 1 mM DNP or potassium cyanide on proluminal movement of ^{3}H-androgen into caput epididymal tubules. Both metabolic inhibitors significantly reduced ($p<0.05$) proluminal ^{3}H-androgen movement.

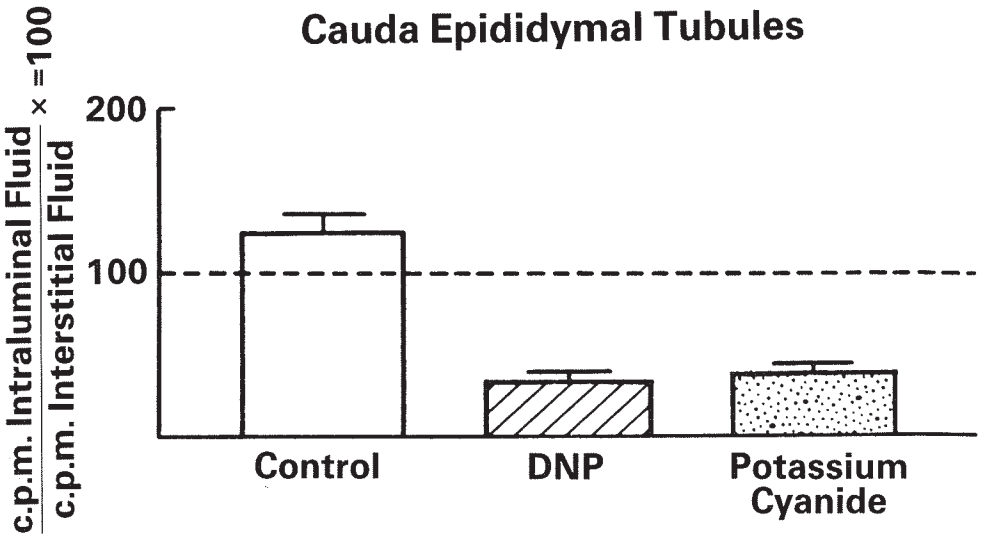

Fig. 4. Effect of 0.1 mM DNP or potassium cyanide on proluminal movement of ^{3}H-androgen into cauda epididymal tubules. Both metabolic inhibitors significantly eliminated ($p<0.05$) proluminal ^{3}H-androgen movement at levels greater than those expected from diffusion alone (dashed line).

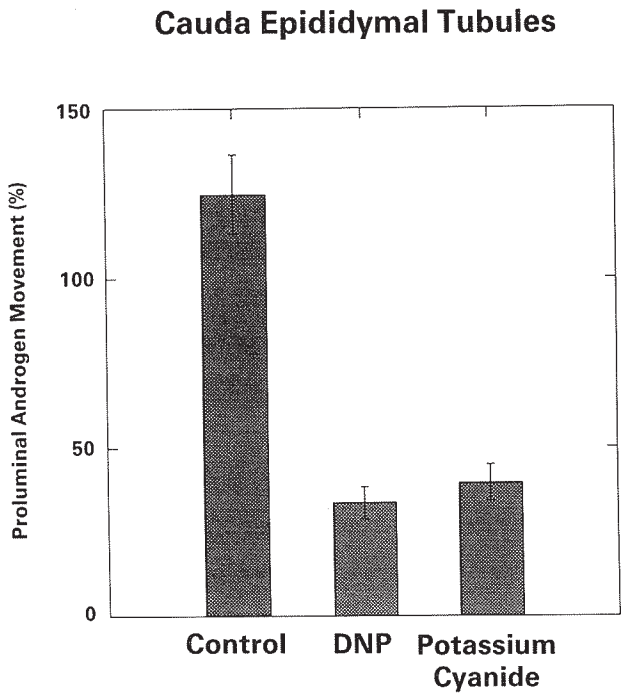

Fig. 5. Effect of 1 mM DNP or potassium cyanide on proluminal movement of ^{3}H-androgen into cauda epididymal tubules. Both metabolic inhibitors significantly reduced ($p<0.05$) proluminal ^{3}H-androgen movement.

Assay for Epididymal Tissue ATP Concentration

The ATP concentrations of normal unperifused caput epididymal tissues were 2.73 ± 0.2 ng/mg (wet weight) (Fig. 6). These values were significantly reduced by addition of 0.1 mM DNP and 0.1 mM potassium cyanide to the perifusion fluid (0.58 ± 0.09 ng/mg and 0.27 ± 0.07 ng/mg, respectively) (Fig. 6).

The ATP concentrations of normal unperifused cauda epididymal tissues were 4.08 ± 0.66 ng/mg (Fig. 7). These values were significantly decreased by addition of 0.1 mM DNP and 0.1 mM potassium cyanide to the perifusion fluid (0.37 ± 0.14 ng/mg and 0.68 ± 0.12 ng/mg, respectively) (Fig. 7). ATP concentrations in the caput and cauda epididymal tissues were significantly decreased after exposure to perifusion fluid with metabolic inhibitor.

^{3}H-DHT Binding to Intraluminal Androgen-binding Protein in the Caput Epididymis

Intraluminal androgen-binding protein activity in the caput epididymal fluid after exposure to MEM perifusion fluid with or without metabolic inhibitor is shown in Fig. 8. Each fluid was analyzed at least three times. The Mean ± SEM area under the androgen-binding protein peak after exposure to MEM perifusion fluid alone was 8.8 ± 1.1 cm^2. This value was not significantly different from that after exposure to MEM perifusion fluid containing 0.1 mM DNP or 0.1 mM potassium cyanide (7.8 ± 0.9 cm^2 or 6.1 ± 0.62 cm^2, respectively). Relative intraluminal androgen-binding protein concentrations were not altered by addition of metabolic inhibitor to the perifusion fluid.

Bound vs Free Androgen in the Interstitial Fluid in the Caput Epididymis

The Bound vs free ^{3}H-androgen ratio in the interstitial fluid of the caput epididymis at 1 h

after perifusion with or without metabolic inhibitor is shown in Table 1. The Bound vs ^{3}H-androgen ratio in the interstitial fluid after perifusion with MEM perifusion fluid alone was 0.07 ± 0.01. This value was not significantly different from that after perifusion with MEM perifusion fluid containing 0.1 mM DNP or 0.1 mM potassium cyanide (Table 1). Bound vs free ^{3}H-androgen ratio in the interstitial fluid was not altered when metabolic inhibitor was added to the perifusion fluid.

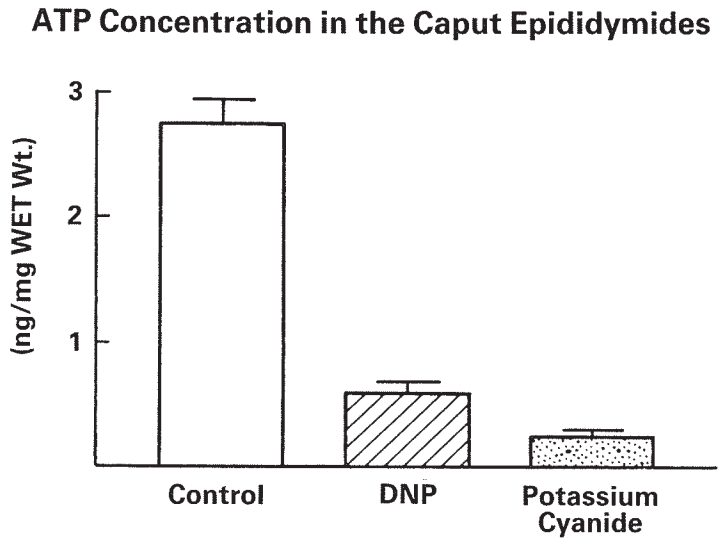

Fig. 6. ATP concentrations in the caput epididymis after 1 h exposure to perifusion fluid with or without 0.1 mM DNP or potassium cyanide. Both metabolic inhibitors significantly decreased ($p<0.05$) tissue ATP concentrations.

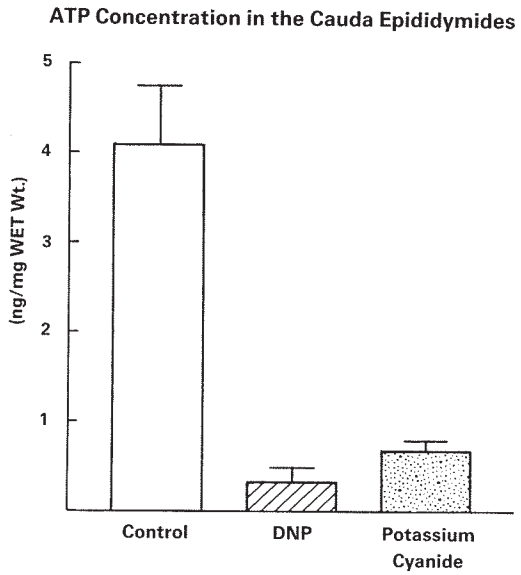

Fig. 7. ATP concentrations in the cauda epididymis after 1 h exposure to perifusion fluid with or without 0.1 mM DNP or potassium cyanide. Both metabolic inhibitors significantly decreased ($p<0.05$) tissue ATP concentrations.

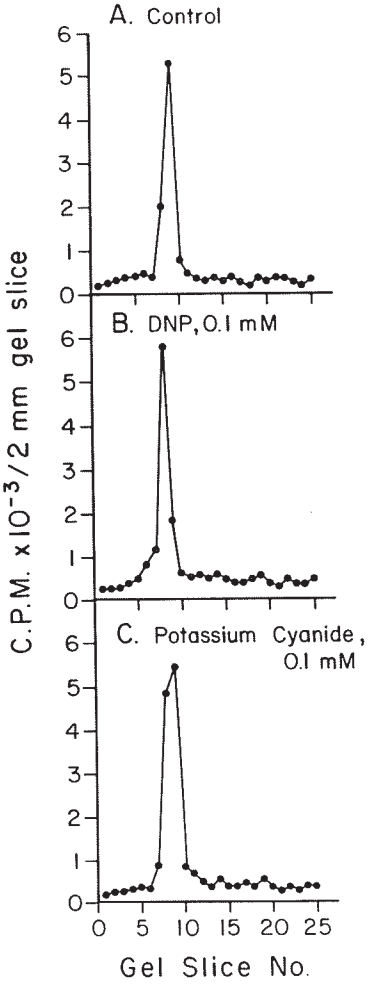

Fig. 8. Effect on intraluminal ABP content of perifusing caput tubules with metabolic inhibitors for 1 h. (A) Control; B) DNP; (C) Potasium cyanide. Perifusion with metabolic inhibitors had no significant effect on intraluminal ABP concentrations. Sample volume was 1 μl lumen fluid.

Table 1. Bound vs Free ^{3}H-androgen Ratio in the Interstitial Fluid of Caput Epididymis 1 h after Perifusion with or Without Metabolic Inhibitor.

	Bound/free ^{3}H-androgen
Control	0.07 + 0.01
DNP	0.08 + 0.01
Potassium Cyanide	0.07 + 0.02

Metabolic inhibitors had no effect on androgen binding to interstitial protein.

DISCUSSION

In vivo epididymal microperifusion and tubule micropuncture demonstrated that the proluminal movement of ^{3}H-androgens in the epididymis occurs against a concentration gradient.[1,2] This uphill, antigrade proluminal movement of ^{3}H-androgens is subject to competitive inhibition (unlabelled testosterone in the perifusion fluid at 10X and 100X the concentration of ^{3}H-testosterone significantly reduced proluminal movement of the isotope).[3] In the present study, proluminal movement of ^{3}H-androgen was inhibited by 0.1 mM or 1 mM DNP, a metabolic inhibitor effective in even micromolar ranges in a variety of tissues.[12–14] Potassium cyanide, another metabolic inhibitor that blocks ATP production at another point in the metabolic pathway,[15] completely eliminated uphill proluminal movement of ^{3}H- androgens at 0.1 mM or 1 mM concentration. Such studies as these have never been done at the level of the epididymal tubule, and with the knowledge that proluminal ^{3}H-androgen movement occurs against a concentration gradient. Therefore, suppression of proluminal antigrade movement of ^{3}H-androgen by metabolic inhibitor provided an interesting insight to fully characterized proluminal ^{3}H-androgen movement.

This finding is certainly inconsistent with accepted dogma that ^{3}H-androgens move across the epididymal epithelium by simple diffusion where the ^{3}H-androgen is then bound to intraluminal androgen-binding protein. Such a hypothesis accounts for the antigrade movement of ^{3}H-androgens into the tubule lumina without the need for active transport and also accounts for the competitive inhibition of ^{3}fH-androgen movement.[3]

It is true that ^{3}H-androgen movement in the epididymal tubule was inhibited by increasing concentrations of unlabelled testosterone in the perifusion fluid; however, the highest concentrations of unlabelled testosterone in the perifusion fluid caused intraluminal ^{3}H-androgen concentrations to fall below 100% of interstitial fluid ^{3}H-androgen concentrations.[3] This should not have happened if simple diffusion across the epididymal epithelium was the complete answer for ^{3}H-androgen movement into the epididymal tubule. If the hypothesis of simple diffusion followed by intraluminal binding is correct, then competitive inhibition of ^{3}H- androgens to intraluminal androgen-binding protein should not have driven intraluminal ^{3}H-androgen concentrations below the result expected from diffusion alone (100%, i.e., unity between intraluminal and interstitial ^{3}H-androgen concentrations). The ability of an uncoupling agent to reduce significantly the uphill ^{3}H-androgen movement into the epididymal tubules indicates that an energy-requiring system for ^{3}H-androgen movement exists in the rat epididymal tubules. This finding can be explained by the presence of a high affinity androgen transport receptor in the epididymal epithelial cell.

0.1 mM or 1 mM DNP and 0.1 mM or 1 mM potassium cyanide significantly reduced proluminal ^{3}H-androgen movement. Therefore, subsequent experiments were conducted using 0.1 mM metabolic inhibitors. DNP or potassium cyanide uncouples oxidative phosphorylation from cell respiration. This deprives the epithelial cells of ATP. The results of the assay for epididymal tissue ATP concentrations demonstrated that caput and cauda epididymal tissue ATP concentrations were 2.7 ng/mg and 4.08 ng/mg, respectively. There have been no previous reports of ATP measurement in the epididymal tissues. These ATP levels were much less than those in other tissues (for example, muscle, 2358 ng/mg[16]; liver, 550 ng/mg[17]; and kidney, 130 ng/mg[17]). DNP or potassium cyanide significantly reduced ATP concentrations. These results suggest that phosphate bond energy is required to maintain the transport system.

Energy deprivation may have inhibited androgen-binding protein secretion or water resorption by principal cells and altered the intraluminal androgen-binding protein concentration, which has previously been shown to play an important role in the maintenance of antigrade,

proluminal ^{3}H-androgen movement.[3,6,7] However, relative intraluminal androgen- binding protein concentrations were not altered by addition of metabolic inhibitor to the perifusion fluid.

It is also possible that a metabolic inhibitor interfered with the capillary endothelium and then interstitial capillary vessels became more leaky; thus, more labelled steroid bound to interstitial protein and proluminal androgen movement was decreased. However, the bound vs free ^{3}H-androgen ratio in the interstitial fluid was not increased when a metabolic inhibitor was added to the perifusion fluid. All of these findings are consistent with an energy-requiring mechanism in the rat epididymal androgen uptake. Although the role of intraluminal androgen-binding protein is still important,[3,7] this energy-dependent mechanism of androgen transport into the epididymal bubules should be scrutinized.

In conclusion, transepithelial movement of ^{3}H-androgen into the caput and cauda epididymal tubules was significantly decreased by exposure of epididymal tubules to metabolic inhibitors. ATP concentrations in the caput epididymal tissues were significantly decreased after exposure to perifusion fluid with metabolic inhibitor. Relative intraluminal androgen-binding protein concentrations were not altered by addition of a metabolic inhibitor to the perifusion fluid. These data suggest the presence of an energy-dependent component in the mechanism by which ^{3}H-androgens appear in the epididymal lumen. This demonstration of an energy-dependent ^{3}H-androgen transport in the epididymal tubule is the first report. It also introduces a heretofore unsuspected mechanism into the process of testicular physiology.

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