Nagoya J. Med. Sci. **54.** 00 ~ 00, 1992

THE EFFECT OF HYPOPHYSECTOMY ON PROLUMINAL MOVEMENT OF ^{3}H-ANDROGENS ACROSS THE EPIDIDYMAL EPITHELIUM IN THE RAT

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ABSTRACT

The effect of hypophysectomy on transepithelial movement of ^{3}H-androgen in the rat epididymis was examined by using in vivo microperifusion of ^{3}H-testosterone followed by in vivo micropuncture to obtain peritubular and intraluminal fluids. Experiments were performed on aminals without hypophysectomy or on aminals 5-6 days after hypophysectomy and 9-10 days after hypophysectomy. 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 the intraluminal fluid by peritubular fluid. Radioactivity of isotopes in the interstitial and intraluminal fluid was determined at 1 and 2 hours after perifusion and the percentage of peritubular isotopes appearing in the intraluminal fluid was determined. A sperm concentration microassay was performed on micropuncture samples from the epididymal tubules to assess testicular contribution to the lumen content. Proluminal movement of ^{3}H-androgen and intratubular sperm concentrations in the caput epididymal tubules of rats 9-10 days after hypophysectomy were significantly decreased. Proluminal movement of ^{3}H-androgen and intratubular sperm concentrations in the cauda epididymal tubules of rats 9-10 days after hypophysectomy were significantly increased. These results suggest that proluminal androgen movement is controlled by the presence of some testicular product in the epididymal lumen.

Key Words: Testosterone, Epididymis, Hypophysectomy, Micropuncture, Microperifusion.

INTRODUCTION

The epididymis serves as a conduit and storage depot for spermatozoa, but beyond that the organ maintains sperm maturation processes that result in the sperm cell's obtaining progressive motility and fertility. Many of the details for the mechanisms underlying these biologically significant functions remain to be elucidated; however, it is now clear that the physiological funcitons of the epididymis are very complex.

Although the mechanisms by which the epididymis performs this function of sperm maturation are not fully understood, the general consensus is that this process is affected by the fluid milieu within the epididymal lumen and by the epithelial cells which produce it. It has long been accepted that the epididymis is an androgen target tissue, that epididymal metabolism and epididymal epithelial secretion depend on the presence of androgens, and that, consequently, epididymal sperm maturation does not occur in the absence of androgens.[1,2,3] However, there is little determination about local mechanisms that regulate androgen availability to either that basal or apical aspects of their respective epithelia.

Received for Publication in August 7, 1991

Recently, by using in vivo microperifusion followed by in vivo micropuncture we have shown that transepithelial movement of ^{3}H-androgen is against a concentration gradient.[4,5] It has been demonstrated that follicle-stimulating hormone (FSH) can stimulate an increase of androgen concentration in the caput epididymis.[6] It is known that FSH stimulated the secretion of androgen binding protein (ABP) by Sertoli cells[7] and that ABP is concentrated in the epididymal lumen.[8] Thus, we have hypothesized that ABP is important in regulating intraepididymal androgen concentrations. These facts have also led us to investigate the effect of hypophysectomy on transepithelial movement of ^{3}H-androgen into epididymal tubules.

MATERIALS AND METHODS

In Vivo Perifusion of Epididymal Tract and Subsequent Micropuncture

Adult male Sprague-Dawley rats (450-670 g) were maintained in a constant temperature (25°C), constant humidity (50%) vivarium on a 12L:12D cycle. Food and water were available ad libitum. Animals were anesthetized and prepared for microperifusion and micropuncture of the epididymal tubules as previously described.[4,5,9] Briefly, a 100-μm-tip micropipette was placed in a micromanipulator 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 was 3 ml of lissamine-green-dyed Minimum Essential Medium (pH 7.0-7.4; Gibco Laboratories, Grand Island, NY) containing 80-μCi ^{3}H-Testosterone (specific activity 55.2 Ci/mM; New England Nuclear, Boston, MA) and 4.0-μCi ^{14}C-polyethyleneglycol (^{14}C-PEG, specific activity 15.0 mCi/g, New England Nuclear). The filled syringe was attached to a perifusion pump (model 341B, Sage Instruments, Cambridge, MA). Priming and sustaining perifusion rates for the caput and cauda epididymal tubules were performed as previously described.[4] The micropipette was inserted directly through the caput epididymal tunica, and the pipette tip was left in the interstitial space as described previously.[4] Samples of fluid from the interstitial space and intraluminal fluids of the tubule were collected by micropuncture at one and two hours after initiation of the sustaining perifusion. After the samples were collected, cell-free fluids were obtained by centrifugation and divided into three aliquots which were transferred into vials containing 3 ml of scintillation fluid, and ^{3}H- and ^{14}C-radioactivity was determined by scintillation spectrophotometry.

Since ^{14}C-PEG is excluded by the blood epididymal barrier, it was included in the perifusion fluid as a marker for contamination of the intraluminal fluid by blood or peritubular fluid.[4] All intraluminal ^{3}H-androgen data were corrected for contamination following ^{14}C-PEG. Radioactivity in the tubule lumina was expressed as the percentage of radioactivity appearing in the same volume of interstitial fluid surrounding the same tubule at that same time period (intraluminal fluid counts per minute [CPM] divided by interstitial fluid CPM × 100).

Intratubular Sperm Concentrations

Sperm concentrations in micropunture samples of fluid from the caput and cauda epididymis were determined with a sperm concentration microassay. Briefly, spermatozoa were obtained by in vivo micropuncture, put through a microdilution procedure with 1% hyaluronidase, and counted on a hemocytometer (× 3) using standard techniques.

Treatment Groups

In addition to a group of nine intact, untreated control rats there were two groups as described below.

1) Five-six day hypophysectomy (5-6d Hypox) group: Animlas were hypophysectomized by entering hypophyseal fossa through a cervical incision on Day 0. These animals were maintained on water containing 1% sodium chloride and 5% dextrose. On Day 5 or 6, they were subjected

to studies as described above of proluminal ^{3}H-androgen movement into the caput and cauda epididymidis and of sperm concentrations in the caput and cauda lumina.

2) Nine-ten day hypophysectomy (9-10d Hypox) group: Animals were hypophysectomized on Day 0. On Day 9 or 10, they were subjected to the same studies as the 5-6d Hypox animals.

After completion of each experiment, the hypophyseal fossa was examined for presence of pituitary tissue. Animals with residual pituitary tissue were excluded from the study.

Data Analysis

Chauvenet's criterion was applied to all the data.[10] The data 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 ($p < 0.05$).

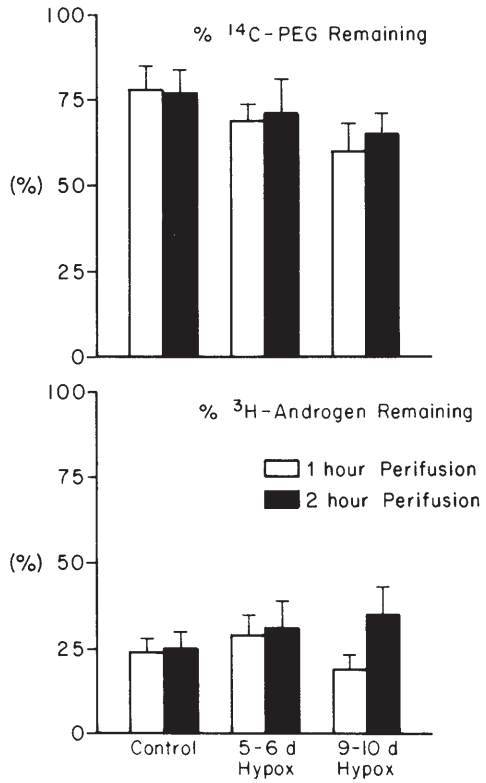

Fig. 1. Characterization of radiolabeled compounds in peritubular fluid from caput epididymal interstitial space in control animals and those hypophysectomized after one and two-hour perifusion. Values were expressed as a percentage of original perifusion fluid ^{14}C-PEG or ^{3}H-androgen concentration remaining in the fluid from the peritubular space at either one or two hours after perifusion. No significant differences in isotope concentrations remaining around the caput tubules were found between groups or times.

RESULTS

Proluminal movement of ^{3}H-androgen and intraluminal sperm concentrations in the caput epididymal tubules

Isotope concentrations remaining in the perifusion fluid around the caput tubules after one and two hours were not significantly different whether originating from ^{14}C-PEG or ^{3}H-T (Figure 1). This was true within the control and within both hypophysectomized groups. Additionally, there were no significant differences in the isotope concentrations between experiments (Fig.1). These data demonstrate that the radiolabeled compounds available to the tubules were relatively similar from group to group and hour to hour.

After one and two hours perifusion of the caput epididymal tubules of the control rats, 315.3 ± 74.5% and 275.8 ± 51.5% of peritubular ^{3}H-androgen concentrations, respectively, appeared in the intraluminal fluid (Fig.1). These values were not altered in the 5-6d Hypox group, but the 9-10d Hypox animals demonstrated a significantly reduced net proluminal movement of ^{3}H-androgen (91.5 ± 39.1% and 44.44 ± 9.1% at one and two hours, respectively), and the caput tubules lost their capacity to move ^{3}H-androgen against a concentration gradient (Fig.2).

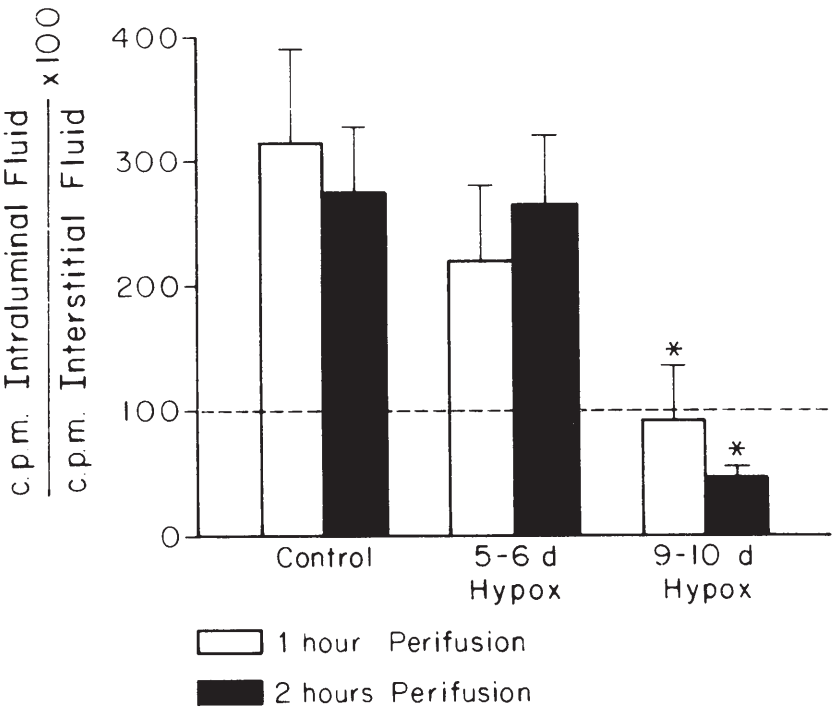

Fig. 2. Proluminal movement of ^{3}H-androgen into the tubules of the caput epididymidis in control animals, those 5 or 6 days after hypophysectomy (5-6d Hypox) and those 9 or 10 days after hypophysectomy (9-10d Hypox). Asterisk shows significant difference ($p < 0.05$).

Intraluminal sperm concentrations in the control caput tubules were $0.72 \pm 0.07 \times 10^{9}$ sperm/ml. These values were not significantly affected in the 5-6d Hypox group, but they were significantly reduced int eh 9-10d Hypox group (Fig. 3).

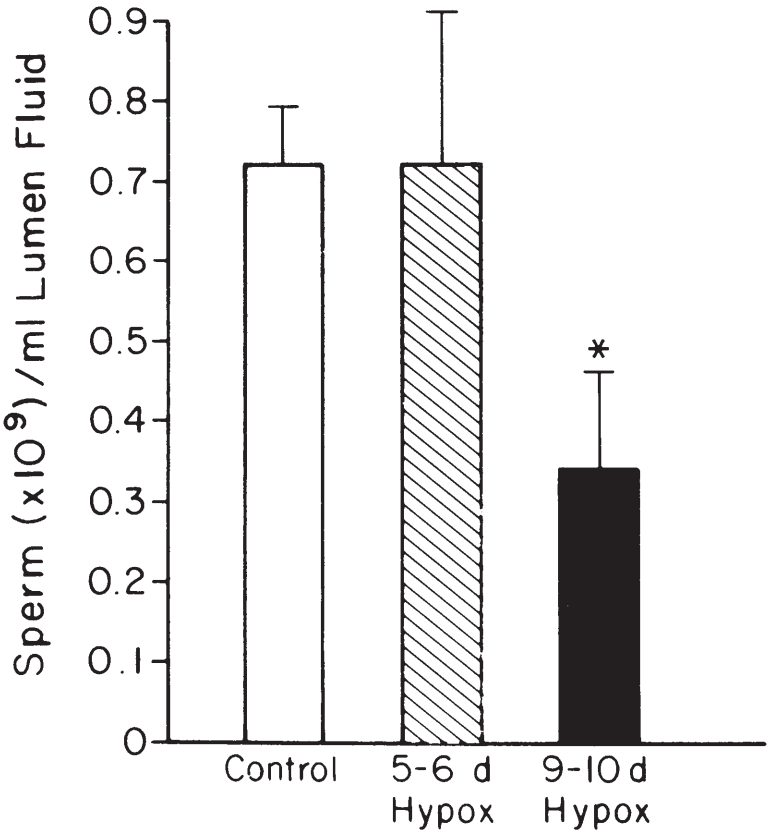

Fig. 3. Intratubular sperm concentrations of lumen fluids in the caput epididymidis of control group, 5-6d Hypox group and 9-10d Hypox group. Treatment group data are compared statistically with the control data. Asterisk shows significant difference ($p < 0.05$).

Proluminal movement of ^{3}H-androgen and intraluminal sperm concentrations in the cauda epididymal tubules

Isotope concentrations remaining around the cauda tubules after one and two hours perifusion were stable in all experimental groups (Fig.4). After one and two hours perifusion of the cauda epididymal tubules of the control rats approximately 120% of peritubular ^{3}H-androgen

concentrations appeared in the intraluminal fluid (Fig.5). These values were significantly increased in both the 5-6d and 9-10d Hypox groups, but the one hour value in the 5-6d Hypox group was not significantly different from that of the control group (Fig.5). Intraluminal sperm concentrations in the control cauda tubules were $1.85 + 0.045 \times 10^9$ sperm/ml. These values were significantly increased in both 5-6d and 9-10d Hypox groups (Fig.6).

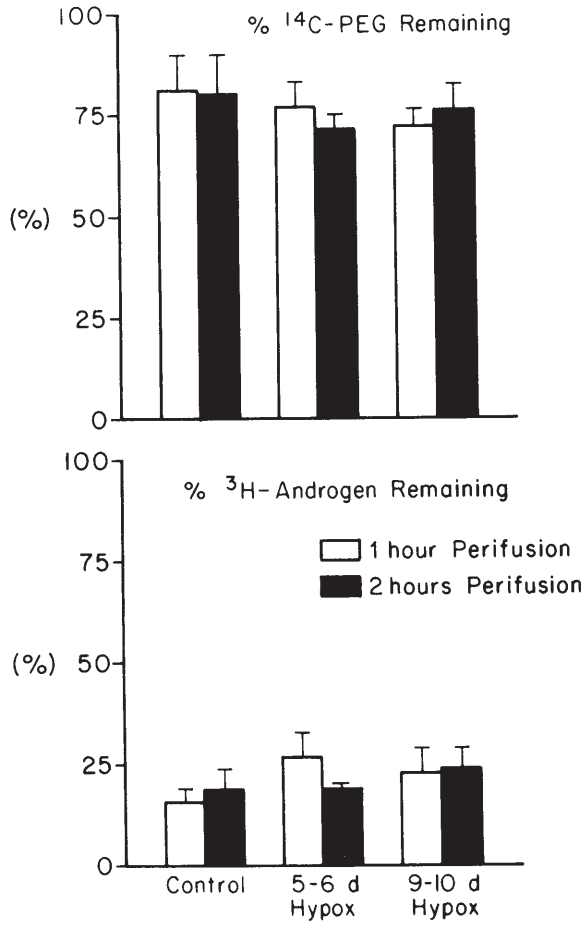

Fig. 4. Characterization of radiolabeled compounds in peritubular fluid from cauda epididymal interstitial space in control animals and those hypophysectomized after one and two-hour perifusion. Values were expressed as the percentage of original perifusion fluid ^{14}C-PEG or ^{3}H-androgen concentration remaining in the fluid from the peritubular space at either one or two hours after perifusion. Isotope concentrations remaining around the cauda tubules after one and two-hour perifusion were stable in all experimental groups.

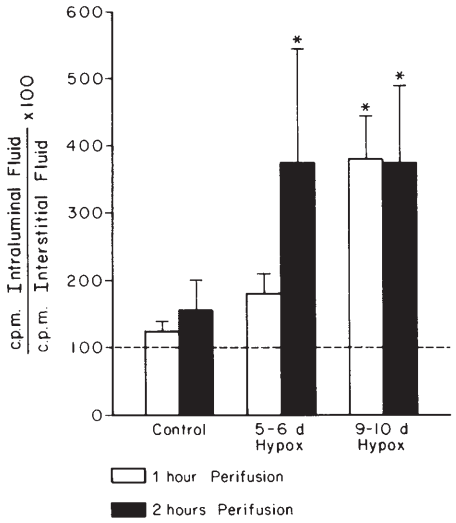

Fig. 5. Proluminal movement of ^{3}H-androgen into the tubules of the cauda epididymidis in control animals, those 5 or 6 days after hypophysectomy (5-6d Hypox) and those 9 or 10 days after hypophysectomy (9-10d Hypox.). Asterisk shows significant difference ($p < 0.05$).

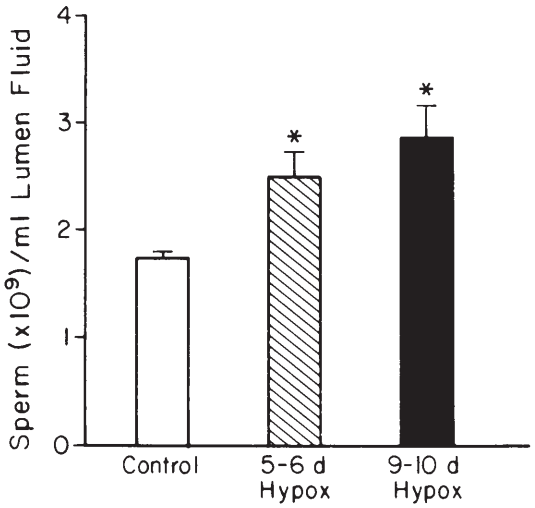

Fig. 6. Intratubular sperm concentrations of lumen fluids in the cauda epididymidis of the control group, 5-6d Hypox group and 9-10d Hypox group. Treatment group data are compared statistically with the control data. Asterisk shows significant difference ($p < 0.05$).

DISCUSSION

Antigrade, transepithelial ^{3}H-androgen movement was maintained in the caput epididymis at 5 to 6 days but not at 9 to 10 days after hypophysectomy (Fig.1). Likewise, intraluminal caput sperm concentrations were still normal in the 5-6d Hypox group, but were significantly decreased in the 9-10d Hypox rats (Fig.3). These data suggest that a period of ten days after hypophysectomy is sufficient time for clearance of pre-hypophysectomy testicular contribution from the caput epididymal lumen, and that there is a loss of antigrade proluminal androgen movement coincident with the loss of testicular contribution. It has been demonstrated that treatment of hypophysectomized rats with FSH can stimulate an incrase of androgen concentration in the caput epididymal tissues greater than that seen with luteinizing hormone (LH) alone.[6] FSH stimulates the secretion of ABP by Sertoli cells[7], and ABP is concentrated in the epididymal lumen.[8] Thus, we have hypothesized that some testicular product in the epididymal lumen, most likely ABP, is needed to maintain normal proluminal androgen movement.

After one and two-hour perifusion of the cauda epididymal tubules of control rats approximately 120% of peritubular ^{3}H-androgen concentrations appeared in the intraluminal fluid (Fig.5). These values were significantly increased in both the 5-6d and 9-10d Hypox groups (Fig.5). It is speculated that if the epididymal flow rate was decreased in the Hypox rats, but some epididyaml absorption continued, and increase in intraluminal concentrations of testicular product resulting in an increase in proluminal androgen movement might occur. Increase in intraluminal sperm concentrations in the cauda tubules in both the 5-6d and 9-10d Hypox groups may be associated with increase of testicular protein and hormone. Although intraluminal fluid volumes have been insufficient to allow analysis of intraluminal ABP in any of the hypophysectomized groups, these results are consistent with the speculation that intraluminal ABP is an important factor in regulating transepithelial androgen movement in the epididymis.

ABP concentrations are high in rat caput epididymal tissue extracts and intraluminal fluids, and these values decline in the more distal epididymis.[12,13,14] Attramadal et al.[15], Pelliniemi et al.[16], and Feldman[17] independently demonstrated that ABP is reabsorbed by the epithelial cells of the rat initial segment and caput epididymis, and Gerrard et al.[18] have recently provided evidence that the ABP internalized by receptor-mediated endocytosis still retains its androgen. This was the first direct evidence that ABP and adgrogens are internalized together in the caput epididymal epithelium. Turner et al.[8] showed that large amounts of ABP are reabsorbed between the rete testis and caput epididymis. The intraluminal ABP: androgen ratio remains approximately 1:1 from the proximal to the distal epididymis[8], and this result is consistent with our speculation that ABP sets the intraluminal androgen concentration.

Earlier reports[19,20,21] have indicated that vascular androgens alone are not sufficient to maintain the caput epithelial function, and that some other intraluminal, nonsteroidal testicular factor, is required. That this factor is ABP must be considered, especially since Anthony et al.[22,23] found that epididymal ABP concentrations were significantly correlated with sperm fertility, presumably through a role in affecting epididymal epithelial function.

Net proluminal movement of ^{3}H-androgens across the tubule epithelia in the in vivo perifusion studies occurs in the order of caput, corpus epithelium > cauda epithelium > seminiferous epithelium.[4,5] Intraluminal, radioimmunoassayable ABP concentrations follow a similar pattern.[8] This apparent association between intraluminal ABP and proluminal ^{3}H-androgen movement and the absence of evidence of intraluminal androgen binding in either sperm cells or intraluminal fluid provide evidence for the speculation that intraluminal ABP is the responsible molecule for the proluminal, antigrade, transepithelial movement of ^{3}H-androgens, and therefore, for the androgen microenvironment in the epididymis. Subsequent experiments will

determine whether proluminal movement of ^{3}H-androgen across the caput epididymal epithelium is returned to normal by supplementation with FSH or LH and whether proluminal movement occurs in the absence of spermatozoa.

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