The Ability of the Rat Epididymis to Concentrate Spermatozoa

Responsiveness to Aldosterone

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Experiments have been performed to determine if aldosterone is involved in the control of water reabsorption from the epididymal lumen *in vivo*. Micropuncture samples of lumen content were collected from the epididymides of control rats and those receiving aldrenalectomy, adrenalectomy + 25 μg aldosterone/day, 10 mg spironolactone/kg body weight/day, 10 mg spironolactone + 1 mg testosterone/kg body weight/day, 5 mg desoxycorticosterone acetate (DOCA)/day, 50 μg aldosterone/day, or 0.1 ml vehicle alone. The treatment period was three days. Seminal vesicles weights and testis weights were obtained. Sperm concentrations (SEM) in the caput, corpus, and cauda epididymidis of normal rats were 0.75 ± 0.05, 1.24 ± 0.13, and 1.99 ± 0.15 × 10^9 sperm/ml, respectively. Both inhibition and removal of aldosterone caused significant reduction ($P < .01$) of intraluminal sperm concentrations. Sham treatment had no effect. Sperm concentrations were normal in animals receiving aldrenalectomy plus aldosterone replacement. It is concluded that water resorption in the rat epididymis is responsive to aldosterone.

Key words: aldosterone, epididymal spermatozoa, spironolactone, adrenalectomy.

One of the functions of the mammalian epididymis is to concentrate spermatozoa as they pass through the organ. In the rat, sperm are concentrated primarily by the efferent ducts and initial segment of the caput epididymidis, but intraluminal sperm concentrations continue to increase progressively from the caput through the cauda epididymidis (Levine and Marsh, 1971; Turner et al, 1977). Increases in epididymal sperm concentrations are due to the resorption of water from the epididymal lumen (Levine and Marsh, 1971; Wong, et al, 1978a).

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Levine and Marsh (1971) used *in vivo* micropuncture techniques to demonstrate that water resorption from the epididymal lumen was concomitant with the resorption of sodium against an electrochemical gradient. These authors inferred that the resorption of water was secondary to active transepithelial transport of sodium out of the epididymal lumen. Turner and Howards (1977) found that sodium ion concentrations in the caput epididymidis could be increased by treating rats with an inhibitor of active sodium resorption, and Wong et al (1978a, b) used *in vivo* microperfusion studies to show that water resorption in the rat cauda epididymidis was indeed due to antiluminal active transport of sodium. It should be noted, however, that these latter investigators suggested that active chloride, not sodium, transport was the driving force for water resorption in the rat caput epididymidis.

Spironolactone is a competitive antagonist of aldosterone, the mineralocorticoid stimulator of sodium reabsorption from the distal renal tubule. The fact that spironolactone also caused increased sodium concentrations in the rat caput epididymal lumen (Turner and Howards, 1977; Jenkins, et al, 1980) indicated that aldosterone-sensitive, active sodium reabsorption had been inhibited in the caput epididymidis. Also, Au, et al (1978) demonstrated aldosterone-sensitive sodium resorption from the perfused rat cauda epididymidis *in vitro*.

The present experiments were performed to test the hypothesis that aldosterone inhibition with spironolactone or aldosterone removal by adrenalectomy significantly inhibits water resorption *in vivo*. Water resorption was observed indirectly by

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determination of intraluminal sperm concentrations. The nondegraded, nonsecreted, nonreabsorbed sperm cells serve as an intraluminal fluid space-marker in the epididymal tubule.

Materials and Methods

Mature, male, Sprague-Dawley rats were obtained from the university vivarium and housed at 25 C with a 12 hour:12 hour light-dark cycle.

At the end of the treatment periods described below for each experiment, all groups were subjected to conventional *in vivo* micropuncture of the caput, corpus, and cauda epididymidis (Howards et al, 1975; Turner, 1979). The micropipette containing the sample of epididymal lumen content (sperm plus fluid) was attached to a vertical transfer apparatus (Bunton Instruments, Rockville, MD) immediately after collection and the sample was expressed under water-equilibrated mineral oil in a glass mini-beaker. A precalibrated 100 nl microvolumetric pipette was used to aspirate duplicate 100 nl aliquots of each micropuncture sample collected. The 100 nl aliquot was transferred to an adjacent mini-beaker and inserted into a mineral oil-covered, 25 μl droplet of 1% hyluronidase in saline. The dilute hyluronidase solution prevented sperm-to-sperm adhesion and facilitated the even mixing of spermatozoa in the small diluent drops. The mixed 25 μl drop of diluted epididymal sample was then aspirated into a precalibrated 300 μl pipette. The remainder of the 300 μl pipette was filled with more 1% hyaluronidase solution. Each pipette was vigorously vibrated for 90 seconds on a Yankee® automatic pipette shaker (Clay-Adams, NY) to completely mix the sample. Drops of the pipette contents were applied under the cover slip of a red-cell hemocytometer to determine the sperm concentration of the fluid in the 300 μl pipette. Each counting procedure was performed in quadruplicate, and sperm concentrations in the original micropuncture samples were calculated with use of the appropriate dilution figures.

Sperm concentrations were determined for the lumen fluids of the caput, corpus, and cauda epididymidis of each animal in each group. After micropuncture of each animal was completed, the testes and seminal vesicles were removed and weighed. Comparisons of data between groups were done by the nonparametric Kruskal-Wallis test (Kruskal and Wallis, 1952) and the Wilcoxin two-sample test.

Experiment 1

This experiment was performed to test the hypothesis that both adrenalectomy and spironolactone treatment reduce epididymal sperm concentrations.

Five animals were assigned to each of five groups: control group (no treatment), sham-injected group (vehicle alone), and groups receiving either 10 mg spironolactone/kg body weight/day for three days, administered subcutaneously in 1 ml corn oil; or 10 mg spironolactone plus 1 mg testosterone/kg body weight/day for three days in 1 ml corn oil. A final group was adrenalectomized and maintained for three days with saline drinking water and normal diet.

Experiment 2

This experiment was performed to confirm the effect of spironolactone plus testosterone and to test the hypothesis that desoxycorticosterone acetate (DOCA) increases epididymal sperm concentrations.

Additional animals were assigned to a control group, a group receiving 10 mg spironolactone plus 1 mg testosterone/kg body weight for three days, and a group of five animals receiving subcutaneous injections of DOCA (5 mg/ml in sesame oil at a dose of 5 mg/animal/day for three days). Epididymal sperm concentrations and organ weights were determined and data analysis was performed as previously described.

Experiment 3

This experiment was performed to test the hypothesis that aldosterone replacement or supplementation will maintain or increase epididymal sperm concentrations, respectively.

Epididymal sperm concentrations were determined in a group of concurrent control animals, in animals that were adrenalectomized and injected with 25 ug aldosterone in 0.1 ml corn oil/day, subcutaneously for three days, and in a group of intact animals injected with 50 μg aldosterone in 0.1 ml corn oil/day, subcutaneously for three days.

Compounds

Spironolactone (SC9420) was obtained from Searle and Co. (San Juan, PR), testosterone and aldosterone were obtained from Sigma Chemical Co. (St. Louis, MO) and DOCA (Percortin®) was obtained from Ciba Pharmaceutical Co. (Summit, NJ).

Results

Experiment 1

Sperm concentrations (SEM) in the normal rat epididymis were $0.75 \pm 0.05 \times 10^9$, $1.24 \pm 0.13 \times 10^9$, and $1.99 \pm 0.15 \times 10^9$ sperm/ml of lumen fluid in the caput, corpus, and cauda epididymidis, respectively (Fig. 1). Sperm concentrations in the epididymis of sham-injected animals were similar and are not shown. Animals receiving either 10 mg spironolactone/kg body weight or 10 mg spironolactone plus 1 mg testosterone/kg body weight had significantly reduced epididymal sperm concentrations ($P < .01$; Fig. 1). Mean caput epididymidal sperm concentrations in these treated animals ranged from $0.41 \pm 0.04 \times 10^9$ to $0.45 \pm 0.03 \times 10^9$ sperm/ml; mean corpus sperm concentrations ranged from $0.69 \pm .02 \times 10^9$ to $0.91 \pm .03 \times 10^9$

sperm/ml, and mean cauda sperm concentrations were 1.21 ± .04 × 10^9 to 1.28 ± .16 × 10^9 sperm/ml. Adrenalectomy produced similar results (Fig. 1).

Body weights and testis weights were similar in all groups (Fig. 2). Seminal vesicle weights of animals treated with 10 mg spironolactone/kg body weight were significantly less ($P < .01$) than seminal vesicle weights in control animals. Seminal vesicle weights in animals receiving 10 mg spironolactone plus 1 mg testosterone/kg body weight were normal, but adrenalectomized rats had significantly elevated seminal vesicle weights ($P < .01$).

Experiment 2

Control group sperm concentrations rose progressively from the caput through the cauda epididymidis (Fig. 3) and 10 mg spironolactone plus 1 mg testosterone/kg body weight again caused a significant reduction ($P < .01$) in epididymal sperm concentrations. Animals receiving 5 mg DOCA/day had mean intraluminal sperm concentrations that were similar to controls (Fig. 3). There were no differences in body weights, testis weights, or seminal vesicle weights among any of the groups in this series of experiments (Fig. 4).

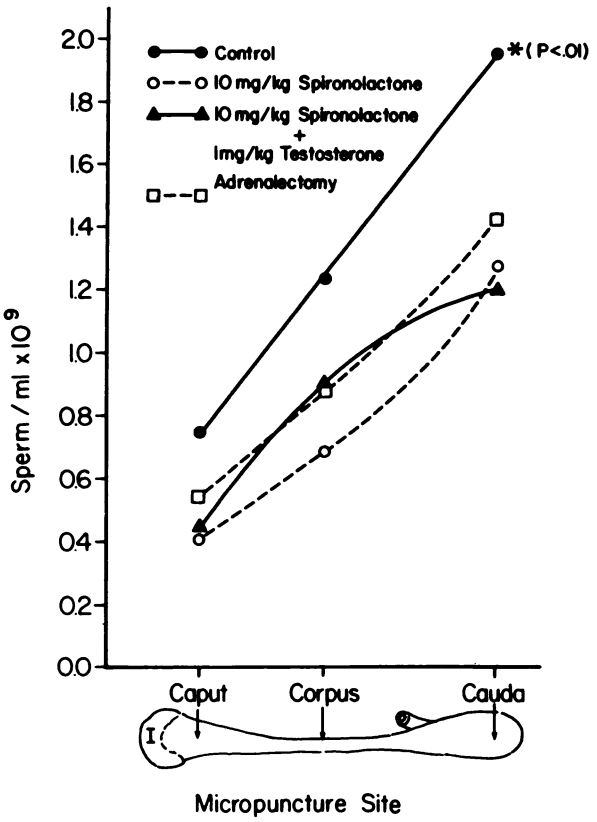

Fig. 1. Sperm concentrations in the different regions of the rat epididymis. All anti-aldosterone treatments caused significant reductions in intraluminal sperm concentrations.

Experiment 3

Epididymal sperm concentrations in animals from this experiment are shown in Table 1. No significant differences in sperm concentrations existed within zones between control animals and adrenalectomized animals receiving aldosterone replacement or intact animals receiving aldosterone supplementation. Mean body weights were 525, 608, and 604 g for control, adrenalectomy plus 25 μg aldosterone/day, and 50 μg aldosterone/day groups, respectively.

Discussion

Turner and Howards (1977) and Jenkins et al (1980) presented evidence that sodium reabsorption from the caput epididymidal lumen is inhibited by the aldosterone inhibitor, spironolactone. Au et al (1978) demonstrated that sodium resorption and water resorption from the rat cauda epididymidis perfused *in vitro* could be inhibited by removal of aldosterone prior to their *in vitro* perfusion procedure. In the present study, it was postulated that if the sodium pump controls water resorption and, consequently, sperm concentration within the epididymal lumen, treatment by adrenalectomy, spironolactone, or spironolactone plus testosterone should result in a decrease in sperm concentration within three days. This is exactly what happened. This indicates that the aldosterone-sensitive component of the previously reported sodium transport system in the rat epididymis is of sufficient magnitude that anti-aldosterone treatment significantly inhibits water resorption *in vivo*, and thus impairs the ability of the epididymis to concentrate spermatozoa.

A potential complication in this study was that spironolactone is known to have anti-androgenic activity (Bassinger and Gittes, 1974; Corvol et al, 1975; Loriaux et al, 1976). These effects are usually demonstrable only after administration of the drug at much higher dose rates or over a longer time period than used in this study. Still,

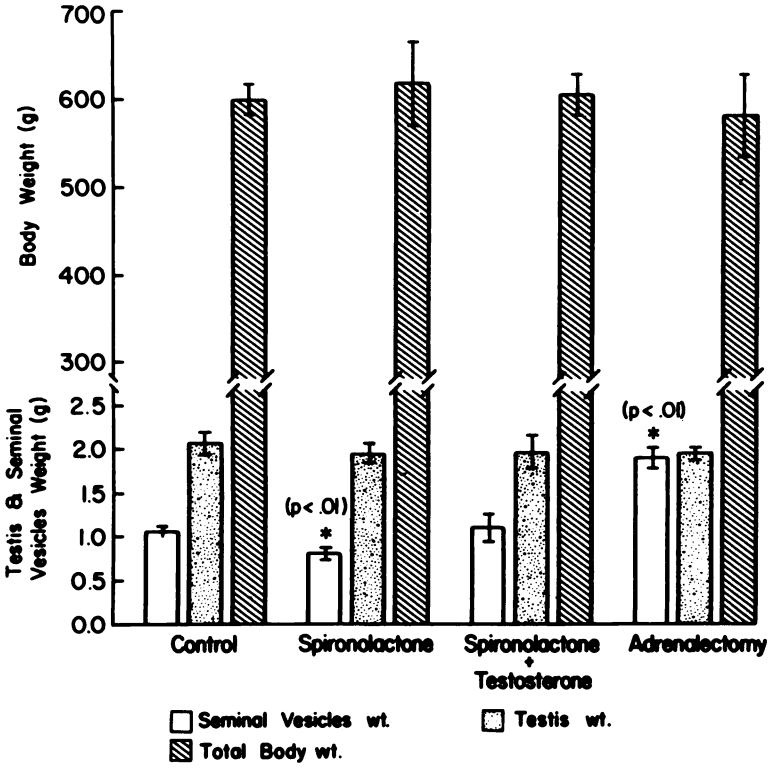

Fig. 2. Body and tissue weights in animals of Experiment 1. Spironolactone treatment significantly reduced seminal vesicle weights. Adrenalectomy significantly increased seminal vesicle weight.

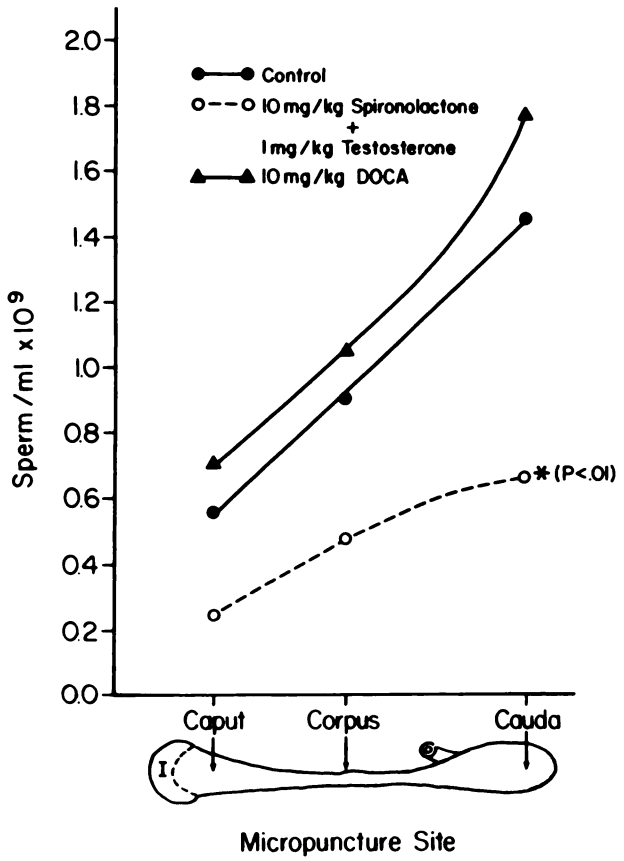

Fig. 3. Sperm concentrations in different regions of the rat epididymis. Treatment with spironolactone plus testosterone reduced intraluminal sperm concentrations. Treatment with DOCA did not significantly alter sperm concentrations.

in order to eliminate this potential side effect as a cause of reduced epididymal sperm concentrations, one group of rats in the first series of experiments was administered testosterone in conjunction with spironolactone. The testosterone dose used was equal to or greater than that previously used to maintain the male rat reproductive tract (Flickinger, 1977; Fawcett and Hoffer, 1979). Testosterone supplementation to spironolactone-treated rats maintained seminal vesicle weights (Fig. 2), yet epididymal sperm concentrations in those same rats remained significantly reduced (Fig. 1). The fact that testosterone administration returned seminal vesicle weights to normal but did not alter the effect of spironolactone on epididymal sperm concentrations indicates that the decreased sperm concentrations were not due to anti-androgen effects on the testis. Additionally, if lower epididymal sperm concentrations were due to fewer sperm being released from the testis, one would not expect to see similar effects of spironolactone in all epididymal zones after only three days of treatment. In the present study, reductions in sperm concentrations were similar in all zones (Fig. 1).

Three days after surgery, adrenalectomy also caused a significant reduction in epididymal sperm concentrations (Fig. 1). The removal of aldosterone by adrenalectomy had an effect on epididymal sperm concentrations that was vir-

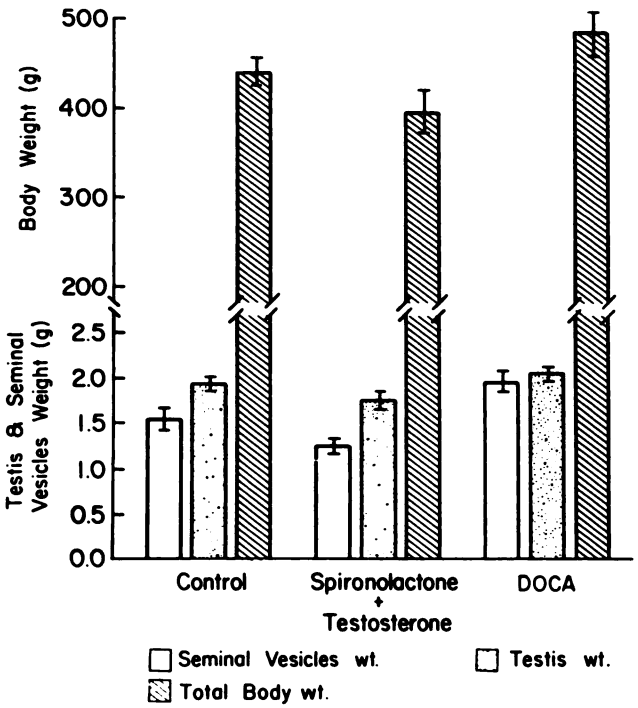

Fig. 4. Body and tissue weights of animals in Experiment 2. No significant differences existed between groups.

tually identical to the inhibition of aldosterone with spironolactone. This indicates that spironolactone did act by the inhibition of aldosterone-sensitive water resorption. Adrenalectomized rats had significantly higher seminal vesicle weights than did control animals. While the reason for this is not known, an untested explanation is that the absence of aldosterone might have inhibited also an aldosterone-sensitive, antiluminal sodium pump in the seminal vesicle epithelium, thus leading to water retention in the organ and the observed increase in weight. The results of spironolactone plus testosterone treatment in Experiment 1 were repeated in Experiment 2 (Figs. 3 and 4).

Animals receiving three-day DOCA treatment had consistent mathematical increases in mean intraluminal sperm concentrations that approached statistical differences from controls (Fig. 3). As a precursor of aldosterone, DOCA mimics the activity of aldosterone but has only about one-third of its biologic activity (Gilman et al, 1980). It was predicted that if DOCA were acting on the epididymal epithelium in its role as a stimulator of sodium resorption, it would cause an increase in water resorption from the epididymis and a concomitant rise in intraluminal sperm concentrations. The failure to achieve this effect in DOCA-treated animals may have been due to the low biologic activity of the molecule; thus, a third experiment was performed wherein intact and adrenalectomized animals were administered a large dose of supplemental aldosterone.

Aldosterone replacement in adrenalectomized rats did maintain normal sperm concentrations (Table 1). The results obtained in separate experiments in which adrenalectomy significantly reduced rat epididymal sperm concentrations (Fig. 1) and aldosterone replacement in adrenalectomized animals maintained normal epididymal sperm concentrations (Table 1) provided further evidence that the ability of the epididymis to concentrate spermatozoa (resorb water) is sensitive to aldosterone. As was the case with DOCA treatment, however, aldosterone supplementation in intact animals failed to significantly elevate epididymal sperm concentrations above controls. It does not appear that water resorption can be stimulated above normal rates.

The explanation for these latter results is not immediately clear; however, it is known that aldosterone stimulates target cell activity by binding to cytoplasmic receptors. From this fact and the results of the present study one can speculate that the response of the epididymal epithelium to aldosterone is modulated by the limited number of cytoplasmic receptors available for binding to aldosterone, not by the concentration of aldosterone in circulating plasma. It is well established that plasma aldosterone concentra-

TABLE 1. Sperm Concentrations in Fluids Collected Directly From the Rat Epididymal Tubule by *In Vitro* Micropuncture*

Group	Number	Epididymal Sperm Concentrations (sperm/ml × 10^9) Caput	Corpus	Cauda
Control	6	0.80 ± 0.80	1.32 ± 0.11	2.03 ± 0.07
Adrenalectomy + 25 μg aldosterone/day	7	0.93 ± 0.08	1.33 ± 0.08	2.16 ± 0.17
Intact, 50 μg aldosterone/day	5	0.87 ± 0.04	1.33 ± 0.03	2.23 ± 0.14

* Samples were collected from normal adults and from two groups of adults subjected to a three-day treatment.

tions vary in response to several specific factors relative to whole-body water balance, not to epididymal sperm concentrations.

The putative epididymal aldosterone receptors may sparsely populate the entire epithelium or they may densely populate the cytoplasm of a specific cell type of limited population. In either case, the flow rate of fluid from the testis or the rate of appearance of some "messenger" from the testis might regulate the production of the putative aldosterone receptors in the epididymal epithelium. More product arriving from the testis would signal a greater need for water resorption. In this scenario the population of aldosterone receptors at any given time would be close to that needed for the correct rate of water resorption; thus, administration of additional aldosterone would not stimulate further water resorption due to the lack of free cytoplasmic receptors.

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