Antiandrogenic Effects of Topically Applied Spironolactone on the Hamster Flank Organ

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• **The effects of topically applied spironolactone on the sebaceous glands of flank organs in adult male golden hamsters were investigated. Daily treatment with spironolactone (0.3 mg and 3 mg) on one side only significantly reduced the size of the treated flank organs, while the contralateral flank organs remained unchanged. Lower doses of spironolactone and the vehicle had no effect. Cyproterone acetate therapy resulted in the bilateral reduction of flank organ sizes. In vivo measurement of the palpable bulk of the flank organs correlated with flank organ volumes as determined by computer-assisted planimetry of serial histologic sections. Dry weights of seminal vesicles in animals treated with spironolactone did not differ significantly from those of the control group, while topically applied cyproterone acetate significantly reduced seminal vesicle weight. Topically administered spironolactone appears to have only local antiandrogenic effects, as indicated by the lack of changes in the untreated contralateral flank organs and in the weights of seminal vesicles.**

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The aldosterone antagonist spironolactone has been used for many years as an antihypertensive and a diuretic. Its side effects include decreased libido, impotence, and gynecomastia.[1,2] Subsequent investigations have partially identified the mechanisms by which spironolactone acts as an antiandrogen. It was found to interfere with adrenal and testicular steroid synthesis by blocking cytochrome P-450-dependent enzyme systems[3,4] and increasing the conversion of circulating testosterone to estrogen.[3] In addition, spironolactone was reported to be a potent competitive inhibitor of dihydrotestosterone (DHT) at its receptor sites in both experimental animals and humans.[5-7]

In clinical trials, orally administered spironolactone (50 to 200 mg/day) decreased hirsutism in women with elevated or normal androgen levels.[8-12] Acne and seborrhea improved in some hirsute patients who were treated with spironolactone.[8,12] Luderschmidt et al[13] reported a substantial reduction in sebaceous gland size of the Syrian hamster ear after subcutaneous injections of spironolactone.

Because we knew of no studies of topically applied spironolactone, we investigated its effects on the sebaceous glands of the hamster flank organ, a reliable model for studying the effects of pharmacologic agents on sebaceous glands.[14-17]

For editorial comment see p 55.

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MATERIALS AND METHODS

Animals

Adult male Syrian golden hamsters, weighing 80 to 110 g, were exposed to artificial light 14 hours daily for ten days before and throughout the experiment. They were confined in separate cages, at constant temperature and humidity, and fed a standard ad lib diet of commercially prepared hamster food and water. Three hamsters were castrated by the scrotal route using intraperitoneally administered phenobarbital (8 mg/100 g body weight) as an anesthetic agent. The costovertebral regions of all of the hamsters were closely shaved with an electric hair clipper before and every four days throughout the experiment.

Treatment

With a micropipet, 100 μL of each test solution was slowly applied directly to the flank organs under a steady stream of compressed air to enhance evaporation of the vehicle. Groups of four hamsters received daily applica-

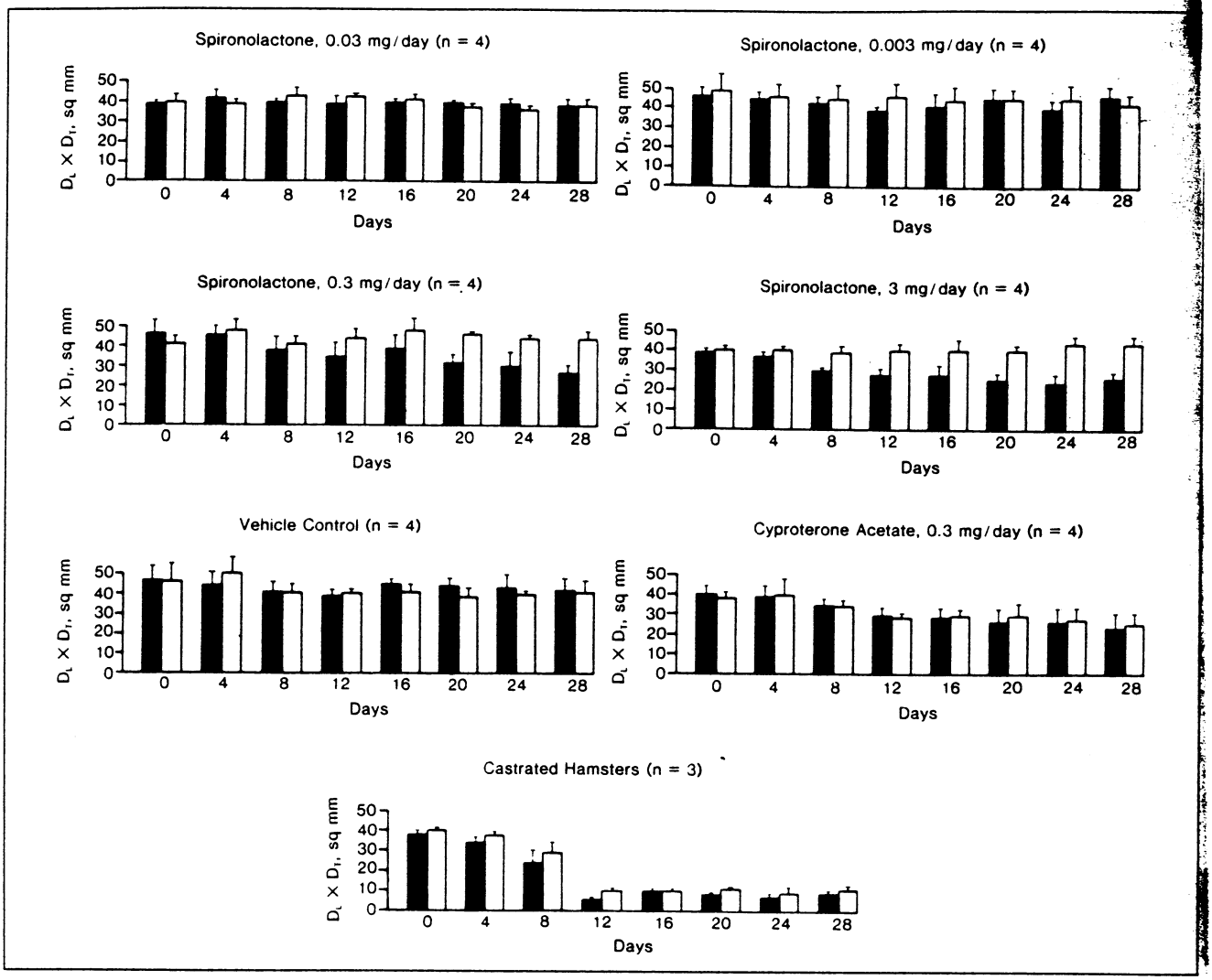

Fig 1.—In vivo measurements of flank organ sizes. D_L indicates greatest longitudinal diameter; D_T, greatest transverse diameter; solid (black) bars, left (treated) flank organs; open bars, right (untreated) flank organs.

tions of 0.003 mg, 0.03 mg, 0.3 mg, or 3 mg of spironolactone (Sigma) in 5% isopropyl myristate in isopropyl alcohol to the *left* flank organ for 27 consecutive days. The right flank organ was untreated. One group of four hamsters received 0.3 mg of cyproterone acetate applied daily to the flank organs, and another group of four animals were treated unilaterally with the vehicle. The three castrated hamsters received no treatment.

Gross Measurements

At the start of the experiment and every fourth day thereafter, the visible or palpable bulk of the flank organs (rather than the area of pigmentation) was measured as described previously.[17] The greatest longitudinal diameter (D_L) and the greatest transverse diameter (D_T) were measured, and flank organ size was expressed in square millimeters by multiplying these two diameters. All measurements were taken by the same investigator, without referring to previous measurements.

Morphometric Techniques

Hamsters were sacrificed on the 28th day of the experiment with halothane. All flank organs were excised and processed as previously described.[17] Specimens were fixed in Karnovsky's fixative, embedded in paraffin, and cut serially into 10-micron sections. Every tenth section was mounted and stained with hematoxylin and eosin. Because an investigator's bias had not been completely ruled out in the gross measurements of flank organ bulk, the slides were labeled with randomly assigned code numbers to permit "blind" evaluations. Flank organ volumes were determined by computerized planimetry.[17]

Dry Weights of Seminal Vesicles

En bloc specimens of seminal vesicles, prostate, bladder, vas deferens, and urethra were removed immediately following the excision of the flank organs. Specimens were fixed in 10% buffered formaldehyde solution, seminal vesicles were separated under a stereomicroscope, and several incisions were made in the organs to drain fluid. The seminal vesicles were then placed in a drying oven at 60 °C for 12 hours and allowed to cool to room temperature in a vacuum desiccator before being weighed. The drying procedure was repeated three or four times, until there was no further decline in weight.

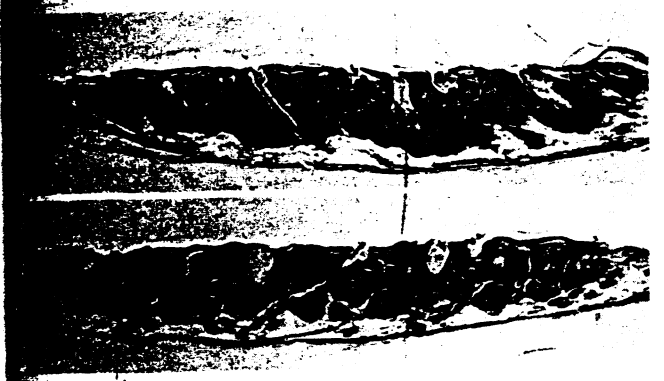

Fig 2.—Left (top) and right (bottom) flank organs of hamster treated with vehicle to left flank organ. Sebaceous glands are of similar size and are well developed, and densely packed in both specimens (hematoxylin-eosin, original magnification ×18).

Fig 3.—Left (top) and right (bottom) flank organs of hamster treated with 3 mg/day of spironolactone to left flank organ. Left flank organ is thinner, with smaller longitudinal diameter than contralateral flank organ. Individual sebaceous glands are smaller and separated from each other. Right flank organ has similar morphologic features as those of the hamster in Fig 1 (hematoxylin-eosin, original magnification ×18).

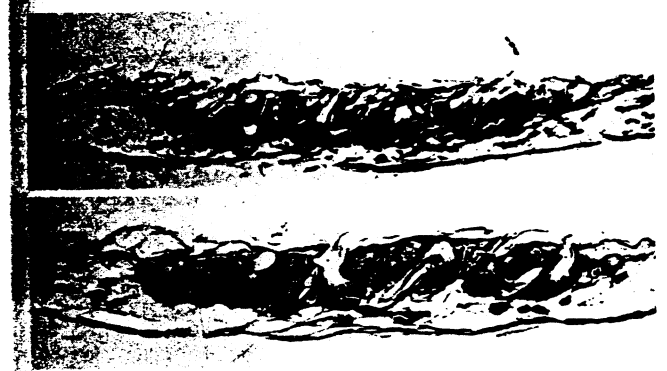

Fig 4.—Left (top) and right (bottom) flank organs of hamster treated with 0.3 mg/day of cyproterone acetate to the left flank organ. Both flank organs have small longitudinal diameters and are composed of small and clearly separated sebaceous glands, similar in appearance to flank organ treated with spironolactone in Fig 2 (hematoxylin-eosin, original magnification ×18).

Fig 5.—Left (top) and right (bottom) flank organs of castrated hamster. Both contain atrophied sebaceous glands that are separated by and replaced with fat and connective tissue (hematoxylin-eosin, original magnification ×18).

Statistical Analysis

The results of in vivo measurements were evaluated using an analysis of variance test with a completely fixed design. A Student's t test was performed to determine the statistical significance of differences in flank organ volumes.

RESULTS

In Vivo Observations

Topical treatment with 0.3 or 3 mg/day of spironolactone decreased the size of the treated flank organs in all eight hamsters, while the untreated sides remained unchanged. This difference was manifested as a clearly visible shrinkage of bulk. There were no changes in flank organ size in animals receiving 0.03 or 0.003 mg/day of spironolactone or vehicle. Cyproterone acetate (0.03 mg/day) resulted in a reduction in size of the treated and contralateral untreated flank organs. A dramatic reduction in the size of all flank organs occurred in the castrated hamsters. Changes in those organs that responded to treatment became noticeable between the eighth and 12th days of the experiment.

Measurements of flank organ size ($D_L \times D_T$) are illustrated in Fig 1. Treatment with 0.3 mg/day of spironolactone resulted in a 39.3% average reduction in flank organ size after 28 days. Mean reduction of flank organ size in animals receiving 3 mg/day of spironolactone was 29.5% compared with pretreatment values. These changes are significant ($P < .01$) in comparison with pretreatment values of the same organs, contralateral organs, and vehicle-treated controls. No changes occurred in contralateral untreated organs, or in hamsters treated with 0.03 and 0.003 mg/day of spironolactone or the vehicle. Average bilateral flank organ reductions of 39.7% on the treated side and 30.3% on the contralateral untreated side compared with pretreatment values ($P < .02$) was produced in those hamsters receiving 0.3 mg/day of cyproterone acetate. Castration

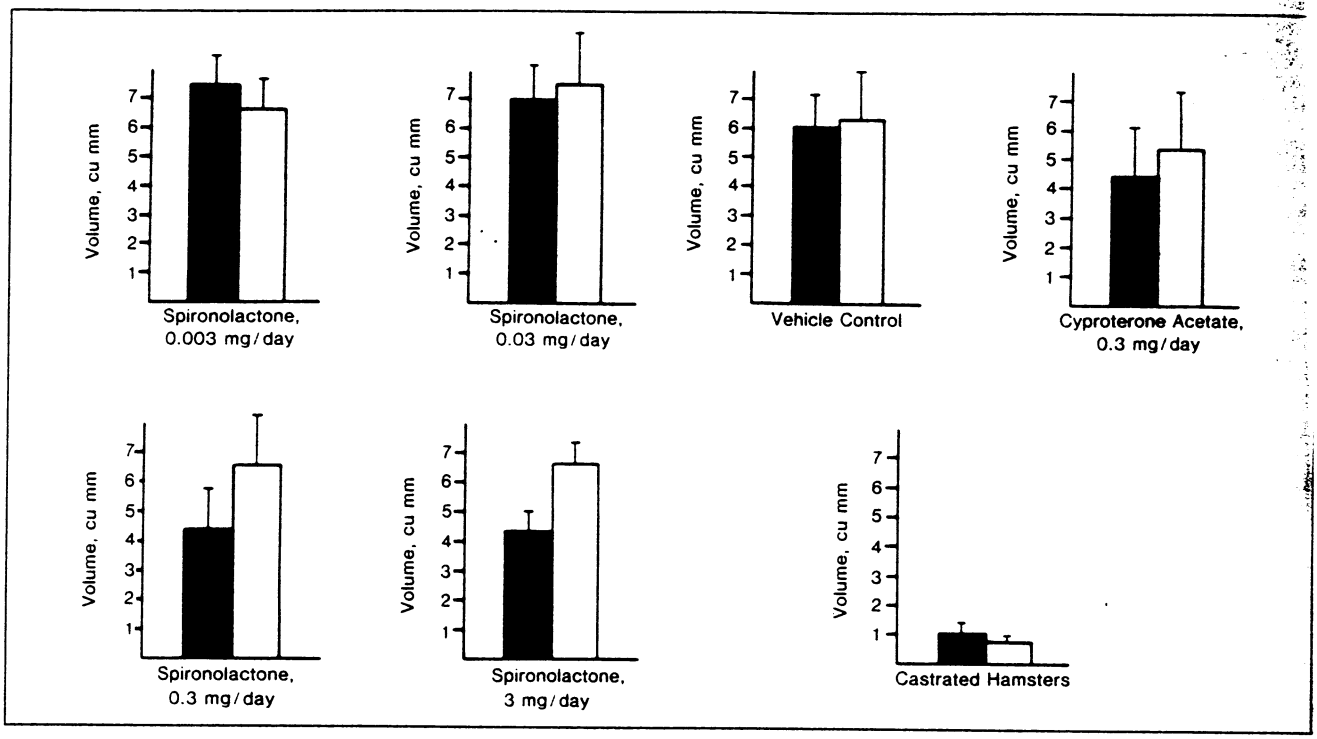

Fig 6.—Sebaceous gland volumes of entire flank organs (day 28) as determined by planimetry. Solid (black) bars represent left (treated) flank organs; open bars, right (untreated) flank organs.

resulted in a mean reduction in flank organ size of 74.6% compared with flank organ size before castration.

Morphometric Studies

Representative photomicrographs of histologic sections from the center of the flank organs (largest cross-sectional area), as determined by planimetry, are shown in Figs 2 through 5.

Planimetrically determined sebaceous gland volumes are summarized in Fig 6. Volumes of all flank organs treated with 0.03 or 3 mg/day of spironolactone were significantly smaller than the volumes of untreated contralateral flank organs ($P < .03$). The average difference was 32.3% and 34.3%, respectively. Compared with the volumes of the left flank organs in the control group, this average difference was 26.7% ($P < .05$) and 27.1% ($P < .05$), respectively, but there was no difference (3.7% and 0.4%) between volumes of the untreated organs and those on the right side of the control group. No significant difference in volume was found between the left and right organs of hamsters treated with a vehicle or 0.03 mg/day of spironolactone. Flank organs treated with 0.003 mg/day of spironolactone were found to be 11.0% greater in volume than the untreated contralateral organs ($P < .05$).

Flank organ volumes of hamsters treated with 0.3 mg/day of cyproterone acetate were 25.0% smaller on the treated side and 16.0% smaller on the untreated side than in the control group. Considerable variation of sebaceous gland volume was seen within the group, as is shown by the large standard deviations (Fig 6). Statistical analysis revealed no significant difference between volumes of treated and untreated organs or between these organs and those of the control group.

Dry Weights of Seminal Vesicles (Day 28)

Variable	Mean Weight (±SD), mg	P
Control (n = 4)	407 ± 40	...
Castrated (n = 3)	163 ± 46	.0007
Cyproterone acetate, 0.3 mg (n = 4)	330 ± 30	.0217
Spironolactone, 0.03 mg (n = 4)	373 ± 90	.5157
Sprionolactone, 0.3 mg (n = 4)	352 ± 79	.2605
Spironolactone, 3 mg (n = 4)	386 ± 80	.6552

Dry Weights of Seminal Vesicles

Seminal vesicle weights in hamsters treated with 3, 0.3, and 0.03 mg/day of spironolactone did not differ significantly from those of the control group (Table). Topical administration of 0.3 mg/day of cyproterone acetate resulted in the significant reduction of dry weights of seminal vesicles (approximately one third of the reduction found in castrated hamsters) (Table). Data on seminal vesicle weights of the hamsters treated with 0.003 mg/day of spironolactone were not included, because these hamsters were used to improve dissecting skills.

COMMENT

The discovery of the antiandrogenic properties of spironolactone may lead to an important addition to

e forms of therapy available for treating conditions in which excess androgen production, blood vels, or target-organ sensitivity play a role. Systemically administered spironolactone has been eported to be effective in the treatment of polycystic vary syndrome,[18] hirsutism,[8-12] acne,[8,12] and, more ecently, adrenal androgenic female pattern alopecia.[19] As with the antiandrogen cyproterone acetate,[20-22] adverse effects may accompany the systemic se of spironolactone in treating skin conditions. Spironolactone is known to cause impotence and gynecomastia in men[1,2] and may, theoretically, lead to feminization of male fetuses in pregnant women, as with all drugs that reduce androgen levels.[23] An "ideal" antiandrogen for the treatment of androgen-related skin conditions should affect the target tissue, without affecting other hormone-dependent structures either directly or through interference with central feedback mechanisms.[14] The search for such a compound has been unsuccessful to date.

We have found topically administered spironolactone is effective in reducing sebaceous gland size by approximately one third when applied to the flank organ of the noncastrated male hamster, while the contralateral untreated flank organ remained unchanged. This reduction is presumably due to a local antiandrogenic effect of spironolactone. Significant systemic antiandrogenic effects of topically applied spironolactone did not occur, as indicated by the dry weights of seminal vesicles.

In the sebaceous glands of both rodents and humans, testosterone is converted into DHT by the enzyme 5α-reductase.[24-30] It then reacts with a cytoplasmic androgen receptor that has a higher affinity for DHT than for testosterone.[31,32] This receptor has been identified in sebaceous glands of hamsters[33,34] and in human skin.[31,32,35] Local antiandrogenic action can occur either by inhibition of 5α-reductase, as with progesterone[14] and 4-androsten-3-one-17β-carboxylic acid,[16] or by competitive inhibition of the androgen receptor, as with cyproterone acetate.[36] Spironolactone does not interfere with 5α-reduction,[5] but several investigators have demonstrated that it acts as a potent competitive inhibitor of androgens at their receptor site, in both animals[5,6] and humans.[7] The affinity of spironolactone for the androgen receptor of prostate cytosol in rats[5,6] and of the foreskin and prostate in humans[7] was approximately one tenth of that of DHT and was equal to the affinity of the androgen receptors to cyproterone acetate.[5] In our experiment, the reduction of flank organ size as a result of treatment with 0.3 mg/day of topically applied cyproterone acetate was of the same magnitude as that produced by an equal dose of spironolactone.

Apart from blockage of androgen receptors in target tissues, spironolactone when given systemically decreases the level of circulating androgens in humans,[37] dogs,[38] and hamsters.[13] It is a result of increased peripheral conversion of testosterone into estrogen[2] and decreased synthesis of androgenic steroids in the adrenals and testes, in which spironolactone interferes with cytochrome P-450, a coenzyme for 17-β-hydroxylase, an enzyme necessary for the conversion of progesterone into testosterone.[3,4]

The metabolism of spironolactone is not completely understood. When radioisotope-labeled spironolactone is given to experimental animals, the highest concentrations of radioactivity are found in the liver, kidneys, adrenal glands, and testes.[39,40] In these organs, spironolactone is rapidly converted to sulfur containing metabolites, which are thought to be involved in the destruction of cytochrome P-450.[41] A later metabolite, canrenone, accounts for the majority of the parent compound in the peripheral circulation.[42,43] Although canrenone is highly effective as an aldosterone antagonist at the distal tubule of the kidney,[44] it differs considerably from spironolactone in regard to antiandrogenic activity. Canrenone does not decrease adrenal cytochrome P-450 content,[45] and its affinity for the DHT receptor is approximately 100 times less than that of spironolactone.[5,6]

Therefore, we believe that systemically administered spironolactone exerts its antiandrogenic effects mainly by reducing levels of circulating androgens and only slightly, if at all, as a peripheral antagonist of androgen receptors. In contrast, we postulate that topically applied spironolactone acts locally by competitively inhibiting DHT receptors. Topically applied cyproterone acetate, also a blocker of DHT receptors, exhibits systemic effects, as has been shown by other investigators[45,46] and as is suggested in our study, in which bilateral reduction of flank organ size and decrease in seminal vesicle weight occurred. We observed no systemic effects of topically administered spironolactone in our animal model, which suggests that the drug may not have been absorbed in amounts sufficient to produce effects at distant sites. Another explanation could be that spironolactone is metabolized to other compounds, such as canrenone, with properties that are less antiandrogenic before entering the circulation.

We conclude that topically administered spironolactone functions as a local antiandrogen in male hamsters. Systemic antiandrogenic effects did not occur with the doses used in our experiment.

Sidney Bernstein of Sci Medicorp, Fort Lauderdale, Fla, provided the Zeiss Videoplan System used in this study; Ken Bawer of Karl Zeiss Inc, Thornwood, NY, provided technical assistance; and Hasik Kasiroglou performed the statistical analyses.

The cyproterone acetate used in this study was provided by Schering AG, Berlin.

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