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TESTOSTERONE-DEPENDENT SEXUAL DIMORPHISM OF THE MOUSE KIDNEY IS MEDIATED BY POLYAMINES

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Male mice exhibit higher levels of kidney ornithine decarboxylase and polyamines and excrete larger amounts of polyamines into the urine than female mice. Orchiectomy elicits the female pattern and testosterone induces the male pattern of polyamine synthesis and excretion. α-Difluoromethylornithine, a specific inhibitor of ornithine decarboxylase, suppressed the testosterone-induced increase in kidney ornithine decarboxylase and polyamines and urinary excretion of polyamines. It also suppressed the testosterone-induced increment in kidney cytochrome c oxidase, lysosomal hydrolases and lysosomal enzymuria, and attenuated the renal hypertrophy. Administration of exogenous putrescine increased kidney polyamine levels and abolished the inhibitory effect of α-difluoromethylornithine. These data indicate that enhanced ornithine decarboxylase activity and polyamine synthesis are essential in mediating androgenic effects on proximal tubule mitochondria and lysosomes. Testosterone-induced kidney growth is at least partially mediated by ornithine decarboxylase and the polyamines.

The decarboxylation of L-ornithine to putrescine by ornithine decarboxylase (EC 4.2.1.17) is the first and rate-limiting reaction in the biosynthesis of the polyamines spermidine and spermine (1,2). Marked increases in ODC[1] and polyamine levels characteristically accompany rapid tissue growth, replication and differentiation (1,2). Augmented ODC activity has been observed after administration of many polypeptide and steroid hormones (3) and has been widely used as a marker of the growth-promoting effects of a hormone. It is unclear, however, whether enhanced polyamine synthesis is essential for hormone-induced growth processes or any of the other biological effects of hormones in target cells.

[1] Abbreviations: ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine; TP, testosterone propionate.

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The mouse kidney is a valuable model for investigating the mechanism of androgenic hormone action as it displays a vigorous anabolic response to testosterone that requires receptor occupancy (4) and is characterized by marked cellular and organ hypertrophy, augmented RNA and protein synthesis, and increases in β-glucuronidase and several other specific proteins (4,5). Recently we showed that the androgenic response in mouse kidney involves enhanced activity of the lysosomal-vacuolar system in proximal tubule cells which is manifested morphologically as a striking autophagy, an accumulation of hypertrophied, membrane-filled lysosomes (myeloid bodies), and augmented exocytosis of these lysosomes into the tubule lumen; and biochemically in elevated kidney activities of numerous lysosomal enzymes, a dramatic lysosomal enzymuria and proteinuria (6,7), and decreased enzyme latency and membrane stability of kidney lysosomes (8,9). Testosterone also induces changes in proximal tubule mitochondria that are reflected in alterations in size, fine structure (7) and equilibrium density (unpublished observations), an associated increase in the inner mitochondrial membrane enzyme, cytochrome c oxidase (7), and augmented cortical tissue respiration (G. Blume, A. Goldstone & H. Koenig, in preparation). We now show that polyamine synthesis plays an essential role in mediating the lysosomal and mitochondrial effects of testosterone in mouse kidney proximal tubules, and is partially involved in mediating the growth-promoting effect of the hormone.

Materials and Methods

L-[1-^{14}C]Ornithine and Aquasol were purchased from New England Nuclear, Boston, MA. Putrescine dihydrochloride, spermidine phosphate, and enzyme substrates were obtained from Sigma Chemical Co., St. Louis, MO. DL-α-Difluoromethylornithine, a specific, irreversible inhibitor of ODC (10), was provided by Dr. W.L. Albrecht of Merrell-National Laboratories, Cincinnati, OH. A/J mice were housed in groups of 4 in metabolic cages. Male mice were orchiectomized transcrotally or subjected to a sham operation under light trichloroethylene inhalation anesthesia 24 h before commencing treatment. One group of orchiectomized mice received a maintenance dose of testosterone propionate (25-100 μg in 0.05 ml ethyl oleate) by subcutaneous injection every other day for a total of four doses. A second group of orchiectomized mice was administered DFMO[1] as a 3% solution in the drinking water during the period of TP[1] treatment. Sham-operated and orchiectomized controls and female mice received ethyl oleate vehicle. Urines were collected under mineral oil into 0.1 ml of 10% sodium azide during the last day or two of the experiment. Mice were killed between 9:00 a.m. and 10:00 a.m. to eliminate circadian variations in ODC and polyamines, generally 24 h after the fourth injection of TP or vehicle (or 8 days post-operatively). Tissue and urine

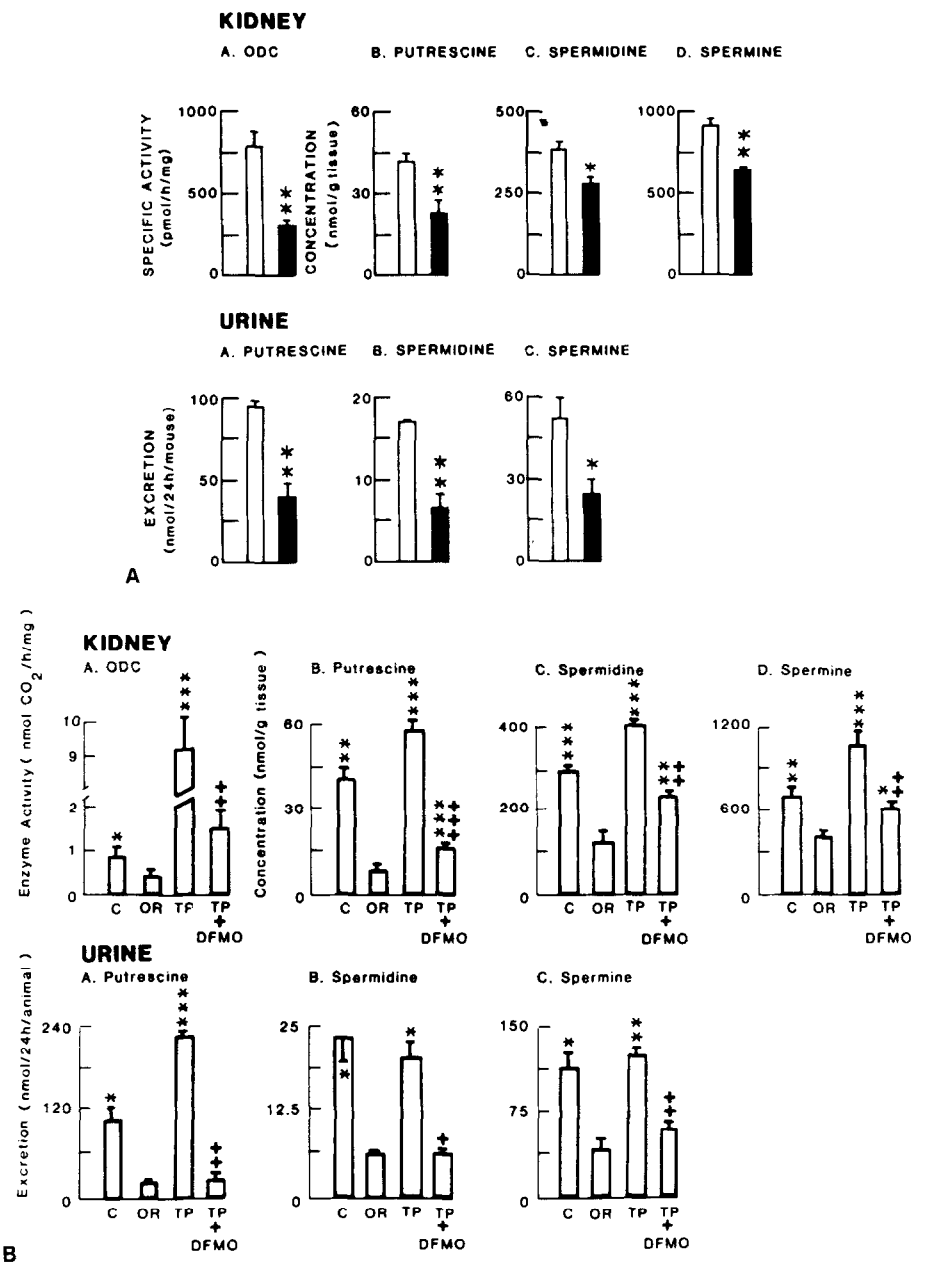

Fig. 1. Effects of sex (A), orchiectomy, testosterone and DFMO (B) on mouse kidney ODC and polyamine levels and the urinary excretion of polyamines. A. Open bar, male, closed bar, female. Results are means ± SEM (n=4 for kidney and 3 for urine). *,**: p<.05, .01. B. Male mice were orchiectomized or sham-operated (C) and 24 h later began receiving TP (100 µg in 0.05 ml ethyl oleate x 4 in 8 d), TP + DFMO (3% in drinking water for 8 d), or ethyl oleate vehicle (Or). Mice were killed 24 h after the last injection. Results are means ± SEM (n=4 for kidney, 3 for urine). *,**,***: p<.05, .01, .001 (vs OR). †,††,†††: p<.05, .01, .001 (vs TP).

samples were stored at -70° C until assayed for protein (11), the inner mitochondrial membrane enzyme cytochrome c oxidase (EC 1.9.3.1) (12), the lysosomal hydrolases β-glucuronidase, hexosaminidase (β-N-acetylhexosaminidase,

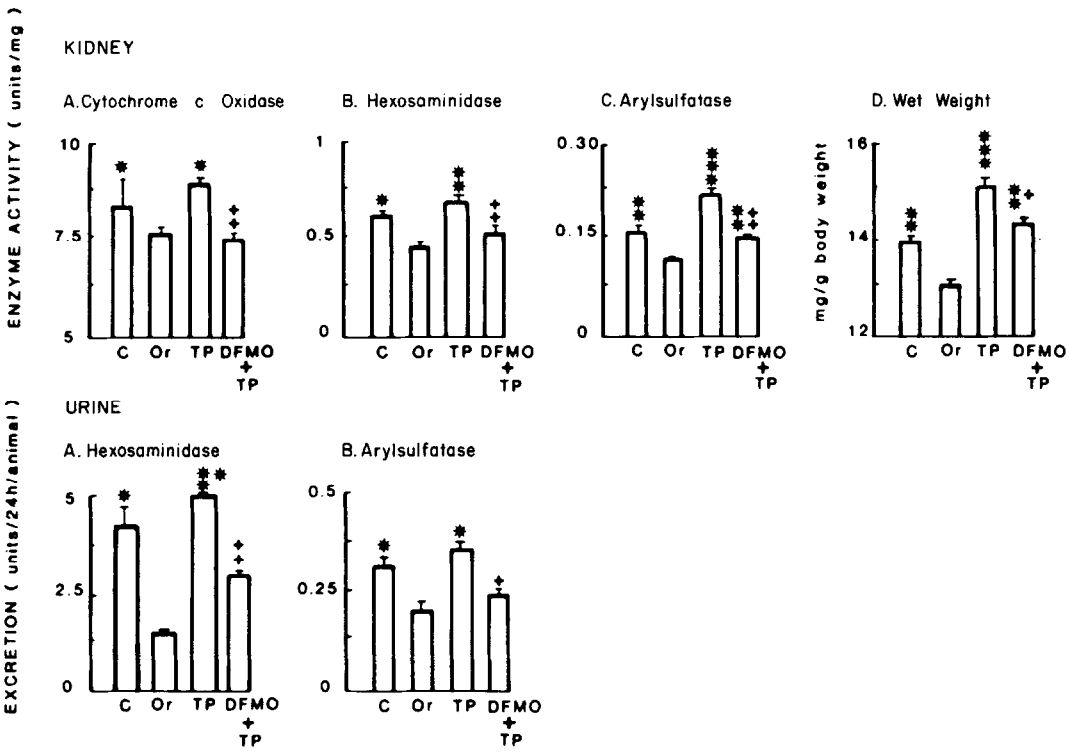

Fig. 2. Effect of orchiectomy, TP and DFMO on mouse kidney weight, cytochrome c oxidase and lysosomal enzymes and the urinary excretion of lysosomal enzymes. Additional details are given in the legend to Fig. 1B and in the text.

EC 3.2.1.30), and arylsulfatase (EC 3.2.1.6.1) (13,14), and ODC (15). For enzyme assays urines were dialyzed against distilled water (for three 2-hour periods against 1000 volumes). Polyamines were determined in 0.2 M perchloric acid extracts of tissues and undialyzed urine by spectrophotofluorimetry of the dansylamide derivatives after separation by thin layer chromatography (15).

Results and Discussion

Kidney ODC activity and polyamine concentrations were substantially higher in male mice than in female mice (Fig. 1A). Male mice also excreted much larger quantities of the polyamines than female mice. Orchiectomy produced a marked decrease in kidney ODC and polyamine levels, and sharply reduced the urinary excretion of the polyamines (Fig. 1B). Conversely, TP administration in orchiectomized male mice and intact female mice (data not shown) induced a sharp increase in kidney ODC, putrescine, spermidine and spermine. It is noteworthy that spermine and spermidine are the major polyamines in the urine. As reported previously (7), orchiectomy decreased kidney wet weight, cytochrome c oxidase and lysosomal hydrolases, and TP re-

placement prevented these orchiectomy-induced changes (Fig. 2). Orchiectomy also decreased the urinary excretion of the lysosomal hydrolases and this decrease was prevented or reduced by TP.

The ODC inhibitor DFMO inhibited the TP-induced increment in kidney ODC by 88-96%, and the concomitant increment in putrescine, spermidine and spermine concentration by 85-87%, 62-100% and 66-81% respectively in two separate experiments (Fig. 1B). DFMO virtually abolished the TP-induced increase in the urinary excretion of the polyamines. At the same time DFMO markedly suppressed the other biochemical effects of TP in the kidney (Fig. 2). DFMO inhibited the TP-increase in kidney cytochrome c oxidase by greater than 100%, kidney lysosomal hydrolases by 60-70%, urinary hydrolases by 70-85%, and kidney weight by 32-52% in four separate experiments. The body weight of the mice in all four treatment groups remained constant during the 8-day period of treatment.

Exogenous putrescine, when administered together with TP and DFMO, raised kidney polyamine concentrations to values equal to or greater than those induced by TP alone (Fig. 3). At the same time putrescine induced an increase in kidney wet weight, cytochrome c oxidase, and lysosomal hydrolases (Fig. 3) and enhanced the urinary excretion of lysosomal hydrolases (data not shown). These data show that administration of a small amount of putrescine nullified the inhibitory effect of DFMO on the androgenic response of mouse kidney. Furthermore, administration of putrescine (dihydrochloride salt, 0.5 mg x 4 in 8 d) or spermidine (phosphate salt, 1 mg x 4 in 8 d) without TP or DFMO evoked similar changes in mouse kidney (data not shown), suggesting that these polyamines exert andromimetic effects in mouse kidney.

Our observations have revealed a sexual dimorphism in polyamine metabolism in mouse kidney. This dimorphism is dependent on endogenous testosterone as orchiectomy resulted in a marked decrease in kidney ODC activity and a depletion of the kidney and urinary polyamines, and TP administration in orchiectomized male mice and female mice reversed this effect. The in-

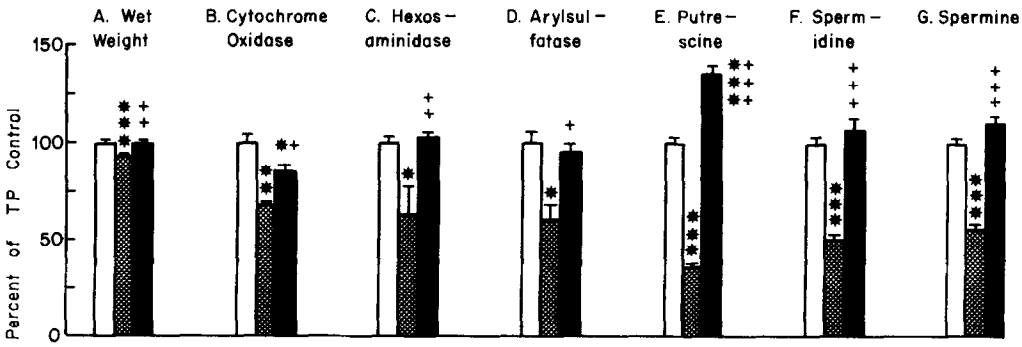

Fig. 3. Exogenous putrescine reverses the inhibitory effect of DFMO on the androgenic response of mouse kidney. Male mice were orchiectomized and 24 h later began receiving TP (50 μg x 4 in 8 d) (open bar), TP + DFMO (3% in drinking water for 8 d) (cross-hatched bar), or TP + DFMO + putrescine dihydrochloride (0.5 mg in 0.1 ml H_2O x 4 in 8 d subcut.). TP control values were: wet weight, 15.6 ± 0.14 mg/g body weight; cytochrome c oxidase, 4.2 ± 0.13 units/mg; hexosaminidase, 0.62 ± 0.017 units/mg; arylsulfatase, 0.20 ± 0.011 units/mg; putrescine, 54 ± 1.6 nmol/g; spermidine, 465 ± 14 nmol/g; spermine, 893 ± 30 nmol/g. Results are means ± SEM (n=4-8). *,**,***: p<.05, .01, .001 (vs TP control). †,††,†††: p<.05, .01, .001 (vs TP + DFMO).

creases in kidney ODC and polyamine levels are already well-developed 20 min after TP administration (unpublished observations), and therefore precede increases in RNA and protein synthesis (4,5). These findings confirm and extend earlier reports that testosterone enhances kidney ODC and polyamines in female and orchiectomized mice (16,17), and imply that ODC and the polyamines are involved in the kidney response to testosterone.

Direct proof that ODC and the polyamines play an indispensable role in the mediation of androgenic hormone action in mouse kidney comes from our experiments on the effects of DFMO and exogenous polyamines. These data show that DFMO, an irreversible inhibitor of ODC (10), effectively suppresses the TP-mediated stimulation of polyamine synthesis and attenuates or abolishes the hormonal response in the kidney. DFMO was somewhat less effective in inhibiting the renal hypertrophy than the lysosomal-vacuolar and mitochondrial effects of testosterone. The inhibitory effects of DFMO are wholly attributable to polyamine depletion, as they can be reversed by administration of a small amount of putrescine. Further, administration of exogenous putrescine and spermidine mimics the actions of testosterone in the kidney. These results establish that enhanced polyamine synthesis is essential in mediating

the action of testosterone on the lysosomal-vacuolar system and the mitochondria of the proximal tubules, and in partially mediating the renal hypertrophy. Polyamines have been implicated in mediating the action of testosterone in rat salivary gland (18). The large fluctuations in the urinary polyamines that occur in response to changes in androgenic state probably reflect comparable alterations in plasma levels of the polyamines that originate from various androgen-responsive tissues, possibly via excretion (or secretion) into the extracellular compartment. This conclusion is supported by the finding that testosterone augments ODC and polyamine levels in the accessory sex glands (19), and in a number of extragenital target tissues in the mouse, namely, heart (20), skeletal muscle (20), aorta (unpublished findings), brain (21), and kidney (this paper). Moreover, rapidly growing mammalian fibroblasts in vitro excrete appreciable amounts of putrescine into the external medium (22). The mechanism and physiological significance of the testosterone-mediated excretion of polyamines by androgen target cells are the subject for future research.

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