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INHIBITION OF POLYAMINE SYNTHESIS BLOCKS URINARY SECRETION OF β-GLUCURONIDASE FROM MOUSE KIDNEY

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SUMMARY. The effect of inhibition of polyamine synthesis on castrated male mouse kidney β-glucuronidase induction and secretion by testosterone was studied. Inhibition of the activities of polyamine synthesis key-enzymes, L-ornithine and S-adenosyl-L-methionine decarboxylases, was performed with the combined treatment of 2-difluoromethylornithine and methylglyoxal bis(guanylhydrazone). Blockage of polyamine synthesis did not affect testosterone-induced increase in renal β-glucuronidase but blocked its secretion into the urine. After withdrawal of inhibitor-treatment β-glucuronidase secretion normalized, and repeated testosterone administration produced undisturbed β-glucuronidase secretion peak in urine suggesting that blockage of β-glucuronidase secretion was not due to the tissue damage produced by inhibitors. These results indicate that the stimulation of renal polyamine synthesis by testosterone is not necessary for the induction of β-glucuronidase but is required for the urinary secretion of this protein.

Testosterone stimulates the proximal convoluted tubules of mouse kidney resulting in renal hypertrophy (1), enhanced RNA and protein synthesis (2,3), and increased activities of several enzymes (4-10), including β-glucuronidase (EC 3.2.1.31)(4) and ornithine decarboxylase (EC 4.1.1.17)(5). Both of these enzymes, β-glucuronidase which is a secretory protein (11,12), and ornithine decarboxylase, have been proposed to serve as specific markers of anabolic androgen response in mouse kidney (13,14). Since ornithine decarboxylase, which is the rate-limiting enzyme

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Abbreviations: DFMO, 2-difluoromethylornithine; MGBG, methylglyoxal bis(guanylhydrazone).

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in polyamine synthesis, is induced faster than β-glucuronidase by androgens (14), and direct evidence for an essential role of polyamines in the macromolecular synthesis (15,16) has generally been confirmed by results obtained with inhibitors of polyamine synthesis, the present study was designed to determine the effect of inhibition of polyamine synthesis on mouse kidney β-glucuronidase induction and secretion into the urine after testosterone administration. Inhibition of polyamine synthesis was performed with the combined use of DFMO, which is known to act as a potent irreversible inhibitor of ornithine decarboxylase *in vivo* (17), and MGBG, a competitive inhibitor of S-adenosylmethionine decarboxylase (18,19).

MATERIALS AND METHODS

Animals and their treatment. Mature male NMRI mice were used. The animals were maintained on 12-h light, 12-h dark schedule and housed in metabolism cages provided with food and water dispensers and capable of housing four mice each. Urine was collected daily into flasks containing 1 ml of 0.2 % (w/v) sodium azide as preservative, and stored frozen at -20°C. Castration was performed under light ether anesthesia. Testosterone was dissolved in 0.2 ml of 10 % (v/v) ethanol/sesame oil and administered intraperitoneally at a single dose of 240 mg/kg and MGBG at a daily dose of 70 mg/kg in 0.15 M NaCl. DFMO was given as a 2 % (w/v) solution in the drinking water. The animls were killed by cervical dislocation between 8 and 10 a.m. to eliminate the influence of diurnal variation on the enzyme activities.

Reagents. L-[1-^{14}C]Ornithine (56 mCi/mmol) and S-adenosyl-L-[carboxyl-^{14}C]methionine (54.9 mCi/mmol) were purchased from the Radiochemical Centre (Amersham, Bucks, United Kingdom). L-[1-^{14}C]Ornithine was treated with 0.1 N HCl before use (20). DFMO was a generous gift from the Centre de Recherche Merrell Inernational (Strasbourg, France). MGBG was purchased from Aldrich-Europe (Beerse, Belgium). Other chemicals were either from E. Merck (Darmstadt, West Germany) or Sigma Chemicals Co. (Saint Louis, MI, USA) and were of the highest available purity grade.

Analytical methods. β-Glucuronidase activity was assayed by a fluorometric method with 4-methylumbelliferyl-β-D-glucuronide as the standard (12). One unit of activity is that amount of enzyme which will hydrolyze 1 μmol of substrate/h at 37°C. The assay conditions for ornithine and S-adenosylmethionine decarboxylases were essentially as described by Jänne and Williams-Ashman (20, 21). Determination of putrescine, spermidine and spermine was conducted as described by Dreyfuss *et al.* (22). Protein measurements were performed using method of Bradford (23) with bovine

serum albumin as the standard. Means of different experimental groups were compared using Student's *t* test (two tailed, *t* independent).

RESULTS AND DISCUSSION

All the mice used in the experiments were castrated three weeks before use to minimize circulating androgen level. As previously described (24), castration results within a few days in a marked decrease in nuclear androgen receptor content, and concomitantly with the changes in nuclear androgen receptor concentration in the kidney, renal ornithine decarboxylase and β-glucuronidase activities decline to the low level of females (14). A single dose of testosterone stimulates dramatically ornithine decarboxylase activity in castrated mouse kidney (14). This stimulation, however, was totally blocked by the combined treatment with DFMO and MGBG (Fig.1). Renal S-adenosylmethionine decarboxyl-

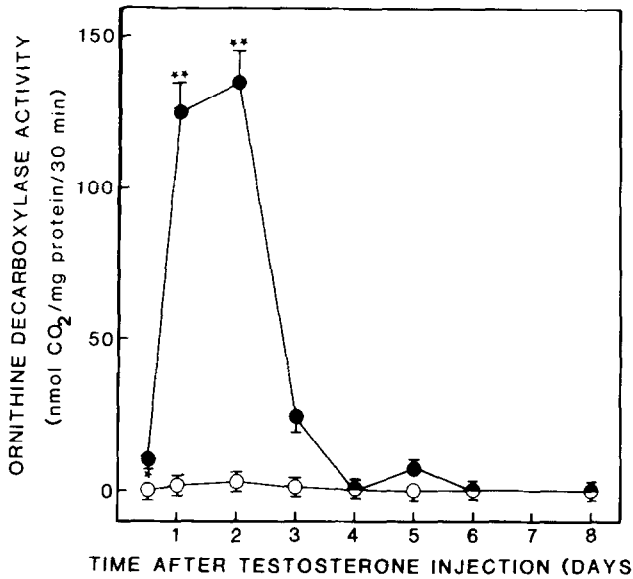

Fig.1. The effect of the combined treatment with DFMO and MGBG on testosterone-induced stimulation of ODC activity in mouse kidney. Inhibitor-treatment was started two days before testosterone injection. Testosterone and inhibitors were given as described in Materials and Methods. Testosterone (●); testosterone with inhibitors (○). Each symbol with a vertical bar shows the mean ± S.E. for a given experimental group comprising at least 4 animals. Asterisks indicate significantly different values from the control ODC level which was 0.76 ± 0.25 nmol CO_2/mg protein/30 min. $^{*}P<0.01$, $^{**}P<0.001$.

Table 1. The effect of the combined use with DFMO and MGBG on S-adenosylmethionine decarboxylase activity in mouse kidney after testosterone injection.

Time after testosterone injection (days)	S-adenosylmethionine decarboxylase activity[a]	
	T[b]	T + I[c]
0.5	0.15 ± 0.03	0.11 ± 0.02*
1	0.15 ± 0.02*	0.11 ± 0.02*
2	0.09 ± 0.02*	0.19 ± 0.07*
3	0.20 ± 0.02	0.08 ± 0.02**
4	0.13 ± 0.02*	0.08 ± 0.01**
5	0.11 ± 0.01*	0.09 ± 0.01*
6	0.15 ± 0.02	0.08 ± 0.02*
8	0.16 ± 0.02	0.09 ± 0.03
Control	0.22 ± 0.02	

[a] nmol CO_2/mg protein/30 min
[b] testosterone alone
[c] testosterone with inhibitors
Values are means ± S.E. for 4-6 animals
*P<0.01; **P<0.001 (as compared to the control)

ase activity, which is not affected markedly by testosterone (14, 25), decreased in inhibitor-treated animals to about one half of that in control mice (Table 1), and even testosterone alone tended to decrease slightly enzyme activity in mouse kidney during the first five days post-injection (Table 1). This can be explained by the accumulation of putrescine (Table 2) and the finding that putrescine acts as a negative regulator in S-adenosylmethionine synthesis (26). In concert with the changes in ornithine and S-adenosylmethionine decarboxylase activities, there was only slight, if any, accumulation of polyamines after testosterone injection in the kidneys of inhibitor-treated animals (Table 2). The blockage of polyamine synthesis, however, did not abolish the induction of renal β-glucuronidase but moved the peak value of the activity one day later (Fig.2). This can be explained by the blockage of urinary secretion of β-glucuronidase in inhibitor-treated animals (Fig.3,B). The blockage appears to be not due to the tissue damage by inhibitors, since the withdrawal of inhibitor-treatment restored the secretion of β-glucuronidase (Fig.

Table 2. The effect of the combined use with DFMO and MGBG on renal putrescine, spermidine and spermine concentrations after testosterone injection.

Time after testosterone injection (days)	Putrescine[a]		Spermidine[a]		Spermine[a]	
	T[b]	T + I[c]	T	T + I	T	T + I
0.5	84.7±17.3*	100.1±15.5**	335.5±50.1	279.5±66.6	713.4±67.9	618.9±54.5**
1	168.9±35.7**	69.3±15.0	432.7±98.3	405.4±119.7	819.3±152.8	679.4±201.5
2	45.5±22.2	65.1±11.4	404.7±82.3	350.9±48.9	849.2±112.2	697.9±151.7
3	49.5±15.9	66.9±10.2	393.0±46.4	380.5±119.3	957.9±115.4	749.4±152.5
4	65.6±19.2	75.9±5.9**	362.9±53.5	332.1±77.4	759.8±55.9	745.3±206.8
5	81.0±18.9	63.9±8.8	438.0±75.6	344.6±53.3	985.7±180.3	672.8±164.8
6	76.3±9.2*	77.9±19.9	471.8±33.4*	372.3±37.9	1052.6±139.6	554.0±83.2**
8	49.7±10.9	89.4±14.7*	282.1±48.6	343.5±72.8	753.3±59.9	761.8±195.8
Control	52.4±1.2		375.2±15.9		834.6±23.2	

[a] nmoles/g wet wt.

[b] testosterone alone

[c] testosterone with inhibitors

Values are means ± S.E. for at least 4 animals

*P<0.01; **P<0.001 (as compared to controls)

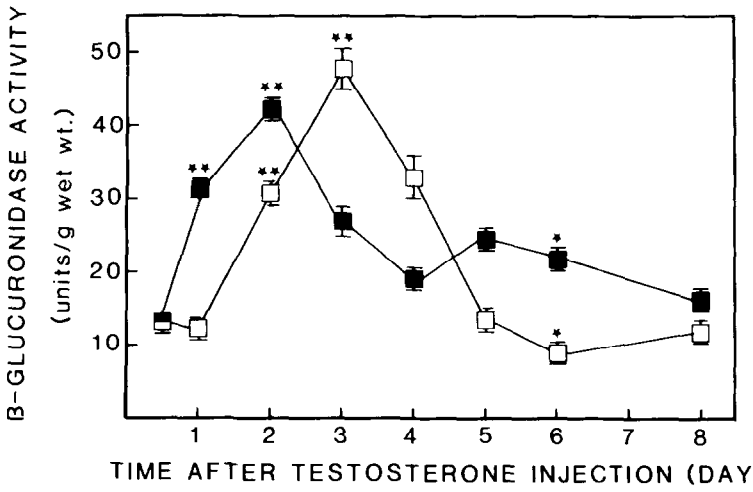

Fig.2. The effect of the combined treatment with DFMO and MGBG on testosterone-induced stimulation of β-glucuronidase activity in mouse kidney. Treatment of animals as described for Fig.1. Testosterone (■); testosterone with inhibitors (□). Each symbol with a vertical bar shows the mean ± S.E. for a given experimental group comprising at least 4 animals. Asterisks indicate significantly different values from the control β-glucuronidase level which was 14.2 ± 0.8 units/g wet wt. * P<0.01; ** P<0.001.

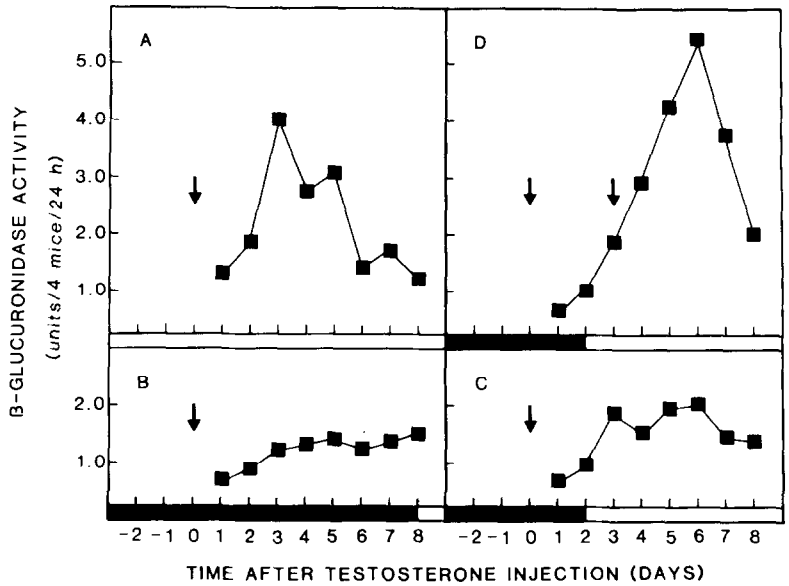

Fig.3. Urinary secretion of β-glucuronidase after testosterone injection. A. Without inhibitors. B. With DFMO and MGBG. C. After withdrawal of inhibitor-treatment. D. After withdrawal of inhibitor-treatment and repeated testosterone injection. Testosterone injection point is indicated by the arrow and inhibitor-treatment period by the black horizontal bar. Doses of testosterone and inhibitors as described for Fig.1 and 2.

3,C), and repeated testosterone administration produced secretion peak (Fig.3,D) comparable to that achieved in mice which were not treated with inhibitors (Fig.3,A). These results indicate that polyamines are involved in the process of extruding lysosomal β-glucuronidase (27) through the plasma membrane of the kidney tubule cells. This is also supported by the similar cellular localization of β-glucuronidase (28) and ornithine decarboxylase (29), the rate-limiting enzyme of polyamine synthesis.

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