Differential Sensitivity of Parameters of Androgen Action to Metabolic Inhibitors: Arginase and β-Glucuronidase*

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An earlier study (Frieden, et al, 1964) showed that some of the metabolic effects of testosterone propionate (T.P.) in mice could be dissected into actinomycin-sensitive (increase in renal β-glucuronidase) and actinomycin-insensitive (stimulation of amino acid incorporation into kidney protein in vitro) components. These experiments have now been extended to include studies of the effect of actinomycin D upon another T.P.-stimulable enzyme, arginase (L-arginine ureohydrolase: 3.5 3.1); in addition, the effects of two other metabolic inhibitors, DL-ethionine and cycloheximide, upon the β-glucuronidase and arginase responses to T.P. have been examined. DL-ethionine inhibits the response of both enzymes to T.P. The increase in renal arginase activity induced by T.P., like the T.P.-induced increase in amino acid incorporation rate, is completely insensitive to actinomycin. In contrast, the arginase response, but not that of β-glucuronidase, is inhibited by cycloheximide.

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EXPERIMENTAL

Female A. Cloudman mice were obtained from the Jackson Laboratories, Bar Harber, Maine. Most of the data reported here were obtained using animals which were 6-10 weeks old at the beginning of the experiment; in a few instances older (6 months) animals were used. Actinomycin D was generously provided by Merck, Sharpe & Dohm, Inc.; testosterone propionate, cycloheximide, and DL-ethionine were purchased from Nutritional Biochemicals. T.P. was dissolved in sesame oil; the dose schedule was 1 mg daily or 2.5 mg every other day, injected intramuscularly, and the animals were killed 5 days after the first T.P. injection. Actinomycin D (200 ugm/Kg/day), cycloheximide (30 mg/Kg/day), and DL-ethionine (50 mg/Kg/day) were administered intraperitoneally twice daily.

After sacrificing the animals, the kidneys were removed, weighed, and homogenized in 9 volumes of ice-cold 0.9% NaCl solution. Arginase activity was determined by incubating aliquots of the homogenate with 0.27 M L(+) arginine, pH 9.5, at 30°. The reaction was terminated by the addition of 2/3 volume of 15% HPO_3, the suspension was centrifuged, and the urea in the filtrate was determined colorimetrically by the method of Coulombe and Favreau (1963). β-Glucuronidase was determined on aliquots of the same homogenate by the method of Fishman (1964), using phenolphthalein β-D-glucuronide as substrate.

RESULTS

In untreated A Cloudman female mice, kidney arginase normally ranges between 11 and 14 units per gram, while kidney

ß-glucuronidase averages about 1100 Fishman units per gram*. When these animals are given T.P., kidney arginase increases slowly at first, then more rapidly; after five days, the increase is approximately 100% in kidneys of young (6-12 weeks) animals and about 200% in older (6 months) animals. Kidney β-glucuronidase increases 16-20 fold during this interval. For both enzymes, the responses are the same whether the animals have received 3 or 30 mg. steroid during this interval.

There is ample evidence (Pettengill and Fishman, 1962) that the marked elevation in activity of renal β-glucuronidase in mice receiving androgenic steroids is due to increased synthesis of enzyme protein. This conclusion is supported by the results of our experiments with ethionine, an agent which has been shown to inhibit protein synthesis in mice as well as rats (Simpson, et al, 1950). If given early enough, ethionine inhibits the β- glucuronidase response by as much as 85-90%, and completely abolishes the arginase response (Fig. 1). The similarities in effects of ethionine upon the two enzymes suggest that T.P. increases the synthesis of arginase as well.

Actinomycin D had no effect upon the rise in arginase concentration resulting from administration of T.P. (Fig.2); in contrast, the increase in β-glucuronidase was almost completely blocked if treatment with the inhibitor was begun early enough. If the first actinomycin injection

*A 'unit' of arginase will catalyze the decomposition of 1 µMole of arginine per minute at 30°, using the assay conditions described above. A 'unit' of β-glucuronidase will effect the release of 1 µgm. of phenolphthalein per hour (pH 4.5, 37°, 0.001M substrate).

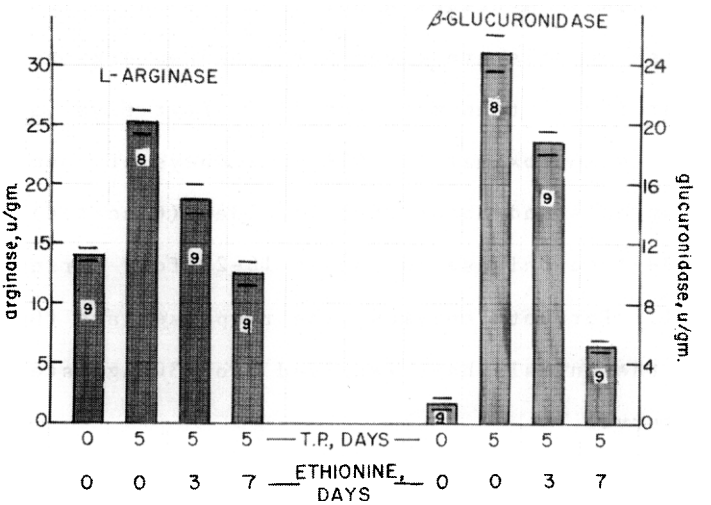

Fig.1: Effect of DL-ethionine (50 mg./kg./day) upon T.P.-induced increase in activities of mouse kidney enzyme. One mg. T.P. was given daily for five days. Ethionine was given twice daily, beginning either two days before or two days after the first T.P. injection. The number of animals in each group, and the standard errors (short horizontal lines) are indicated for each set of data.

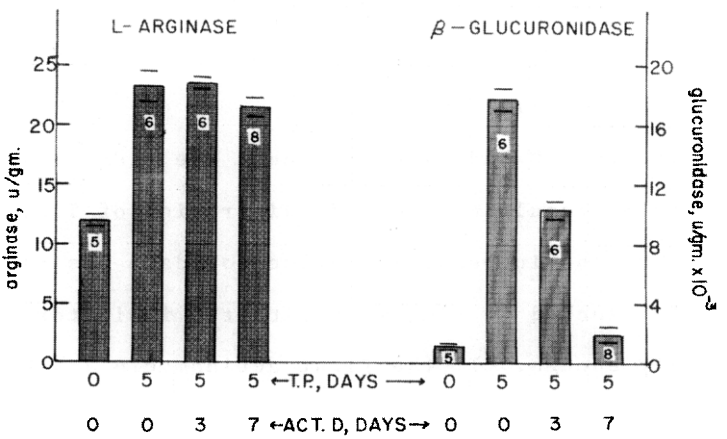

Fig.2: Effect of actinomycin D (200 µgm./kg./day) upon T.P.-induced increase in activities of mouse kidney enzymes. 2.5 mg. T.P. was given on alternate days. Other details as in Fig.1.

was delayed, only partial inhibition was observed (see also Frieden, et al, 1964).

When cycloheximide was given to animals which were also receiving T.P., the effects upon the two enzymes were the reverse of those seen with actinomycin (Fig.3). Cycloheximide, even when given for a total of 7 days, failed to diminish the β-glucuronidase response significantly; indeed, the data suggest a slight potentiation of the T.P. effect. On the other hand, the increase in arginase concentration was reduced by 80% when cycloheximide was given during the last three days of a 5-day T.P. regimen, and was abolished essentially completely when cycloheximide injections began two days earlier.

In the absence of T.P., neither actinomycin nor cycloheximide was able to reduce the concentration of either enzyme below that observed in kidneys of untreated animals.

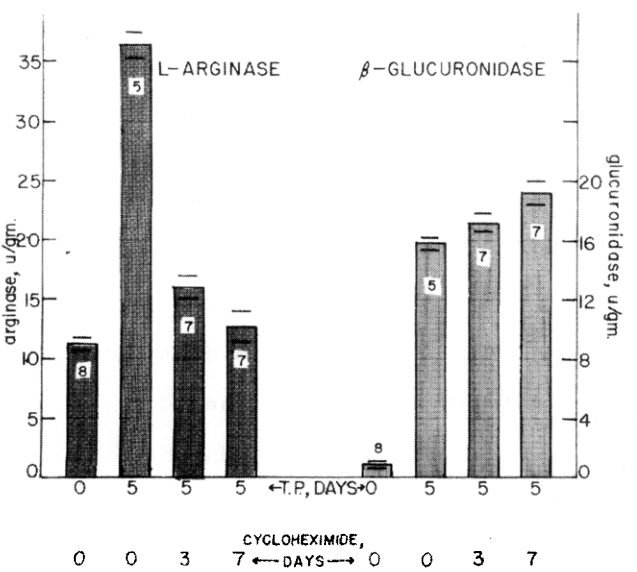

Fig.3: Effect of cycloheximide (300 mg./kg./day) upon T.P.-induced increase in activities of mouse kidney enzymes. One mg. T.P. was given daily for five days. Other details as in Fig.1.

Our data on the comparative effects of actinomycin D upon the response of the two enzymes to T.P. is not inconsistent with the assumption that the primary aspect of the action of androgenic steroids upon protein metabolism in the mouse kidney involves stimulation of DNA-directed RNA synthesis. In experiments of even shorter duration than those reported here, actinomycin D was found to significantly inhibit the incorporation of C^{14}-uridine and C^{14} orotic acid into mouse kidney RNA *in vivo* (Frieden and Fishel, unpublished). The failure of actinomycin D to affect the synthesis of arginase, in contrast to its ready inhibition of β-glucuronidase, would then be explained by a difference in sensitivity of the two DNA primers to this agent (reflecting, perhaps a difference in number or accessibility of guanosine residues). It is also possible to accommodate the cycloheximide data within the framework of this hypothesis, in view of the reported ability of this antibiotic to interfere selectively with the synthesis of different kinds of RNA (Fiala and Davis, 1965; Fukuhara, 1965). On the other hand, the possibility that androgens affect the synthesis of protein at more than one locus cannot be foreclosed, and may, in fact, represent a more reasonable interpretation of the data. More information is needed, especially concerning the effects of cycloheximide upon protein and nucleic acid metabolism in mouse kidney.

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