Acute Effects of Testosterone Propionate upon RNA Synthesis in Mouse Kidney[1] (35737)

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Although several metabolic parameters have been explored in attempts to define the action of anabolic steriods upon the mouse kidney in molecular terms, information on nucleic acid synthesis *in vivo* is confined to the chronic (7 days or longer) effects of testosterone propionate (T.P.) in castrated males (1). On the basis of present knowledge, it would be expected that the action of this hormone would bring about a rapid change in the rate of RNA synthesis. We have therefore studied the acute effects of single injections of T.P. upon the incorporation of labeled orotic acid into the ribonucleic acids of mouse kidney. We have also examined the effects of several metabolic inhibitors (actinomycin D, cycloheximide, and DL-ethionine) upon orotic acid incorporation in androgen-treated and androgen-spared mice.

Experimental Procedures. Female mice of the A Cloudman ('Ajax') strain were purchased from the Jackson Laboratories and used when 14–17 weeks old. Treated animals each received 1 mg T.P., injected intramuscularly in 0.1 ml sesame oil. At intervals thereafter ranging from 6–68 hr, groups of six to eight animals received 0.10 or 0.125 μCi (160,000–200,000 cpm) of orotic acid-6-^{14}C, given intraperitoneally in 0.25 ml water. Food was withheld for the last 2 hr of the preinjection period. Exactly 2 hr later, the animals were sacrificed. The kidneys were removed, homogenized separately in cold 0.9% NaCl solution, and the nucleic acids precipitated by the addition of 0.5 vol of 2.1 *N* perchloric acid. Ribonucleotides were liberated from the acid-insoluble fraction by brief (2.5 hr) digestion with 0.3 *M* KOH at 37° (2). The alkaline digest was acidified with dilute perchloric acid and the solution separated from the resulting precipitate by centrifugation. The precipitate was washed with dilute (0.7 *N*) perchloric acid and discarded. The filtrate (plus washings) was diluted to volume (7.5–10.0 ml); duplicate 0.50-ml aliquots were dissolved in 10 ml Bray's solution (3) and counted in a Packard Tri-Carb liquid scintillation spectrometer. The nucleotide concentration in each filtrate was determined by measuring the absorption at 260 mμ.

In some experiments, homogenates were prepared in 0.25 *M* sucrose and separated by differential centrifugation into nuclear (700*g*, 10 min), particulate (100,000*g*, 60 min; this includes both mitochondria and microsomes) and soluble (100,000*g* supernatant) fractions before precipitation and hydrolysis of nucleic acids.

Solutions of ribonucleotides prepared by alkaline hydrolysis of kidney RNA were fractionated into their constituent nucleotides by chromatography on 8.5 × 0.6-cm columns of Dowex 1 × 8 chloride. The hydrolysate was applied as a dilute solution containing 40–80 OD units (at 260 mμ) in 250 ml; the column was eluted with HCl, the concentration of the latter being increased stepwise through the sequence: 0.001 *N*, 0.003 *N*, 0.005 *N*, and 0.007 *N* (4).

The column effluent was monitored by measuring the optical densities of alternate tubes at 260 mμ. After locating the nucleotide peaks, aliquots from the peak tubes, to-

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TABLE I. Effect of Testosterone Propionate upon Incorporation of Orotic Acid into Mouse Kidney RNA.[a]

Expt. no.	Number of animals	Δ T (hr)[b]	Specific activity[c]	% Change
1	6	—	461 ± 10[d]	—
	6	6	612 ± 21	+33
	6	12	581 ± 24	+26
	6	24	607 ± 7	+31
2	6	—	450 ± 11[d]	—
	7	6	608 ± 12	+35
	7	18	613 ± 19	+36
	7	68	450 ± 12	0

[a] All animals received 0.125 μCi orotic acid-6-^{14}C and were killed 2 hr later. For other details, see text.

[b] Lapsed time between T.P. and orotic acid injections.

[c] cpm/E_{260} (± SE) of RNA hydrolysate.

[d] These animals received no T.P.

gether with samples from the trailing and leading edges, were removed for counting and for determination of spectral properties over the range 220–300 mμ (Cary 14 or Perkin-Elmer 202 spectrophotometer). Specific radioactivities (cpm/μmole) of each radioactive nucleotide were calculated, using optical densities at appropriate wavelengths (5).

For inhibition experiments, groups of animals were maintained on actinomycin D, cycloheximide, or DL-ethionine for 4–5 days preceding a single injection of T.P. Half of the total daily dose of each drug (4.0 μg actinomycin D, 0.6 mg cycloheximide, or 1.0 mg DL-ethionine) was injected each morning and evening. Thirteen to 16 hr after the injection of T.P., orotic acid was given and the animals were killed 2 hr later. A series of animals which received inhibitor alone served as controls.

Testosterone propionate, cycloheximide, and DL-ethionine were purchased from Nutritional Biochemicals, orotic acid-6-^{14}C was obtained from Amersham-Searle, and actinomycin D was the generous gift of Merck, Sharpe and Dohm, Inc.

Results. Significant enhancement of RNA synthesis was detected as early as 6 hr after a single injection of T.P. The stimulation persisted for at least 24 hr but disappeared completely after 68 hr (Table I).[2] In these experiments, the maximum increase seen was 36%; however, increases of 50–75% were found in other experiments in which orotic acid incorporation was measured 15–18 hr after T.P.

When homogenates of kidneys from untreated and T.P.-treated animals were fractionated before separation of RNA, radioactive nucleotides could be isolated from all three fractions (Table II). The specific activity of microsomal RNA was significantly less than that of RNA from other fractions; it is apparent, however, that all three fractions share in a significant T.P. effect.

In RNA prepared from unfractionated kidney homogenate, the most abundant nucleotides were guanylic and uridylic acids, representing approximately 33 and 26 mole %, respectively, of the nucleotide total. The remainder consisted almost entirely of cytidylic and adenylic acids in about equal proportions. No marked departures from these proportions could be detected in ribonucleotides derived from nuclear, microsomal, or soluble RNA. In T.P.-treated animals, the relative proportion of UMP increased and that of AMP decreased; the significance of these changes is, however, uncertain, since pooled samples were used to obtain these data and

[2] When orotic acid incorporation was measured 18 hr after the last of a series of daily T.P. injections (1 mg/day, 7 days) the effect of T.P., although much reduced (14% instead of 73%), was still evident. This is contrary to the results reported by Kochakian and Hill (1), who found that prolonged treatment of castrated male mice with T.P. reduced the incorporation rate by 60%.

TABLE II. Testosterone Propionate and Cell Fraction RNA.[a]

Fraction	Specific activity (cpm/E_{260}) Control	T.P. treated	% Change
Nuclear	331	519	+57
Microsomal	200	354	+77
Soluble	319	460	+44

[a] Treated animals received 1.0 mg T.P. in 0.1 ml sesame oil. Sixteen hours later both groups received 0.10 μCi orotic acid-6-^{14}C. After 2 hr, the mice were killed. Other details in text.

the extent of individual variation is unknown.

As expected, essentially all of the radioactivity of orotic acid-labeled mouse kidney RNA was recovered either as UMP or CMP; only trace amounts were found in the guanylic and adenylic acid fractions. In RNA prepared from the kidneys of untreated mice, the specific activity of UMP was about 2.7 times that of CMP. Although the specific activities of both nucleotides increased in androgen-treated mice, the increase for CMP was nearly twice that for UMP. When specific activities of nucleotides from each cellular fraction were compared, a similar pattern emerged; in each, UMP was more intensely labeled, but after androgen treatment the increase was more pronounced for CMP (Table III).

The failure of either ethionine or cycloheximide to affect RNA synthesis or its stimulation by TP was not unexpected (Table IV). It was surprising, however, to find that mice treated chronically with actinomycin D actually incorporated more orotic acid into kidney RNA than did their untreated counterparts. In these animals, moreover, a distinct TP effect was still apparent, although it was reduced to about half that seen in otherwise untreated animals. In other experiments, it was found that single doses of actinomycin D (200–250 μg/kg) would reduce the incorporation of orotic acid by 25–30%, but only if the interval between drug and labeled compound was decreased to 6 hr or less. Even in this instance, however, a definite TP effect (+20%) was retained.

Discussion. The earliest detectable metabolic response of the mouse kidney to an anabolic steroid appears to be a transitory increase in nuclear, Mg^{2+}-, and DNA-dependent RNA polymerase; following the injection of 0.4 mg T.P., the concentration of this enzyme increases about 50% within 2 hr, then de-

TABLE III. Testosterone Propionate and Labeling of RNA Pyrimidines.[a]

Expt. no.	Source	Nucleotide	Specific activity (cpm/μmole) Control	T.P. treated	% Change
1	Homogenate, unfractionated	UMP	7,520	13,150	+ 75
		CMP	2,075	4,830	+133
2	Nuclear fraction	UMP	21,350	25,600	+ 20
		CMP	7,085	9,750	+ 38
	Microsomal fraction	UMP	8,310	13,150	+ 58
		CMP	1,650	4,000	+142
	Soluble fraction	UMP	14,950	20,600	+ 38
		CMP	4,110	7,225	+ 73

[a] All animals received 0.100 μCi orotic acid-6-^{14}C and were killed 2 hr later. Treated animals had received 1.0 mg T.P. 16 hr before. In experiment 1, pyrimidine ribonucleotides were isolated from unfractionated homogenate; in experiment 2, homogenates prepared in 0.25 *M* sucrose were fractionated before hydrolysis and chromatography. For other details, see text.

TABLE IV. Inhibitors and the Response to Testosterone Propionate.[a]

Expt. no.	Number of animals	Inhibitor and dose	T.P.	Specific activity of kidney ribonucleotides (cpm/E_{260} ± SE)	% Change
1	26	None	—	466 ± 7	
	13	None	+	597 ± 12	+28
2	14	DL-Ethionine, 1.0 mg/day	—	473 ± 17	
	7	DL-Ethionine, 1.0 mg/day	+	595 ± 20	+26
3	13	Cycloheximide, 0.6 mg/day	—	400 ± 11	
	7	Cycloheximide, 0.6 mg/day	+	575 ± 9	+44
4	12	Actinomycin D, 4 μg/day	—	568 ± 13	
	7	Actinomycin D, 4 μg/day	+	661 ± 14	+16

[a] Drugs were given twice daily for 5 days (4 days for actinomycin D-treated animals). A single injection of 1 mg T.P. was given at the same time as the last drug injection. Orotic acid (0.125 μCi) was injected 13–16 hr later. For other details, see text.

creases to control levels within another hour or so (6). As shown here, stimulation of orotic acid incorporation into RNA occurs within 6 hr and persists for 24–36 hr. These events are followed by increases in the rates of incorporation of labeled amino acids into kidney protein *in vitro* (7, 8) and elevations of the concentrations of various renal enzymes; these phenomena occur after periods of 1–5 days (9, 10). Significant increases in total RNA, DNA, and protein content are apparent after 5–10 days.

The fact that UMP isolated from hydrolysates of RNA shortly after giving labeled orotic acid is of higher specific activity than CMP prepared from the same source probably reflects the more remote position of the latter relative to orotic acid in the biosynthetic sequence. Explanation of the observation that labeling of CMP increases more in T.P.-treated animals than does UMP labeling is more difficult. One possibility is that T.P. stimulates the synthesis of a unique species of RNA of high cytidine content. If this were so, it might be expected that (1) the CMP content of RNA from treated animals would be higher than that from untreated animals, and (2) that the ratio of specific activities (UMP/CMP) would be different in different kinds (nuclear, microsomal, soluble) of RNA. Since neither expectation was supported by the data, this explanation cannot be correct. It is more likely that T.P. has a direct influence upon the size of the CTP or UTP pools, or upon the rate of conversion of UMP to CMP. It is known (1) that the concentrations of uracil and cytosine compounds in mouse kidney change with castration and prolonged T.P. administration, but the rapidity of these changes has not been explored.

It has been established that several enzymatic parameters of androgen action in the mouse (incorporation of labeled amino acid into kidney protein, kidney β-glucuronidase, L-arginase, and D-amino acid oxidase concentrations) respond in different ways to the administration of actinomycin D, cycloheximide, or DL-ethionine (9, 10). Our data suggest that it is possible to separate the effects of T.P. upon RNA synthesis in mouse kidney into actinomycin-sensitive and actinomycin-insensitive components. Recalling that only 4 μg of actinomycin D/day (in a 20–25-g mouse) is sufficient to block completely the elevation of β-glucuronidase which occurs in response to T.P., it is tempting to identify the actinomycin-sensitive component with the synthesis of an RNA with a role in β-glucuronidase synthesis. It remains to be seen whether such a unique RNA species can be identified.

Although comparative data are sparse, it appears that synthesis of RNA in mouse kidney (as measured by orotic acid incorporation) is relatively resistant to inhibition by actinomycin D *in vivo*. As noted earlier, 200–250 μg of actinomycin D/kg reduced labeling by only 30%, and then only if incorporation was measured within 6 hr. In other

experiments, it has been found that a single injection of 25 μg of actinomycin D (about 1 mg/kg) was required to reduce kidney RNA synthesis by 75%. Trakatellis, Axelrod, and Montjar (11) found that the same dose of actinomycin D (1 mg/kg) completely suppressed labeling of mouse liver RNA by orotic acid *in vivo*, and that more than 50% inhibition resulted when the dose was reduced to 250 μg/kg. Incorporation of labeled cytidine into RNA of mouse liver, pancreas, small intestine, and tongue was inhibited by 125–500 μg/kg (12); in another study (13) 8 μg/kg was reported to have inhibited RNA synthesis in mouse ileum by 50% within 2 hr.

Summary. In A. Cloudman female mice a single injection of 1 mg T.P. induces a significant increase in the incorporation of labeled orotic acid into kidney RNA. This increase is detectable within 6 hr and has disappeared after 68 hr. In all circumstances, specific activities of uridine nucleotides isolated from labeled RNA are greater than those of cytidine nucleotides; after T.P., however, CMP labeling increases more than does UMP labeling.

Actinomycin D, in quantities sufficient to inhibit the β-glucuronidase response to T.P., does not diminish (in fact slightly increases) nucleic acid labeling, while reducing the T.P. effect by about 50%.

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