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Inhibition of testosterone stimulation of microsomal hexobarbital metabolism by 17 α-methyl-B-nortestosterone (SK&F 7690)

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TESTOSTERONE administration stimulates some microsomal drug-metabolizing enzymes in livers of female and immature or castrate male rats.[1, 2] Compounds which occupy the testosterone receptor for enzyme stimulation might alter this testosterone effect. 17α-Methyl-B-nortestosterone (SK&F 7690), an antiandrogen, probably competes with testosterone for *extra*hepatic receptors.[3] This consideration and the structural similarities between SK&F 7690 and testosterone suggested that the compounds might compete for *intra*hepatic receptors for enzyme stimulation. Therefore, we studied the SK&F 7690 effect on testosterone stimulation of hexobarbital metabolism by liver microsomes.

METHODS

Testosterone experiment ; intact female rat

Sprague-Dawley rats (80–100 g) were injected with saline, testosterone (2·5 mg/kg), SK&F 7690 (100 mg/kg), or testosterone plus SK&F 7690. Testosterone was suspended in saline; SK&F 7690 was suspended in saline or 1 : 2 propylene glycol : water.* Drugs were injected at different subcutaneous sites to avoid possible interaction. Single daily injections were given for 14 days. On day 15, rats were killed and hepactectomized.

"Hexobarbital oxidase" activity

Livers were homogenized in 0·2 M Na_2HPO_4–NaH_2PO_4 buffer (pH 7·4) and centrifuged at 9,000 *g* for 15 min (0 ± 2°). Hexobarbital oxidation was catalyzed by the microsome-containing supernatant fraction. Five-ml reaction mixtures buffered to pH 7·4 contained : NADP (0·4 μmole), G-6-P† (20 μmoles), $MgCl_2$ (75 μmoles), nicotinamide (100 μmoles), hexobarbital sodium (1 μmole), and 2 ml of microsomal preparation equivalent to 333 mg of wet weight liver. Samples were incubated 60 min. The reaction was terminated by addition of 1 ml of 30% trichloroacetic acid.

Hexobarbital was determined by the Cooper and Brodie method[4] except that we used heptane to extract the barbiturate. SK&F 7690 added *in vitro* had no effect on the enzyme system.

*Data was combined from experiments involving different SK&F 7690 vehicles since its effects were the same in either vehicle.

†G-6-P = glucose-6-phospate (disodium). NADP and G-6-P were bought from the California Corp. for Biochemical Research, Los Angeles, Calif.

TABLE 1. HEXOBARBITAL OXIDASE ACTIVITY IN MICROSOMAL PREPARATIONS OF INTACT FEMALE RATS: EFFECTS OF TESTOSTERONE, SK&F 7690 AND TESTOSTERONE PLUS SK&F 7690

Treatment, 14 days, s.c.	Enzyme activity (μmoles/g liver/hr)*
Vehicle	0·77 ± 0·05 (31)
Testosterone, 2·5 mg/kg	1·68 ± 0·06 (24)†
SK&F 7690, 100 mg/kg	1·04 ± 0·08 (16)†
Testosterone + SK&F 7690	1·41 ± 0·09 (16)†‡

*Mean ± standard error. Animal numbers are in parentheses. In 5 ml, the equivalent of 333 mg of liver was incubated for 1 hr at 37° with 1 μmole hexobarbital and appropriate additives (see Methods).

†Significantly different from vehicle ($P < 0·05$, *t* test).

‡Significantly different from testosterone alone ($P < 0·05$).

RESULTS AND DISCUSSION

SK&F 7690 inhibition of testosterone stimulation of hexobarbital oxidase activity; intact female rats

Testosterone (2·5 mg/kg) and, to a lesser extent, SK&F 7690 (100 mg/kg) stimulated hexobarbital oxidase activity (Table 1). When the agents were given together, stimulation was less than after testosterone alone. The results suggest that a "weak" enzyme inducer (SK&F 7690) may compete with a "strong" inducer (testosterone) for occupation of a common receptor site for enzyme induction. The antiandrogen SK&F 7690 exhibited antitestosterone activity at an enzymatic level in liver. These results are compatible with those of Saunders *et al.*[3] wherein SK&F 7690 competed with testosterone for *extra*hepatic receptors.

Since doses of SK&F 7690 higher than 100 mg/kg were incompletely absorbed from subcutaneous sites, it was difficult to describe accurately the effects of high doses. Such doses of SK&F 7690 stimulated hexobarbital oxidase and inhibited testosterone stimulation, but there was no relationship between dose and effect of SK&F 7690. Fifty mg SK&F 7690/kg stimulated enzyme activity slightly and when given with testosterone produced a small but insignificant reduction of testosterone stimulation.

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α-Aceto-γ-hydroxybutyramide—a new anti-metabolite

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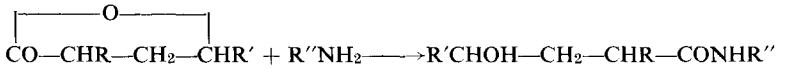
α-ACETO-γ-hydroxybutyramide has been examined in a microbiological system for anti-metabolic activity having shown signs of such action by interrupting pregnancy in mice. Some information on the mode of action of this compound has been obtained using *Escherichia coli* as a test system.

MATERIALS AND METHODS

Chemically, hydroxyamides can often be prepared by the reaction of the appropriate lactone with ammonia or amine—



α-Aceto-γ-hydroxybutyramide. 0·1 mole (12·8g) of α-aceto-γ-butyrolactone was warmed to 30° and a solution of concentrated ammonia (d = 0·880) (0·1 mole, ~ 5·5 ml) dissolved in water (10 ml) was added over a period of 1 hr with vigorous stirring, the temperature being maintained at 30–35°. The reaction product was concentrated by distillation under reduced pressure to obtain the crude solid. Recrystallization from water gave the pure product in 57 per cent yield as a white solid, m.p. 64–65° (Found : N, 9·7 per cent, $C_6H_{11}NO_3$ requires N,9·7 per cent). Compounds prepared similarly are in the appendix.