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Evidence for spironolactone as a possible inducer of liver microsomal enzymes in mice*

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SPIRONOLACTONE, currently employed as a diuretic, has recently been reported to have pharmacological effects in addition to aldosterone-blockade. Selye *et al.*[1] have shown that this agent offers protection against the sedative and anesthetic effects of such steroids as testosterone, progesterone, desoxycorticosterone and hydroxydione, as well as pentobarbital and ethanol. They emphasized that several days of pretreatment were essential for antagonism of this CNS depressant activity. Androgens, estrogens, glucocorticoids, anabolic and progestational steroids have been noted to influence the activity of drug-metabolizing liver microsomal enzymes.[2, 3]

To test the hypothesis that spironolactone was stimulating hepatic drug metabolism, we sought to examine the effects of its pretreatment on pentobarbital and testosterone-potentiated pentobarbita sleeping times and to measure liver microsomal enzyme activities.

Experimental

Animal pretreatment. Male Swiss mice, 18–25 g, were maintained *ad lib.* on a standard laboratory pellet diet and water. Animals were given a 100 mg/kg subcutaneous injection of spironolactone or its corn oil vehicle for 3 consecutive days. These injections, and all others described, were delivered in a volume of 0·1 ml/10 g body weight. Approximately 24 hr after the last injection, control and drug-treated animals were randomly divided into groups for the sleeping time studies and liver microsomal assays. Another group of animals, treated in an identical manner, were employed for the study of hexobarbital metabolism.

Sleeping time experiments. Details of the drug administration schedule are presented in Table 1. The duration of sleeping time was calculated from the time the animal lost its righting reflex until it was able to right itself three times in 30 sec.

Preparation of liver microsomes. Animals were sacrificed and livers excised immediately, rinsed and transferred to ice-cold 0·02 M Tris-HCl buffer, pH 7·4, containing 1·15% KCl. Livers were homo-

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TABLE 1. EFFECTS OF SPIRONOLACTONE PRETREATMENT ON PENTOBARBITAL AND TESTOSTERONE-POTENTIATED PENTOBARBITAL SLEEPING TIME IN MICE*

Group†	Spironolactone (100 mg/kg, s.c.)	Testosterone (300 mg/kg, i.p.)	Pentobarbital (50 mg/kg, i.p.)	Sleeping time ± S.D. (min)	Control (%)
I	−	−	+	63·4 ± 23·1	
II	+	−	+	28·8 ± 11·2	46‡
III	−	+	+	121·6 ± 42·0	192§
IV	+	+	+	44·9 ± 17·6	37‖
					156¶

* Spironolactone or corn oil vehicle was given once daily for 3 consecutive days. Twenty-four hr after the last dose, testosterone proprionate or corn oil vehicle was injected and followed 30 min later by pentobarbital or saline.
† No. = 10.
‡ II vs. I; $P < 0.001$.
§ III vs. I; $P < 0.005$.
‖ IV vs. III; $P < 0.001$.
¶ IV vs. II; $P < 0.025$.

genized in 4 vol. of the buffer with a motor-driven Teflon-glass homogenizer. All subsequent tissue manipulations were carried out on ice. Homogenates were centrifuged at 9000 *g* for 20 min in a refrigerated centrifuge; the 9000 *g* supernatant was then carefully removed and centrifuged at 78,000 *g* for 75 min. The resulting microsomal pellet was resuspended in ice-cold 0·02 M Tris-HCl buffer, pH 7·4.

Enzyme assays. Incubation mixtures contained the following components: 5 mg microsomal protein, 2 μmoles NADPH and 300 μmoles Tris-HCl buffer, pH 7·4, in a final vol. of 3·0 ml. For hexobarbital metabolism, a NADPH-generating system consisting of 1 μmole NADP, 12 μmoles glucose 6-phosphate, 2 units of glucose 6-phosphate dehydrogenase and 15 μmoles $MgCl_2$ was substituted for NADPH. The amounts of substrates present in the mixtures were 20 μmoles aniline, 15 μmoles ethylmorphine and 2 μmoles hexobarbital for the aniline hydroxylase, *N*-demethylase and hexobarbital assays respectively. After the addition of all components, the flasks were transferred from ice to a Dubnoff apparatus and incubated at 37° under air with shaking (90 oscillations/min) for the times indicated in Tables 2 and 3.

TABLE 2. EFFECT OF SPIRONOLACTONE PRETREATMENT ON THE LIVER MICROSOMAL ENZYME ACTIVITY IN MALE MICE*

Treatment†	Liver weight / Body weight (%)	Liver microsomal protein (mg/g liver)	Aniline hydroxylation (mμmoles *p*-aminophenol formed/mg protein/15 min)	Ethylmorphine *N*-demethylation (mμmoles HCHO formed/mg protein/12 min)
Control	4·86 ± 0·41	18·3 ± 2·4	33·6 ± 5·2	95·4 ± 4·7
Spironolactone	5·32 ± 0·30	24·2 ± 1·9	43·4 ± 1·9	120·4 ± 3·1
% Increase	9·5‡	32·0§	29·2§	26·3§

* Livers from three mice in each treatment group were pooled and treated as an individual sample (N). Tabulated values represent the calculated mean ± S.D. for N = 5–6.
† Animals were treated subcutaneously with 100 mg/kg spironolactone or corn oil for 3 consecutive days.
‡ $P < 0.02$.
§ $P < 0.005$.

TABLE 3. EFFECT OF SPIRONOLACTONE ON THE MICROSOMAL METABOLISM OF HEXOBARBITAL IN MALE MICE*

Treatment†	Hexobarbital metabolized (mμmoles disappeared/mg protein/20 min)
Control	34·0 ± 6·4
Spironolactone	57·2 ± 12·8‡

* Livers from five mice in each treatment group were pooled and treated as an individual sample (N). Tabulated values represent the calculated mean ± S.D. for N = 5.

† Animals were treated subcutaneously with 100 mg/kg spironolactone or corn oil for 3 consecutive days.

‡ $P < 0{\cdot}02$.

Analytical methods. Microsomal protein was determined by the method of Lowry *et al.*,[4] employing crystalline bovine albumin as the standard. Aniline metabolism was measured by its conversion to *p*-aminophenol as described by Kato and Gillette;[5] *N*-demethylation of ethylmorphine was determined by formaldehyde production,[6] and hexobarbital disappearance by the method of Cooper and Brodie.[7]

Results and discussion

The duration of sleeping times after the various drug treatment schedules and the statistical analyses (Students *t*-test) are presented in Table 1. Mice treated with spironolactone and/or testosterone exhibited no gross behavioral effects. Testosterone potentiated pentobarbital sleeping time in the absence of spironolactone pretreatment (III vs. I) or in its presence (IV vs. II). Pretreatment with spironolactone significantly reduced pentobarbital sleeping time (II vs. I) and testosterone-potentiated pentobarbital sleeping time (IV vs. III).

The influence of spironolactone pretreatment on the liver microsomal enzyme activities is presented (Tables 2 and 3). As shown in Table 2, prior administration of spironolactone enhanced the microsomal metabolism of aniline and ethylmorphine 29 and 26 per cent, respectively, over control values; microsomal protein per gram liver in spironolactone-treated animals was increased 32 per cent. Further, the liver weights of treated animals expressed as a percentage of total body weight were significantly increased over controls. Moreover, it is clear that hexobarbital metabolism is increased (68 per cent) after spironolactone pretreatment (Table 3), and this provides evidence for a more direct relationship between barbiturate sleeping time and its metabolism.

Selye *et al.*[1] noted that the anesthetic effects of testosterone were markedly reduced after spironolactone-pretreatment in female rats. Since we observed no gross behavioral changes with testosterone in male mice, we gave this compound with pentobarbital to detect CNS depression which was otherwise unobservable; potentiation was noted. The findings that spironolactone-pretreatment reduced testosterone-potentiated, as well as pentobarbital sleeping time, coupled with our biochemical findings suggested that this antagonism of anesthesia may not be a major consequence of a change in CNS responsiveness, as suggested by Selye *et al.*,[1] but rather the result of a stimulation in microsomal enzyme activity by a mechanism similar to other inducers. In this regard, a wide variety of steroid hormones and related substances, including testosterone, are not only metabolized by microsomal enzymes, but have also been shown to stimulate drug metabolism in the liver.[2, 3]

Spironolactone is therapeutically employed as a diuretic, and it may be argued that the effects observed are the result of this pharmacological action. In this regard, there was a small difference in body weight between control and spironolactone-treated animals on the day of testing. Although it is conceivable that the excretion rates of testosterone and pentobarbital were increased in the drug-treated animals, we believe that the reduction in pentobarbital sleeping time is the result of an enhancement of barbiturate metabolism. In view of our results, the administration of other therapeutic agents with spironolactone should be investigated for possible drug–drug interactions.

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