Stimulation of Liver Microsomal Drug Metabolism in Male and Female Mice by Spironolactone and Aldadiene[1] (35489)

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(Introduced by A. M. Burkman)
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Recent findings in our laboratory (1–3) and others (4, 5) have shown that spironolactone (SPL) stimulates the metabolism of drugs by liver microsomes. Stripp and coworkers (5) reported differences in the nature of enzyme induction in male and female rats after SPL pretreatment. The present study was initiated to ascertain whether SPL inductive effects occur in both sexes of mice. Aldadiene (SC-9376), a major *in vivo* metabolite of SPL (6), was included to determine if this Δ^6-dethioacetylated metabolite retains the inductive properties of the parent drug.

Materials and Methods. Nonfasted male and female albino Swiss mice, weighing 20–30 g, were used. Spironolactone and aldadiene (100 mg/kg) or corn oil vehicle were given ip twice daily for 3 consecutive days, in a volume of 1.0 ml/100 g of body weight. Experiments were initiated 24 hr after the last dose.

Sleeping time. After the injection of hexobarbital sodium (120 mg/kg, ip), the duration of sleep was determined to be the time interval between the loss and restoration of the righting reflex.

Microsomal enzyme assays. Procedures for the isolation of liver microsomes and their incubation with hexobarbital and ethylmorphine were conducted as previously described (1). Incubation mixtures contained the following components in a final volume of 3.0 ml: 5 mg of microsomal protein, a NADPH-generating system (consisting of 2 enzyme units of glucose-6-phosphate dehydrogenase, 15 μmoles of glucose-6-phosphate, 1.2 μmoles of NADDP, 150 μmoles of Tris–HCl buffer, pH 7.4), and either 15 μmoles of ethylmorphine or 2 μmoles of hexobarbital.

The extent of hexobarbital metabolism and ethylmorphine *N*-demethylation by liver microsomes was assayed using the procedures described by Cooper and Brodie (7) and Nash (8), respectively.

Cytochrome P-450 was estimated by the method of Omura and Sato (9); NADPH-cytochrome *c* reductase was measured by the procedure of Phillips and Langdon (10) and microsomal protein was assayed by the method of Lowry *et al.* (11). Statistical comparisons were made using Student's *t* test.

Results. The influence of spironolactone (SPL) and aldadiene pretreatment on various hepatic microsomal enzyme systems and related parameters in mice is presented in Tables I and II, respectively. These results reveal that there are no qualitative differences in the induction by either drug in both sexes. It was observed that both SPL and aldadiene increased liver weight (11–27%), liver/body weight (14–20%) and microsomal protein content (17–34%). In addition, the observed reductions in hexobarbital sleeping times (4–18% of the control group) are in agreement with the 2-fold increases noted in the microsomal metabolism of hexobarbital. Similarly, pretreatment with either drug elevated the *N*-demethylation of ethylmorphine (1.3–2.5-fold). In the same series of experiments, components of the hepatic microsomal electron transport system were examined. It was found that both compounds elevated the cytochrome P-450 content (34–57%) and increased the reduction of cytochrome *c* by NADPH (20–75%).

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TABLE I. Effect of Spironolactone Pretreatment on Liver Weight, Liver/Body Weight, Microsomal Protein Content, Sleeping Time, and Various Hepatic Microsomal Enzyme Levels in Mice.

Parameter	Sex	Treatment[a]		% of control
		Corn oil	Spironolactone	
Liver wt (g)[b]	M	0.93 ± 0.04	1.18 ± 0.02	127[e]
	F	1.05 ± 0.03	1.18 ± 0.03	112[e]
Liver/body wt (%)[b]	M	5.39 ± 0.24	6.47 ± 0.27	120[e]
	F	4.93 ± 0.17	5.85 ± 0.27	119[d]
Microsomal protein (mg/g of liver)[c]	M	24.2 ± 1.1	28.3 ± 1.1	117[e]
	F	27.7 ± 2.1	33.6 ± 2.3	121[d]
Hexobarbital sleeping time (min)[b]	M	83 ±19	15 ± 7	18[e]
	F	127 ±39	23 ±18	18[e]
Hexobarbital metabolism *in vitro* (mμmoles/mg of protein/20 min)[c]	M	29.8 ± 6.3	62.3 ± 9.1	209[e]
	F	21.6 ± 6.6	48.6 ± 3.9	225[d]
Ethylmorphine *N*-demethylase (mμmoles/mg of protein/12 min)[c]	M	123 ± 9	312 ±14	254[e]
	F	83 ± 6	164 ±11	198[e]
Cytochrome P-450 (mμmoles/mg of protein)[c]	M	0.75 ± 0.02	1.18 ± 0.01	157[e]
	F	0.74 ± 0.08	0.99 ± 0.04	134[d]
NADPH-cytochrome *c* reductase (mμmoles/mg of protein/min)[c]	M	97 ± 4	170 ± 7	175[e]
	F	108 ± 7	145 ±10	134[e]

[a] Animals were given spironolactone (100 mg/kg) or corn oil ip twice daily for 3 consecutive days.
[b] Values represent the mean ± SD of $N = 10$ for each treatment group.
[c] Livers from 5 mice were pooled and treated as an individual sample (N) for the *in vitro* assays. Tabulated values represent the calculated mean ± SD of $N = 5$.
[d] $p < .01$.
[e] $p < .001$.

In a kinetic study (Table III), it was observed that pretreatment of male mice with SPL significantly increased the maximal velocity (V_{max}) for the *N*-demethylation of ethylmorphine by 2.2-fold, without altering the apparent affinity constant (K_m). Thus, the enhanced microsomal metabolism of ethylmorphine by SPL can be attributed to an increase in the amount of the enzyme rather than an alteration in the existing enzyme system.

Discussion. The data obtained in this study demonstrate that SPL increases the activity of microsomal enzymes concerned with drug metabolism and electron transport in both sexes of mice. These findings differ considerably from those previously reported after SPL pretreatment in rats (5). According to Stripp *et al.* (5), a reduction in hexobarbital sleeping time as well as an increase in the microsomal metabolism of the barbiturate was seen only in female rats. In contrast, they found no appreciable sex differences in the stimulation of NADPH-cytochrome *c* reductase and ethylmorphine demethylase activitites. Furthermore, there was no change in the cytochrome P-450 content in either sex after pretreatment with SPL. In view of these observations, it is evident that the nature of hepatic microsomal enzyme induction by SPL is markedly different in mice and rats.

We have shown that the Δ^6-dethioacetylated metabolite of SPL, aldadiene, is also an inducer of hepatic microsomal drug metabolism. On the basis of this evidence, we are unable to ascertain whether the inductive effects of SPL are mediated directly or via aldadiene. However, because of the similarity in chemical structure, it might be suggested that both drugs are active in this regard. Further, it is of interest to note that aldadiene retains the antimineralcorticoid effects of spironolactone (12).

TABLE II. Effect of Aldadiene Pretreatment on Liver Weight, Liver/Body Weight, Microsomal Protein, Sleeping Time, and Various Hepatic Microsomal Enzyme Levels in Mice.

Parameter	Sex	Treatment[a] Corn oil	Aldadiene	% of control
Liver wt (g)[b]	M	1.51 ± 0.04	1.68 ± 0.06	111[d]
	F	1.27 ± 0.03	1.51 ± 0.03	119[e]
Liver/body wt (%)[b]	M	5.24 ± 0.34	6.08 ± 0.21	116[f]
	F	5.12 ± 0.36	5.86 ± 0.35	114[f]
Microsomal protein (mg/g of liver)[c]	M	23.4 ± 4.7	31.4 ± 2.1	134[e]
	F	25.3 ± 1.4	31.7 ± 1.8	125[f]
Hexobarbital sleeping time (min)[b]	M	50 ± 21	9 ± 7	18[f]
	F	47 ± 32	2 ± 5	4[f]
Hexobarbital metabolism *in vitro* (mμmoles/mg of protein/20 min)[c]	M	30.9 ± 11.4	63.9 ± 9.1	207[f]
	F	21.6 ± 6.0	41.1 ± 3.5	190[f]
Ethylmorphine *N*-demethylase (mμmoles/mg of protein/12 min)[c]	M	83 ± 11	104 ± 5	125[e]
	F	103 ± 9	206 ±16	200[f]
Cytochrome P-450 (mμmoles/mg of protein)[c]	M	1.25 ± 0.20	1.75 ± 0.17	140[e]
	F	1.06 ± 0.04	1.57 ± 0.19	148[f]
NADPH-cytochrome *c* reductase (mμmoles/mg of protein/min)[c]	M	119 ± 8	159 ±22	133[e]
	F	97 ± 10	116 ±11	120[d]

[a] Animals were given aldadiene (100 mg/kg) or corn oil ip twice daily for 3 consecutive days.

[b] Values represent the mean ± SD of $N = 10$ for each treatment group.

[c] Livers from 5 mice were pooled and treated as an individual sample (N) for the *in vitro* assays. Tabulated values represent the calculated mean ± SD of $N = 5$.

[d] $p < .20$.

[e] $p < .05$.

[f] $p < .01$.

TABLE III. Effect of Spironolactone Pretreatment in Male Mice on the K_m and V_{max} Constants for the Microsomal *N*-demethylation of Ethylmorphine.[a]

Parameter[b]	Treatment Corn oil	Spironolactone
K_m (mM)	1.11 ± 0.22	1.15 ± 0.22
V_{max} (mμmoles/mg of protein/min)	17.5 ± 2.9	38.5 ± 7.3[c]

[a] Animals were given spironolactone (100 mg/kg) or corn oil ip twice daily for 3 consecutive days.

[b] Varying concentrations of ethylmorphine (0.05–2.0 mM) were incubated with liver microsomes and a NADPH-generating system as described in Materials and Methods. The kinetic constants (K_m + V_{max}) were found by visually fitting straight lines to double reciprocal plots of the data (5–6 data points/experiment). The results are expressed as the mean value ± SD of 5 experiments.

[c] $p < .001$.

Prior treatment with SPL has been shown to reduce the pharmacological and toxicological actions of many drugs. Whereas, the enhancement in drug metabolism may explain the reduction in toxicity of certain anesthetics and digitoxin (13, 14), it does not satisfactorily explain the attenuation of mercuric chloride poisoning (15).

Summary. Pretreatment with spironolactone (SPL) or aldadiene enhanced the activity of drug-metabolizing enzymes in mouse liver microsomes. Elevations in components of the microsomal electron transport system and other parameters indicative of enzyme induction were also noted. The nature of the induction by these drugs did not differ qualitatively nor was sex-dependent difference observed. Kinetic studies suggest that the microsomal induction by SPL may be mediated by an increase in the amount of enzyme (V_{max}) rather than by an alteration in the apparent affinity (K_m) of the enzyme.

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