Increased Hepatic Microsomal Activity Induced by Spironolactone and Other Steroids[1] (34342)

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Recently it was shown that pretreatment with antimineralocorticoids (*e.g.*, spironolactone, spiroxasone) or anabolic steroids (*e.g.*, norbolethone, methylandrostenediol) protects the rat against otherwise fatal doses of digitalis compounds; it also diminishes the anesthetic effect of steroids and barbiturates as well as the convulsions caused by diphenylhydantoin (1–3). The development of indomethacin-induced intestinal ulcers was similarly inhibited by spironolactone and norbolethone (4). In addition, spironolactone was shown to offer complete protection against dimethylbenzanthracene induced adrenal necrosis (5). This remarkably broad resistance-increasing or "catatoxic" (3, 4) effect of certain steroid hormones and their derivatives raises the possibility, that—among other factors—nonspecific enzyme induction in the hepatic microsomes might be involved. The electron microscopic observation that spironolactone causes extensive proliferation of the smooth-surfaced endoplasmic reticulum in hepatocytes supports this view (6).

To prove directly whether spironolactone or anabolic steroids which possess catatoxic activity, can influence the drug-detoxifying activity of hepatic microsomal enzymes, we have studied the metabolism of pentobarbital in rats pretreated with spironolactone, norbolethone or ethylestrenol.

Materials and Methods. Sixty female Sprague-Dawley rats of the Holtzman Farms (Madison, Wisconsin), averaging 100 g (range 90–110 g) and maintained *ad libitum* on Purina Laboratory Chow and tap water were placed in four equal groups and treated as indicated in Table I. Spironolactone,[2] norbolethone,[3] or ethylestrenol[4] (10 mg in 1 ml of H_2O) were given twice daily by gavage. On the fourth day (2 hr after steroid administration) 10 animals of each group were giv-

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[2] The spironolactone, Aldactone, was donated by G. D. Searle & Co., Chicago, Ill.

[3] The norbolethone, Genabol, was donated by John Wyeth & Bros. (Canada) Ltd., Windsor, Ontario.

[4] The ethylestrenol was donated by Organon Inc., W. Orange, N.J.

TABLE I. Influence of Spironolactone and Other Steroids on Pentobarbital Metabolism (means ± standard errors).

Group	Treatment[a]	Depth of anesthesia (scale: 0–3)	Pentobarbital in blood 90 min after administration (μg/ml)	Pentobarbital oxidized by liver microsomes after 30 min (μg/g)
1	None	1.4	13.4 ± 0.9	200.5 ± 7.6
2	Spironolactone	0.8	8.3 ± 0.9	257.5 ± 6.9
3	Norbolethone	0.6	5.0 ± 0.7	279.0 ± 4.6
4	Ethylestrenol	0.1	1.9 ± 0.5	317.7 ± 8.4

[a] In addition to this treatment, 10 animals of each group received 3 mg of pentobarbital sodium (in 0.5 ml of oil, intraperitoneally) for blood determination; the remaining 5 rats were used for the hepatic detoxification studies.

en pentobarbital sodium[5] (3 mg in 0.5 ml of oil intraperitoneally). Ninety min later, the depth of anesthesia was estimated in terms of an arbitrary scale in which 0 = no change, 1 = manifest sluggishness indicative of sedation, 2 = maintenance of prone position when laid on back, but righting upon pinching the tail, and 3 = deep anesthesia with loss of righting reflex (2). The animals were then exsanguinated by aorta puncture and the serum pentobarbital level determined according to Brodie *et al.* (7). The remaining rats of each group were similarly exsanguinated, after stunning, and the liver was quickly removed and chilled. Samples were taken and processed at 0–3°: homogenates were prepared in 3 vol of 0.1 *M* phosphate buffer (pH 7.4) with a Potter-Elvehjem type of homogenizer. 2 ml of individual liver microsomal + supernatant fractions (9000 × *g*) were incubated for 30 min with 2 μmoles of pentobarbital sodium in a Dubnoff metabolic shaking incubator at 37° using oxygen as the gas phase; 50 μmoles of nicotinamide, 25 μmoles of $MgCl_2$ and 0.26 μmole of TPN were added and 0.1 *M* phosphate buffer (pH 7.4) to a final volume of 4 ml (8). Enzyme activity was determined by measuring the disappearance of pentobarbital (7).

Results. The depth of anesthesia, the concentration of serum pentobarbital and its oxidation rate by the liver microsomal + supernatant fraction are shown in Table I. Pretreatment with steroids reduced the anesthetic effect of pentobarbital. In this respect, spironolactone and norbolethone (Groups 2 and 3) were almost equally active, whereas ethylestrenol (Group 4) was much more potent.

As compared with the controls, spironolactone pretreatment (Group 2) increased the disappearance rate of pentobarbital from the blood ($p<0.001$). Norbolethone (Group 3) proved to be slightly more active than spironolactone ($p<0.001$) and ethylestrenol (Group 4) was even more effective than norbolethone ($p<0.001$) in this respect. A correlation was found between the degree of anesthesia and the concentration of pentobarbital in the blood.

As compared with the controls, spironolactone and norbolethone pretreatment (Group 2 and 3) increased pentobarbital oxidation by the liver microsomal + supernatant fraction to a similar degree ($p<0.01$); ethylestrenol (Group 4) was much more potent ($p<0.01$ as compared with Groups 2 and 3).

Discussion. It is known that pentobarbital is exclusively metabolized in the liver microsomes (8) and the rate of its oxidation can be increased by certain drugs (9). Therefore the study of pentobarbital metabolism seemed suitable to investigate whether spironolactone, norbolethone, and ethylestrenol can induce detoxifying enzymes in the liver. Our results show that pretreatment with these steroids enhances the oxidation of pentobarbital by liver microsomes and accelerates the rate of its disappearance from the blood in proportion to the decreasing depth of anesthesia. Based on these data it is probable that the resistance-increasing effect of these steroids against many drugs is at least partly due to their enzyme-inducing capacity. It is noteworthy that spironolactone and anabolic steroids [unlike many of the previously known enzyme inducers such as hypnotics, sedatives or polycyclic aromatic hydrocarbons (10)] are virtually free of toxic side-effects.

Summary. In the rat, pretreatment with spironolactone, norbolethone, or ethylestrenol increased the oxidation of pentobarbital by liver microsomes, enhanced its disappearance from blood and proportionally decreased the depth of anesthesia.

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