DECREASED HEPATIC MICROSOMAL DRUG METABOLISM AFTER THE INJECTION OF A MIXTURE CONTAINING SOMATOTROPIN, CORTICOTROPIN AND PROLACTIN

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The hepatic microsomal metabolism of several drugs was decreased in rats 31-34 days after implantation of a pituitary mammotropic tumor (MtT)(Wilson, 1968 a,b). In a recent report (Wilson, 1968c), a decrease in the hepatic metabolism of hexobarbital and the formation of formaldehyde from aminopyrine was noted as early as 48 hours after implantation of the MtT. This finding suggested that pituitary hormones (somatotropin (STH), corticotropin (ACTH), and prolactin) contained in the tumor innoculum (Bates et al. 1962) were factors which contributed to the decrease in drug-metabolizing enzyme activity in liver from MtT-injected rats. To test this hypothesis, a mixture of STH, ACTH, and prolactin was administered to rats in an amount similar to that reported by Bates et al (1962) for an earlier transplant generation of the MtT. A decrease in the liver metabolism of hexobarbital and aminopyrine was noted 48 hours after the injection of this hormone mixture.

METHODS

Male Fischer rats (80-90 days old) were injected subcutaneously with one ml of a mixture which contained bovine STH, ACTH and ovine prolactin[1]. A mixture of these three hormones was prepared in 0.9% aqueous saline and then dissolved in 14-16% gelatin. In one study, a boiled hormone preparation was injected. STH, ACTH or prolactin in 0.9% aqueous saline at pH 7.04 was placed in a boiling water bath for 20 minutes. Each hormone solution was alkalinized with 1-2 drops of 0.1 N NaOH after boiling, and a mixture of STH, ACTH, and prolactin was prepared as described for the unboiled hormones.

[1]The author gratefully acknowledges the gift of STH and prolactin from the Endocrinology Study Section of the National Institutes of Health. Armour Laboratories corticotropin (ACTHAR) was used.

Rat livers were excised and homogenized (1 gram liver + 2 ml 1.15% KCl) in a glass homogenizer with a teflon pestle. The liver homogenate was centrifuged at 9000 xg for 20 minutes, and 0.25 ml of the 9000 xg supernatant fraction was added to a reaction mixture which contained (in μmoles/2.5 ml of the mixture): glucose-6-phosphate, 12.5: nicotinamide adenine dinucleotide phosphate, 2.08; $MgSO_4$, 12.5; and, when formaldehyde as measured, semicarbazide HCl, 25. Potassium phosphate buffer (0.1M, pH 7.35) was added to adjust the final volume of the reaction mixture to 2.5 ml. Substrate concentration in μmoles/2.5ml incubation mixture was: hexobarbital sodium, 1.5; aminopyrine, 20; and ethylmorphine HCl, 2.5. The reaction mixture was incubated for 30 minutes at 37° under an atmosphere of oxygen. The side chain oxidation of hexobarbital by the liver 9000xg supernatant fraction was estimated by measuring substrate disappearance (Cooper and Brodie, 1955). The method described by Nash (1955) as modified by Cochin and Axelrod (1959) was used to measure formaldehyde produced by the liver demethylation of aminopyrine or ethylmorphine. Statistical methods for the student "t" test are described in Snedecor (1956), and the level of significance used was $P<.05$.

RESULTS AND DISCUSSION

The hepatic metabolism of hexobarbital and the production of formaldehyde from aminopyrine were decreased 48 hours after rats were injected with a mixture of STH + ACTH + prolactin as compared with rats which received an injection of bovine serum albumin (fig. 1.). The amount of STH (420 mUnits), ACTH (690 mUnits) and prolactin (497 mUnits) administered to these rats was similar to that found in 500 mg wet weight of the MtT (Bates et al, 1962). Thus, 48 hours after the injection of MtT homogenate equivalent to 500 mg wet weight MtT (Wilson, 1968c) or a mixture of pituitary hormones in an amount similar to that reported by Bates et al (1962) to be present in the MtT, the liver metabolism of hexobarbital and aminopyrine was decreased. It may be noted that the liver metabolism of hexobarbital and the demethylation of aminopyrine were not decreased 48 hours after rats were injected with a boiled hormone mixture of STH (420 mUnits), ACTH (690 mUnits) and prolactin (497 mUnits) as compared with bovine serum albumin-injected control rats.

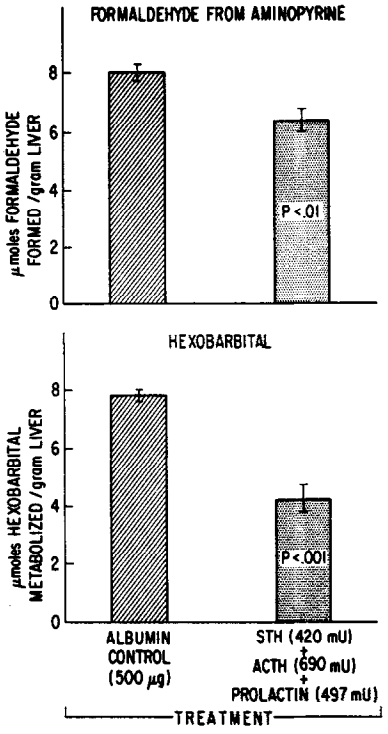

Figure 1. Hepatic metabolism of hexobarbital and aminopyrine 48 hours after the injection of STH + ACTH + prolactin. Each value represents the mean ± S.E. Male rats (5 per group) were injected subcutaneously with bovine serum albumin, or a mixture of STH + ACTH + prolactin dissolved in 16% gelatin. In this study 1.04 μmoles of NADP were added to a reaction mixture which was incubated for 20 minutes.

A significant decrease ($P<.05$) in the hepatic metabolism of hexobarbital, aminopyrine and ethylmorphine did not occur until 48 hours after the injection of a more concentrated pituitary hormone mixture (STH 840 mUnits + ACTH 1380 mUnits + prolactin 700 mUnits)(fig.2). This finding was consistent with the hypothesis that pituitary hormones contained in the MtT innoculum were partly responsible for the decreased metabolism of hexobarbital and aminopyrine, because a significant decrease in the liver biotransformation of these two drugs was not observed until 48 hours after the injection of MtT homogenate into rats (Wilson, 1968c).

The addition of three concentrations of a hormone mixture (STH + ACTH + prolactin) to a reaction flask which contained control rat liver, cofactors and substrate failed to inhibit the *in vitro* metabolism of hexobarbital or the formation of formaldehyde from aminopyrine (Table I). This suggested that the

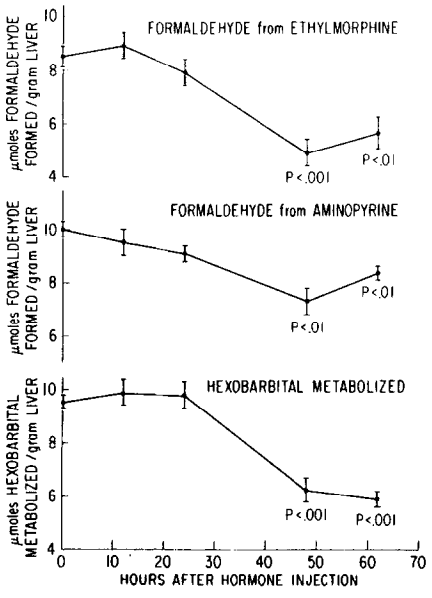

Figure 2. Hepatic metabolism of hexobarbital, aminopyrine and ethylmorphine from 12 to 62 hours after the injection of STH (840 mUnits) + ACTH(1380 mUnits) + prolactin (700 mUnits). Values are depicted as the mean ± S.E., and values shown without P<.05 were not statistically significant as compared with albumin-injected controls. Control rats were injected with bovine serum albumin (1mg/ml of 16% gelatin) 12 hours before determinations were made, and control values are shown at "0" hours. There were six male rats per group.

TABLE I

Substance added	Concentration	Hexobarbital Metabolized (µmoles/gm)	Formaldehyde from aminopyrine (µmoles/gm)
Albumin	2 µg	10.8	9.4
Mixture # 1		10.1	10.2
STH	0.0012 mU		
ACTH	0.019 mU		
Prolactin	0.01 mU		
Mixture # 2		10.6	10.6
STH	0.012 mU		
ACTH	0.19 mU		
Prolactin	0.1 mU		
Mixture # 3		10.3	10.9
STH	1.2 mU		
ACTH	19.0 mU		
Prolactin	10.0 mU		

The in vitro hepatic metabolism of hexobarbital and aminopyrine after the addition of various mixtures of STH, ACTH and prolactin. Liver from male rats was used. Bovine serum albumin or any one of three mixtures of STH, ACTH, and prolactin was dissolved in 0.9% aqueous saline, and 0.1 ml of this saline solution was added to the reaction mixture which contained cofactors, substrate and rat liver 9000xg supernatant fraction. The concentration of albumin or hormone mixture is depicted as µg or mUnits/2.5 ml reaction mixture. The µmoles of hexobarbital metabolized or the µmoles of formaldehyde formed from aminopyrine/gram liver represent a single determination.

presence of STH, ACTH and/or prolactin in the liver of hormone-treated rats was not responsible for the observed decrease in microsomal drug-metabolizing enzyme activity.

In the present study, a decrease in the hepatic metabolism of hexobarbital and aminopyrine was noted after the injection of STH + ACTH + prolactin, and a decrease in the metabolism of these compounds as well as that of ethylmorphine was maximal 48 hours after the injection of this hormone mixture. Addition of these three pituitary hormones to a reaction mixture which contained a control rat liver preparation did not produce a decrease in the metabolism of hexobarbital or aminopyrine. These findings are consistent with the hypothesis that STH, ACTH, and prolactin present in the MtT were some of the factors which decreased hepatic microsomal drug-metabolizing enzyme activity *in vivo*. In liver from phenobarbital-treated rats, a half life of about 2 days (Arias & DeLeon, 1967) or less than 7 days (Wilson & Fouts, 1966) was suggested for some drug-metabolizing enzymes. Microsomal protein from control mouse liver may have a half life of 3.5 days (Shuster & Jick, 1966), and the half life for mouse liver microsomal reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase may be 2.8 days (Jick & Shuster, 1966). Since a decrease in the liver metabolism of hexobarbital, aminopyrine or ethylmorphine appeared to be maximal 2 days after the injection into rats of a STH, ACTH and prolactin mixture, these hormones may decrease the synthesis of some components of the hepatic microsomal drug-metabolizing enzyme system.

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