Biochemical Pharmacology, Vol. 18, pp 2029–2031. Pergamon Press. 1969. Printed in Great Britain

SHORT COMMUNICATIONS

Identification of somatotropin as the hormone in a mixture of somatotropin, adrenocorticotropic hormone and prolactin which decreased liver drug metabolism in the rat*

(*Received* 10 *October* 1968; *accepted* 31 *January* 1969)

HEPATIC microsomal drug-metabolizing enzyme activity may be decreased by one or all of the hormones found in a transplantable pituitary tumor (MtT).[1, 2] Bates *et al.*[3] observed high blood levels of STH, ACTH and LtH† in rats implanted with the MtT, and these hormones were present in the tumor brei. The administration of a mixture of STH, ACTH and LtH in an amount similar to that contained in the MtT inoculum produced a decrease in liver drug metabolism.[4] In the study discussed here, evidence is presented which identifies STH, rather than ACTH or LtH, as the pituitary hormone which decreased the liver metabolism of three drugs in rats.

Male Fischer rats (80–90 days old) were injected with STH, ACTH, LtH or albumin‡ subcutaneously. Hormones or albumin were dissolved in 0·9% saline, and gelatin was added (final concentration 14–15%) in order to retard the absorption rate of the compounds. Forty-eight hr after hormone injection, rat livers were removed and homogenized in the cold with a glass (Teflon pestle) homogenizer. KCl (1·15%) was added before homogenization such that 1 ml of the homogenate was equivalent to $\frac{1}{2}$ g liver. The rat liver 9000 *g* supernatant fraction (0·25 ml), prepared by centrifuging the homogenate at 9000 *g* for 20 min, was added to a reaction mixture which contained (μmoles/2·5 ml of the mixture): glucose 6-phosphate, 25; $MgSO_4$, 25; nicotinamide adenine dinucleotide phosphate (NAPD), 2·08; and, when formaldehyde was measured, semicarbazide HCl 25. Substrates used and their concentration as micromoles per 2·5 ml of the mixture were: hexobarbital sodium, 1·5; ethylmorphine, 10; or aminopyrine, 20. Potassium phosphate buffer (1·3 to 1·45 ml of a 0·1 M, pH 7·35 solution) was added to adjust the final volume of the mixture to 2·5 ml. Incubations were performed at 37° for 30 min under oxygen. At the end of this period of incubation, the amount of nonmetabolized hexobarbital remaining in the mixture was determined by the method of Cooper and Brodie,[5] and formaldehyde formed by the demethylation of aminopyrine or ethylmorphine was measured according to the methods described by Nash[6] and Cochin and Axelrod.[7] Under these reaction conditions and for this period of incubation, there was minimal deviation from linearity with regard to the liver metabolism of hexobarbital or the formation of formaldehyde from aminopyrine or ethylmorphine. A $P \leqslant 0{\cdot}05$ was chosen as the level of statistical significance, and the Student *t*-test for paired differences was used.[8]

Hexobarbital metabolized or formaldehyde formed from aminopyrine or ethylmorphine by rat liver 48 hr after an injection of STH, ACTH or LtH or of all three hormones is depicted in Fig. 1. Only the injection of STH + ACTH + LtH or of STH alone produced a decrease in the metabolism of these three compounds as compared with albumin-injected controls. The injection of high doses of LtH (up to 104 units/rat) failed to produce a decrease in the liver metabolism of hexobarbital or in the formation of formaldehyde from aminopyrine. Intact or adrenalectomized male rats which were injected with ACTH (2·8 units/rat) also failed to show a decrease in the liver metabolism of hexobarbital or aminopyrine 48 hr after injection.

This is perhaps the first report which identifies STH as a pituitary hormone which decreases the

* Part of this study was presented at the Fall 1968 meeting of the American Society for Pharmacology and Experimental Therapeutics.

† Abbreviations for pituitary hormones used in this paper are: STH, somatotropin or growth hormone; ACTH, corticotropin adrenocorticotropic hormone; LtH, prolactin.

‡ The gift of bovine STH and ovine LtH from the Endocrinology Study Section of the NIH is gratefully acknowledged. Lyophilized ACTH and bovine albumin powder (Fraction V from plasma) from Armour Laboratories were used.

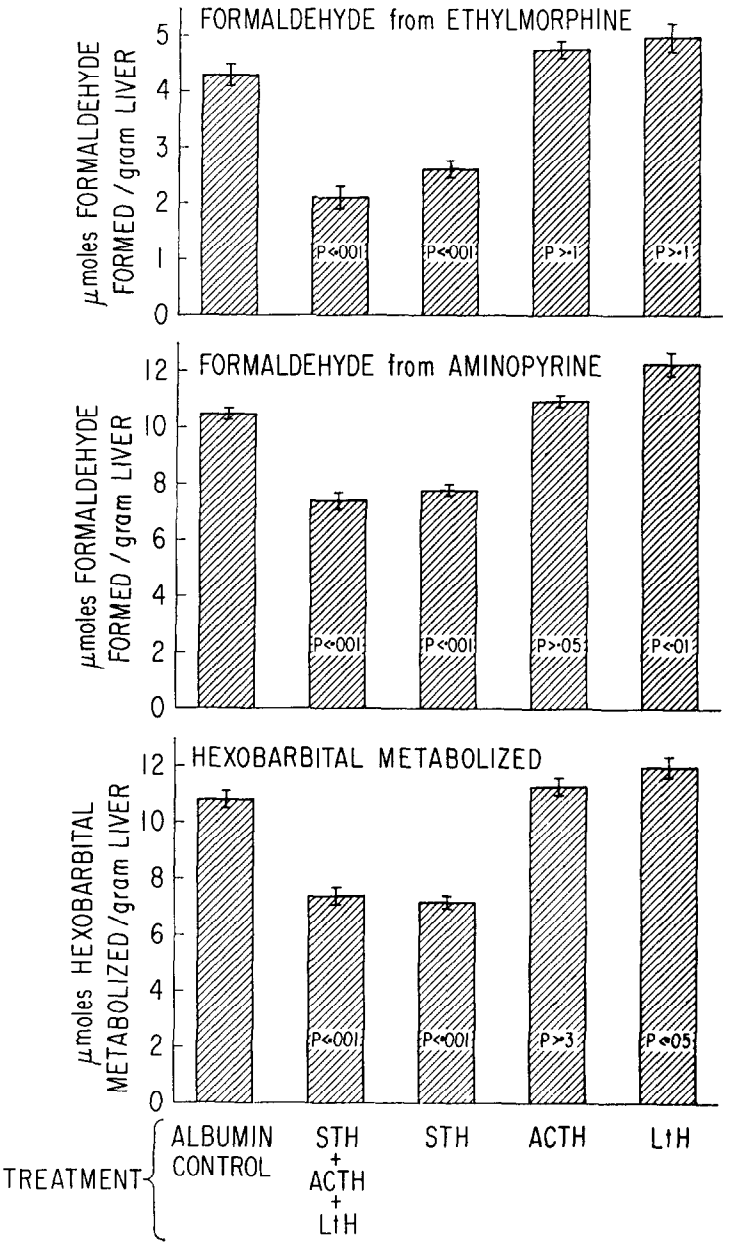

FIG. 1. Rat hepatic drug metabolism after the injection of STH, ACTH or LtH. Male Fischer rats were injected s.c. with STH (1·73 units), ACTH (2·8 units), LtH (1·4 units) or bovine albumin (2 mg) in 14% gelatin 48 hr before determinations were made. Results are depicted as the mean ± S.E., and the level of significance chosen was $P < 0{\cdot}05$. There were six rats in each group.

metabolism *in vitro* of drugs by rat liver. Several studies, however, have suggested that STH or other pituitary hormones may alter the metabolism *in vitro* of carcinogens. Shirasu *et al.*[9] fed MtT-bearing rats the carcinogen *N*-hydroxy-*N*-2-fluorenylacetamide (N-OH-FAA) and noted a decrease in the urinary excretion of some N-OH-FAA metabolites. The rat liver dehydroxylation and deacetylation of N-OH-FAA may have been decreased. Certain metabolic changes (e.g., elevated rectal temperature) were noted with MtT-bearing rats and were also found after rats received high doses of ACTH (80–96 units) for 10 days.[10] The injection of ACTH or of high doses of STH (10–12 mg for 10 days) in rats fed N-OH-FAA produced changes in the urinary excretion of N-OH-FAA metabolites consistent with the suggestion that dehydroxylation and deacetylation of this carcinogen were decreased.[10] In the present study, only the injection of STH was found to decrease the liver metabolism of hexobarbital, aminopyrine and ethylmorphine. The injection of ACTH did not produce a decrease in this metabolism in adrenalectomized or intact rats. Our study differs from the one reported by Shirasu *et al.*,[10] however, because a low dose of STH (1·73 units) or of ACTH (2·8 units) was injected only once. In addition, our measurements were performed *in vitro* and the rats were not fed a drug such as N-OH-FAA. The results of both investigations, however, strongly suggest that STH was one of the factors secreted by the MtT which decreased the liver metabolism of hexobarbital, aminopyrine, ethylmorphine and possibly of N-OH-FAA. Studies are now under way to determine if somatotropin acts directly on the liver cell at the transcription or translation level to decrease hepatic microsomal drug-metabolizing enzyme activity.

Acknowledgement—The author gratefully acknowledges the skilful and excellent technical assistance of Mrs. M. G. Lind.

JOHN T. WILSON

Section of Endocrinology,
Reproduction Research Branch,
National Institute of Child Health and Human Development,
National Institutes of Health,
Bethesda, Md., and

Perinatal Health Center, Department of Pediatrics,
Children's Hospital of San Francisco,
San Francisco, Calif., U.S.A.

REFERENCES

1. J. T. WILSON, *J. Pharmac. exp. Ther.* **160**, 179 (1968).
2. J. T. WILSON, *Biochem. Pharmac.* **17**, 1449 (1968).
3. R. W. BATES, S. MILKOVIC and M. H. GARRISON, *Endocrinology* **71**, 943 (1962).
4. J. T. WILSON, *Biochem. biophys. Res. Commun.* **32**, 903 (1968).
5. J. R. COOPER and B. B. BRODIE, *J. Pharmac. exp. Ther.* **114**, 409 (1955).
6. T. NASH, *Biochem. J.* **55**, 416 (1953).
7. J. COCHIN and J. AXELROD, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
8. G. W. SNEDECOR, *Statistical Methods*, Iowa State College Press, Ames, Iowa (1956).
9. Y. SHIRASU, P. H. GRANTHAM, R. S. YAMAMOTO and J. H. WEISBURGER, *Cancer Res.* **26**, 600 (1966).
10. Y. SHIRASU, P. H. GRANTHAM, E. K. WEISBURGER and J. H. WEISBURGER, *Cancer Res.* **27**, 81 (1967).