Biochemical Pharmacology, Vol. 28, pp. 1801–1803
Pergamon Press Ltd. 1979. Printed in Great Britain.

SHORT COMMUNICATIONS

Precocious development of hepatic glutathione *S*-transferase activity with glucocorticoid administration in neonatal rat

(*Received* 2 *October* 1978; *accepted* 15 *November* 1978)

Since the early work of Fouts and Adamson [1], which demonstrated that neonatal mice, rats and guinea pigs had a very low capacity to metabolize drugs, a voluminous literature has appeared on the perinatal maturation of drug-metabolizing systems (for recent reviews, see Refs. 2 and 3). The mechanism(s) responsible for the postnatal increase in activity of most of these pathways is not clear. Some evidence is available which indicates that the developmental formation of aniline hydroxylase and aminopyrine *N*-demethylase in rats is dependent on the adrenal pituitary axis [4]. Recently, Leakey and Fouts [5] demonstrated that treatment of neonatal rats with glucocorticoid hormones causes precocious development of hepatic cytochrome P-450. Also, recent studies in mutant newborn mice have shown a common deficiency in the appearance of cytochrome P-450 and several other neonatally developing enzymes such as tyrosine aminotransferase and glucose-6-phosphatase [6], thus suggesting a lesion in a common mechanism controlling the development of these enzymes.

Developmental formation of epoxide hydrase and glutathione *S*-transferase, two enzymatic pathways for epoxide biotransformation, has been studied in several laboratories [7–12]. The present report focuses on the precocious induction of glutathione *S*-transferase activity following glucocorticoid administration.

Rats of the CD strain (Charles River Laboratories) were injected i.p. with various hormones (Sigma Chemical Co., St. Louis, MO) suspended in 0.9% NaCl at the ages and doses indicated in the tables or figure. Control animals received an equal volume of NaCl alone. Fetal rats were treated with dexamethasone by i.p. injection to the mother.

Livers were homogenized in 150 mM KCl containing 1.25 mM HEPES buffer (pH 7.6) and centrifuged at 11,000 *g* for 20 min. The resulting supernatant fraction was centrifuged at 176,000 *g* for 45 min and the microsomal supernatant fraction obtained was used for assay of glutathione *S*-transferase activities. Glutathione *S*-transferase activities with styrene oxide (SO) or benzo(a)pyrene 4,5-oxide (4,5-BPO) [13] or 1-chloro-2,4-dinitrochlorobenzene (DNCB) [14] as substrate were assayed. Details of the assays have been described earlier [15]. Protein content was determined according to the procedure of Lowry *et al.* [16] with bovine serum albumin as the reference standard. Statistical evaluation of the results was made using Student's *t*-test.

Administration of the glucocorticoids, dexamethasone acetate, or corticosterone acetate to 2½-day-old neonatal rats precociously stimulated the development of hepatic glutathione *S*-transferase activities assayed with SO, 4,5-BPO or DNCB as substrate (Table 1). Treatment with the two glucocorticoids had no effect on hepatic microsomal epoxide hydrase activity (data not shown). Glucagon administration did not produce any effects on hepatic epoxide hydrase or glutathione *S*-transferase activities.

Dexamethasone treatment of mothers at 19½ days of gestation had no effect on the glutathione *S*-transferase activity of fetal rats (Table 2) but when administered to neonatal rats

Table 1. Effect of hormone treatment on neonatal rat liver glutathione *S*-transferase activities*

Treatment (dosage)	No. of animals	Glutathione *S*-transferase activity (nmoles/min/mg protein) (substrates) SO	4,5-BPO	DNCB
Saline	4	41.7 ± 6.2	5.17 ± 0.74	368 ± 22
Glucagon† (3 × 50 μg/g)	4	39.8 ± 5.9	4.89 ± 0.59	412 ± 42
Saline	4	50.3 ± 8.3	5.19 ± 0.64	413 ± 23
Corticosterone acetate† (3 × 100 μg/g)	5	64.7 ± 7.4‡	6.69 ± 0.57‡	601 ± 29‡
Corticosterone acetate† (3 × 50 μg/g)	3	59.9 ± 5.9‡	6.32 ± 0.61‡	597 ± 49‡
Saline	5	43.6 ± 5.4	5.28 ± 0.54	347 ± 24
Dexamethasone acetate§ (1 × 35 μg/g)	7	62.5 ± 3.8‡	8.13 ± 0.74‡	529 ± 37

*All animals were killed 36 hr after the first hormonal treatment. Results are mean ± S. D.
† Rat (neonates) were treated (i.p.) 2½, 3, and 3½ days postpartum.
‡ Indicates significantly different from controls ($P < 0.05$).
§ Rat (neonates) were treated (i.p.) with a single injection at 2½ days postpartum.

Table 2. Effect of age on dexamethasone-mediated stimulation of rat liver glutathione *S*-transferase activity*

Age at treatment (days)	Treatment	No. of animals	Glutathione *S*-transferase activity (nmoles conjugate/min/mg protein) (substrates) SO	4,5-BPO	DNCB
	Saline	4		3.68 ± 0.47	214 ± 27
19½ (fetal)	Treated	6		3.28 ± 0.59	184 ± 39
	Saline	4		4.10 ± 0.64	386 ± 42
<4 hr	Treated	5		7.13 ± 0.56†	690 ± 130†
	Saline	4	39.7 ± 5.2	4.31 ± 0.39	412 ± 49
2½	Treated	7	58.8 ± 6.1†	7.82 ± 0.51†	801 ± 69†
	Saline	3	42.8 ± 7.3	5.34 ± 0.71	532 ± 68
7	Treated	5	63.5 ± 3.9†	8.12 ± 0.54†	892 ± 39†
	Saline	4	71.4 ± 6.4	5.88 ± 0.48	684 ± 82
12½	Treated	4	99.8 ± 8.7†	7.94 ± 0.81†	806 ± 49†

*Rats were treated either with saline or dexamethasone acetate (35 μg/g body wt) 36 hr before the assay. Fetal rats were treated by i.p. injection to mother. Results are mean S. D.
†$P < 0.05$.

4 hr after birth caused 74 and 79 per cent increases in glutathione *S*-transferase activity when assayed with 4,5-BPO and DNCB respectively. This effect on glutathione *S*-transferase activity was very similar in 2½-day-old neonates. However, in 12½-day-old neonatal rats the effects of dexamethasone treatment on glutathione *S*-transferase activity were less pronounced (40, 35 and 18 per cent increase for SO, 4,5-BPO and DNCB as substrates respectively). Following a single i.p. administration of dexamethasone (35 μg/g) to 3-day-old rats, maximal increases in glutathione *S*-transferase activities with all three substrates were observed within 36–40 hr of treatment (Fig. 1).

Physicians have been concerned for many years with the sensitivity of the newborn human infant to a variety of chemicals. This concern is based largely on the findings that, relative to adults, human neonates have a limited capacity to metabolize xenobiotics. In this respect, perinatal development

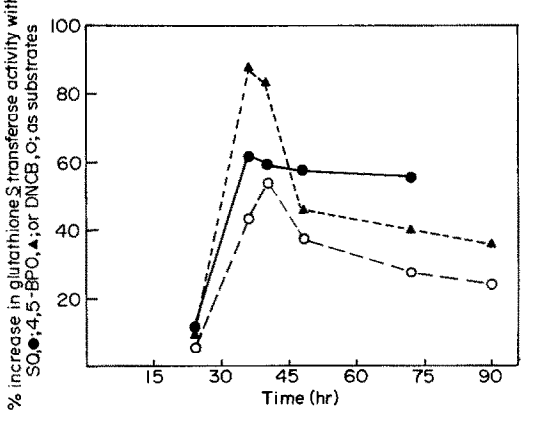


Fig. 1. Per cent increase in hepatic cytosolic glutathione *S*-transferase activity of 3-day-old rats following a single i.p. dose of dexamethasone (35 μg/g body wt). Data represent mean of four experiments. Standard deviation of the mean was always less than 10 per cent of the values.

of enzyme activities has assumed special emphasis. However, relatively few attempts have been made to explore the reasons for the low neonatal enzyme activity or to identify the naturally occurring trophic factors responsible for the development of the relevant enzymes. Glutathione *S*-transferase activity in rat liver appears in early fetal life [8] and attains a significant level around day 15 of gestation. Activation of the adrenal pituitary axis during the second week of postnatal life of the rat [17] coincides with the peak in the developmental pattern of glutathione *S*-transferase activity. Therefore, glucocorticoids appear to be a natural stimulant for glutathione *S*-transferase. Consistent with this hypothesis, we found that administration of dexamethasone to neonatal rats invoked precocious induction of hepatic glutathione *S*-transferase activity. Glucagon, which is known to stimulate some neonatally developing enzymes [18, 19], was ineffective in precociously inducing the glutathione *S*-transferase activity in rats (Table 1). This implies involvement of endogenous glucocorticoids in the natural development of glutathione *S*-transferases.

Dexamethasone failed to cause premature development of glutathione *S*-transferase activity in fetal rat liver, which apparently is not due to the inability of the steroid to reach the fetal liver, since maternal dexamethasone treatment has been shown to stimulate other fetal hepatic enzymes [20]. Also, endogenous glucocorticoid concentrations are higher in late fetal plasma than in neonatal plasma [21, 22], suggesting that endogenous glucocorticoids are not the "trigger" initiating the onset of development of glutathione *S*-transferase activity. It is reasonable to assume that the competence of the perinatal hepatic glutathione *S*-transferase activity is evoked at birth and that, following this, the enzyme develops in response to endogenous glucocorticoid.

Glucocorticoids thus appear to be an important trophic factor in the differentiation of hepatocytes with regard to development of glutathione *S*-transferase activity.

Acknowledgements—We wish to thank Mr. Gary D. Byrd for his excellent technical assistance.

Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, U.S.A.

HASAN MUKHTAR
JULIAN E. A. LEAKEY*
TAHANI H. ELMAMLOUK†
JAMES R. FOUTS
JOHN R. BEND

* Present address: Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.

† Present address: Department of Zoology, Cairo University, Cairo, Egypt.

REFERENCES

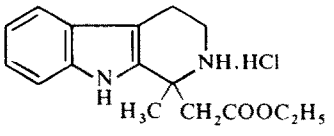
1. J. R. Fouts and P. H. Adamson, *Science* **129**, 857 (1959).
2. C. R. Short, D. A. Kinden and R. Stith, *Drug Metab. Rev.* **5**, 1 (1976).
3. A. H. Neims, M. Warner, P. M. Loughnan and J. V. Aranda, *A. Rev. Pharmac. Toxic.* **16**, 427 (1976).
4. H. Mukhtar, M. K. Sahib and J. R. Kidwai, *Biochem. Pharmac.* **23**, 345 (1974).
5. J. E. A. Leakey and J. R. Fouts, *Biochem. biophys. Res. Commun.*, in press.
6. M. M. Thaler, R. P. Erickson and A. Pelger, *Biochem. biophys. Res. Commun.* **72**, 1244 (1976).
7. T. A. Stoming and E. Bresnick, *Cancer Res.* **34**, 2810 (1974).
8. H. Mukhtar and E. Bresnick, *Cancer Res.* **36**, 937 (1976).
9. B. F. Hales and A. H. Neims, *Biochem. J.* **160**, 231 (1976).
10. M. O. James, G. L. Foureman, F. C. P. Law and J. R. Bend, *Drug Metab. Dispos.* **5**, 19 (1977).
11. J. R. Bend, M. O. James, T. R. Devereux and J. R. Fouts, in *Basic and Therapeutic Aspects of Perinatal Pharmacology* (Eds. P. L. Morselli, S. Garattini and F. Serini), p. 229. Raven Press, New York (1975).
12. A. J. Ryan, M. O. James, Z. Ben-Zvi, F. C. P. Law and J. R. Bend, *Envir. Hlth. Perspect.* **17**, 135 (1976).
13. M. O. James, J. R. Fouts and J. R. Bend, *Biochem. Pharmac.* **125**, 187 (1976).
14. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
15. H. Mukhtar and J. R. Bend, *Life Sci.* **21**, 1277 (1977).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. S. Levine and R. F. Mullins, *Science* **152**, 1585 (1966).
18. O. Greengard, *Biochem. J.* **115**, 19 (1969).
19. F. B. Butcher and V. R. Potter, *Cancer Res.* **32**, 2141 (1972).
20. G. J. Wishart and G. J. Dutton, *Biochem. biophys. Res. Commun.* **75**, 125 (1977).
21. O. Greengard, *J. Steroid Biochem.* **6**, 639 (1975).
22. C. E. Martin, M. H. Cake, P. E. Hartman and I. F. Cook, *Acta endocrin., Copenh.*, **84**, 167 (1977).

Biochemical Pharmacology, Vol. 28, pp. 1803–1806 0006-2952/79/0601–1803 $02.00/0

Serotonergic activity of a novel tetrahydro-β-carboline

(*Received* 3 *August* 1978; *accepted* 27 *November* 1978)

There is much pharmacological interest in tetrahydro-β-carbolines due to their possible endogenous synthesis in brain from serotonin [1–6]. Neurochemical studies *in vitro* indicated that 1,2,3,4-tetrahydro-β-carboline and 6-methoxy-1,-2,3,4-tetrahydro-β-carboline elevated serotonin levels in the brain of mice and rats [7, 8]. Studies *in vitro* indicated that tetrahydro-β-carboline analogs inhibited the metabolism of serotonin by monoamine oxidase (MAO) [9–11] or the neuronal uptake of serotonin [12–14] or increased serotonin syntheses [15]. Here we report the synthesis and activity of (±)1-ethoxycarbonylmethyl-1-methyl-,1,2,3,4-tetrahydro-β-carbolin-2-ium chloride (carbonylmethyl-THBC). This novel tetrahydro-β-carboline increases brain serotonin levels in mice but has little effect on MAO activity. However, carbonylmethyl-THBC does increase the neuronal release of serotonin *in vitro* and has agonist activity at serotonergic sites on the isolated rat fundus preparation.



Carbonylmethyl-THBC

In all studies, male albino mice (20–25 g) and rats (180–200 g) of the Fullinsdorf strain were used. Carbonylmethyl-THBC was dissolved in aqueous-Tween 80 (1%, v/v) for administration to animals. Control animals received an equivalent volume of the aqueous-Tween 80 vehicle. For all neurochemical studies, *in vitro* carbonylmethyl-THBC was dissolved in distilled water.

MAO activity *in vitro* was determined by the method of Jarrott [16] using a 10 vol. mouse brain homogenate in distilled water as the enzyme source. Substrates studied were 5-hydroxy[side chain 2-^{14}C]tryptamine creatinine sulfate ([^{14}C]5-HT, 56 mCi/m-mole, Amersham) and [side chain 2-^{14}C]tyramine hydrochloride ([^{14}C]tyramine, 55 mCi/m-mole, Amersham) over the concentration range 10^{-4} M to 2×10^{-5} M for [^{14}C]5-HT and 2.5×10^{-3} M to 5×10^{-4} M for [^{14}C]tyramine. Carbonylmethyl-THBC and standard substances were preincubated with the enzyme preparation for 5 min prior to addition of substrate.

The neuronal uptake of 10^{-8} M 1-[7-^{3}H]noradrenaline hydrochloride ([^{3}H]NA, 9.1 Ci/m-mole, Amersham) and 5×10^{-8} M 5-hydroxy[G-^{3}H]tryptamine creatinine sulfate ([^{3}H]5-HT, 11.1 Ci/m-mole, Amersham) was measured using a crude synaptosomal (P_2) preparation of mouse or rat brain cerebral cortex. Carbonylmethyl-THBC and standard substances were preincubated with synaptosomes for 5 min at 37° before addition of substrate [17].

The neuronal release of serotonin was studied using a modification of the method of Pylatuk and McNeil [18]. A crude synaptosomal (P_2) preparation from mouse or rat brain cortex was incubated for 15 min at 37° with 10^{-7} M [^{3}H]5-HT (11.1 Ci/m-mole). The synaptosomes were washed twice in cold Krebs phosphate buffer (pH 7.4) to achieve a constant efflux of [^{3}H]5-HT. Carbonylmethyl-THBC was then added to aliquots of washed synaptosomes. Release was studied over a 2-min period of 37° followed by centrifugation at 12,000 *g* for 10 min. Aliquots of the supernatant fluid were sampled and the synaptosomal pellets were dissolved in ethanol for liquid scintillation counting. Results were expressed as a percentage of [^{3}H]5-HT released compared to that retained in the synaptosomal pellet.

Carbonylmethyl-THBC was compared with serotonin for agonist activity on the isolated fundus strip prepared from the stomach of the rat. Contractions of the fundus were recorded against a 2 g weight using a Gould isotonic transducer.

Noradrenaline (NA) and dopamine (DA) concentrations were assayed by the trihydroxyindole fluorimetric method [19] after separation on a Dowex 50-W cation exchange column [20]. The endogenous concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) in mouse brain were determined by measuring native