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Biochemical Pharmacology, Vol. 41, No. 3, pp. 476–478, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Selective stimulation of carboxylesterases metabolizing charged steroid esters by hydrocortisone

(*Received* 2 *July* 1990; *accepted* 27 *September* 1990)

Corticosteroids have gained wide therapeutic use in the treatment of various diseases. In order to prolong the duration of action or to prepare injectable solutions, many steroid hormones and analogous synthetic derivatives are applied as esters. Studies on the metabolism of these compounds have revealed that the hydrolysis of steroid esters to generate the free molecule is accomplished by a group of steroid esterases of the B-type [1, 2]. We have reported earlier the existence of three distinct carboxylesterases involved in the hydrolysis of steroid esters, where two enzymes are responsible for the metabolism of hydrocortisone hemisuccinate (HCHS) at pH 5.5 and 8.0 and a third enzyme for the metabolism of hydrocortisone acetate (HCAC) at pH 8.0, in isolated rat liver microsomes [3]. The free steroid molecule then undergoes hydroxylation via the cytochrome P450-dependent oxidative pathway and is excreted as its glucuronide conjugate [4].

In a recent report evaluating the responses of mixed function oxidases (MFO) to corticosteroids, cortisone acetate and deoxycortisone acetate elicited substrate specific and sex-dependent changes in arylhydrocarbon hydroxylase and aminopyrene *N*-demethylase activity [5]. In fact, adrenalectomized male rats retrieved the MFO-component enzyme level as well as *N*-demethylase activity upon being administered subcutaneous doses of cortisone acetate (5 mg/kg) for 6 consecutive days [6].

Heterogeneous multiple forms of carboxylesterases are known to be involved in the hydrolytic metabolism of xenobiotics in tissues and blood [7–9]. The hepatic microsomal and cytosolic enzymes belonging to this group [10–12] are nonspecifically induced by phenobarbital and carcinogenic polycyclic aromatic hydrocarbons such as 3,4-benzo(*a*)pyrene and 3-methylcholanthrene. In addition, their interactions with a vast array of xenobiotics renders them susceptible to modulation in a qualitative and quantitative manner [13–15]. Considerable work during the past few years on the influence of hormones on hydrolytic metabolism has failed to spell out a clearly defined role for pituitary, adrenal and gonadal systems [16, 17]. Besides, the influence of repeated administration of corticosteroids on the enzymes hydrolysing xenobiotics remains as yet unassessed.

This prompted us to examine the changes occurring in hepatic microsomal hydrolytic metabolism of hemi-succinate and acetate esters of hydrocortisone, acetyl-salicylic acid (ASA), 2-acetylaminofluorene (AAF), acetanilide, *p*-nitrophenylacetate (NPA), procaine and butyrylcholine, and cytosolic thiacetazone esterase under the effect of repetitive oral treatment with charged and uncharged hydrocortisone esters in rat.

Materials and Methods

Chemicals. 2-Acetylaminofluorene, acetanilide, procaine hydrochloride and bovine serum albumin were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Butyrylcholine iodide and *p*-nitrophenylacetate were acquired from Koch-Light Lab. (Colnbrook, Bucks, U.K.) and Sisco Research Lab. Pvt. Ltd (India), respectively. Hydrocortisone and its hemisuccinate and acetate esters were a gift from Glaxo Laboratories (India) Ltd (Bombay) and thiacetazone from Dey's Medical Stores (Manufacturing) Ltd (India). All other chemicals were of analytical grade.

Male albino rats having free access to food and water were used throughout the study. Rats in batches of six to eight animals each were administered equimolar quantities (≈10 mg/kg HC base) of HC (10 mg/kg), HCAC (13.4 mg/kg) and HCHS (11.2 mg/kg) in 1% gum acacia (w/v) orally with the aid of a feeding cannula for 8 consecutive days. Controls received a comparable quantity of the vehicle. The animals were killed by decapitation 18–20 hr after administration of the last dose. Their livers were removed immediately, chopped, washed in ice-cold 1.15% KCl buffered with 0.01 M Tris–HCl to remove blood and homogenized in the same medium with a Potter–Elvehjem homogenizer. Liver microsomes and cytosol were prepared by differential centrifugation by the procedure described earlier [3].

Assay. Enzyme activity was assayed spectrophotometrically by estimating the quantity of product formed or substrate disappeared. Thus, steroid esterases hydrolysing HCHS at pH 5.5 and 8.0 (I and II) and HCAC at pH 8.0, procaine esterase and butyrylcholine esterase were assayed by following the disappearance of substrates using alkaline hydroxylamine-ferric chloride reagent [18, 19]. The quantity of salicylic acid (at pH 5.5 and 7.4), *p*-nitrophenol, aniline and aminofluorene produced/min/mg protein served as a measure of the activity of microsomal B-esterases hydrolysing the substrates ASA, NPA, acetanilide and AAF [3, 20, 21]. In the cytosol thiacetazone esterase was evaluated on the basis of its hydrolytic product 4-aminobenzaldehyde thiosemicarbazone [22].

Protein was determined by the method of Lowry *et al.* [23] using bovine serum albumin as the reference standard. The Student's *t*-test was employed for the analysis of the results.

Results and Discussion

The activities of hydrolases exemplified in Table 1 reveal a markedly selective augmentation of HCHS esterase activity following oral treatment with HCHS, HCAC and

Table 1. Effect of hydrocortisone, hydrocortisone acetate and hydrocortisone hemisuccinate on microsomal esterases/amidases and cytosolic thiacetazone esterase in rat liver

Enzyme	Enzyme activity (nmol product formed or substrate disappeared/min/mg protein)			
	Control	HC	HCAC	HCHS
ASA Esterase I	103 ± 6	92 ± 5	97 ± 8	106 ± 4
ASA Esterase II	79 ± 4	75 ± 4	72 ± 2	85 ± 3
Procaine esterase	3.9 ± 0.32	4.2 ± 0.46	3.5 ± 0.35	3.2 ± 0.31
NPA Esterase	1520 ± 68	1387 ± 105	1496 ± 96	1612 ± 88
Acetanilide *N*-deacetylase	16.4 ± 0.95	17.1 ± 1.2	16.6 ± 0.81	14.7 ± 0.39
AAF *N*-deacetylase	0.91 ± 0.04	0.82 ± 0.07	0.99 ± 0.06	0.94 ± 0.04
HCAC Esterase	350 ± 26	326 ± 14	368 ± 20	348 ± 13
HCHS Esterase I	13.2 ± 0.81	56.7 ± 3.1*	51.4 ± 1.8*	63.5 ± 5.1*
HCHS Esterase II	8.4 ± 0.44	18.4 ± 0.97*	14.2 ± 1.10*	21.8 ± 0.93*
Butyrylcholine esterase	8.7 ± 0.39	9.2 ± 0.61	9.8 ± 0.71	7.3 ± 0.95
Thiacetazone esterase†	5.8 ± 0.42	5.1 ± 0.39	5.6 ± 0.43	5.5 ± 0.48

Values are mean ± SE obtained from 6–8 rats.

* $P < 0.001$.

† Thiacetazone esterase activity was measured in the cytoplasm whereas that of the remaining substrates was measured in the microsomes.

HC (≈10 mg/kg HC), respectively, to rat. Interestingly, the treatment was devoid of any effect on the activities of the enzymes/isozymes hydrolysing ASA, acetanilide, procaine, NPA, AAF and butyrylcholine. Cytosolic thiacetazone esterase also displayed no perceptible response to steroids.

HCHS esterase I exhibited 379.5, 329.5 and 289% increase with HCHS, HC and HCAC, respectively, whereas isozyme II of HCHS esterase displayed an induction of the order of 159.5, 119 and 69% which was approximately 200% less in magnitude as compared to that of HCHS esterase I.

Inasmuch as the rate of cleavage of the charged hemisuccinate ester is sluggish relative to the uncharged HCAC, it seems probable that the prolonged presence of HCHS in the system brings about a higher induction of HCHS esterase than is achieved with HCAC and HC. In contrast, HCAC esterase remained unperturbed, an observation that reflects not only on the highly specific nature of induction but also insinuates distinct genetic regulation of the three forms of steroid esterases. Such specificity of induction corroborates our earlier hypothesis that the hydrolysis of corticosteroid esters of charged and uncharged origin requires three discrete enzymes active at different pH values and discernible by their differential response to inducers and inhibitors [3]. The highly selective nature of induction was apparently responsible for a lack of detectable increase in the total protein content of the microsomes.

In summary, we observed a remarkable augmentation in the rate of hydrolytic breakdown of HCHS following exposure to corticosteroid therapy. This underscores the need for a careful reappraisal of its dosage in long term therapy. In such an event the uncharged ester may be the preferred drug of choice.

Acknowledgements—We thank Glaxo Laboratories (India) Ltd, Bombay, for their generous gift of hydrocortisone and its hemisuccinate and acetate esters and Dey's Medical Stores (Manufacturing) Ltd, Calcutta, for kindly supplying us with thiacetazone.

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