Biochemical Pharmacology, Vol. 43, No. 7, pp. 1473–1477, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

EFFECTS OF ACUTE CARBAMAZEPINE ADMINISTRATION ON HAEM METABOLISM IN RAT LIVER

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(*Received* 21 *November* 1991; *accepted* 9 *January* 1992)

Abstract—The effects of acute carbamazepine (CBZ) administration on haem metabolism in rat liver were examined in relation to the mechanism by which it exacerbates hepatic porphyrias. In a screening test for drug exacerbation of porphyria developed in this laboratory, CBZ at a very small dose (1.5 mg/kg, p.o.) behaved as an exacerbator, potentiating the loss of haem utilized by tryptophan pyrrolase (TP; tryptophan 2,3-dioxygenase; L-tryptophan-O_2 oxido-reductase, decyclizing; EC 1.13.11.11) and the associated induction of activity of the rate-limiting enzyme of haem biosynthesis, 5-aminolaevulinate synthase (5-ALA-S) caused by the experimental porphyrogen 3,5-diethoxycarbonyl-1,4-dihydrocollidine. A larger dose of CBZ (50 mg/kg, i.p.) induced 5-ALA-S activity by 40–100% at 3 hr. This induction was preceded by an increase in the haem saturation of TP, and was abolished when such an increase was prevented by allopurinol. 5-ALA-S induction by CBZ was not associated with decreased turnover of the enzyme, nor with any significant changes in concentration of the major hepatic haemoprotein, cytochrome P450. It is suggested that CBZ may exacerbate the hepatic porphyrias by inducing 5-ALA-S activity secondarily to an increased utilization of haem by TP.

Carbamazepine [CBZ†; 5-carbamyl-5*H*-dibenzo-(*b*,*f*)-azepine] is a first-line anti-convulsant [1] and a widely used psychotropic agent, particularly in affective disorders and alcoholism (for reviews, see Refs 2–4). CBZ has, however, been reported to induce some of the clinical and/or biochemical features of hepatic porphyria in both epileptic patients [5–7] and healthy volunteers [8] [abdominal pains, increased urinary excretion of porphyrins and their precursor porphobilinogen, inhibition of erythrocyte porphobilinogen deaminase and induction of the rate-limiting enzyme of haem biosynthesis, 5-aminolaevulinate synthase (5-ALA-S) in leucocytes], and its therapeutic use is now generally considered unsafe in patients with prophyria [9]. The mechanism(s) by which CBZ disturbs porphyrin metabolism and haem biosynthesis is, however, not clearly understood. Thus, although the drug induces 5-ALA-S (EC 2.3.1.37) in chick embryo liver cell monolayers in culture [10] and in human leucocytes and rat liver after chronic administration [7], its effects on other enzymes of the haem biosynthetic pathway appear to be species-dependent (inhibition of 5-ALA dehydratase in human leucocytes and rat liver after chronic administration, and of uroporphyrinogen decarboxylase and to a lesser extent porphobilinogen deaminase in cultured chick embryo liver cells). 5-ALA-S induction is considered the major biochemical event both in the production of experimental hepatic porphyria and during an acute attack in man. The present experiments were designed, therefore, to examine the effects of acute CBZ administration on 5-ALA-S activity in rat liver in relation to the mechanism by which it exacerbates the human disease.

MATERIALS AND METHODS

Animals and treatments. Locally bred male Wistar rats (150–170 g at the start of experiments) were maintained on cube diet 41B (Oxoid) and water under a natural light : dark cycle and at 22 ± 1°. Rats were starved for 24 hr before death (by stunning and cervical dislocation between 13:00 and 15:15 hr), but were allowed free access to drinking water throughout. CBZ was administered either p.o. (1.5 mg/kg) in arachis oil (10 mL/kg) or i.p. (50 mg/kg) in a mixture of dimethylformamide (DMF) and saline (1 : 3, v/v) (2 mL/kg) and control rats received an equal volume of the appropriate vehicle. No behavioural effects were observed after administration of either dose of CBZ. All other compounds were administered i.p. in the following doses: 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC; 50 mg/kg), cycloheximide (10 mg/kg), allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) (20 mg/kg). The first of these three compounds was dissolved in DMF (1 mL/kg), whereas the other two were dissolved in saline (2 mL/kg each) and control rats received an equal volume of the appropriate vehicle. The allopurinol solution for injection was prepared as described previously [11].

Chemicals. Allopurinol was a gift from the Wellcome Foundation, whereas DDC was purchased from Kodak Ltd and was recrystallized from ethanol before use. All other chemicals were purchased from the Sigma Chemical Co. or BDH Chemicals (both

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† Abbreviations: 5-ALA-S, 5-aminolaevulinate synthase; CBZ, carbamazepine,5-carbamyl-5*H*-dibenzo(*b*,*f*)-azepine; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; DMF, dimethylformamide; TP, tryptophan pyrrolase, L-tryptophan-O_2 oxido-reductase.

Table 1. Effects of DDC, CBZ or both on rat liver 5-ALA-S and TP activities, and cytochrome P450 concentration

Parameter	Control	DDC	CBZ	DDC + CBZ
5-ALA-S	0.49 ± 0.03	1.00 ± 0.14‡	0.69 ± 0.11	1.78 ± 0.27‖
TP				
Holoenzyme	4.1 ± 0.38	3.3 ± 0.27	4.1 ± 0.11	2.5 ± 0.07§
Total enzyme	7.9 ± 0.53	9.7 ± 1.55	7.9 ± 0.18	9.6 ± 0.38*
Haem saturation ratio	1.08 ± 0.06	0.52 ± 0.06¶	1.08 ± 0.07	0.35 ± 0.02¶
Cytochrome P450	56.1 ± 3.0	53.0 ± 2.1	64.3 ± 3.6	66.2 ± 1.2†

Rats were starved for 24 hr and received, 4 hr before death, an i.p. injection of either DDC (50 mg/kg) or an equal volume (1 mL/kg) of the vehicle DMF. The animals also received, 15 min before the above injections, an oral intubation of either CBZ (1.5 mg/kg) or an equal volume (10 mL/kg) of the vehicle arachis oil. Liver 5-ALA-S activity is expressed in nmol of 5-ALA formed/min per g wet wt, whereas that of TP is in μmol of kynurenine formed/hr per g wet wt. Cytochrome P450 concentration is in nmol/g wet wt. Values are means ± SEM for each group of four rats. The results in columns 2, 3 and 4 have been compared with the control data in column 1, and the significance of the differences is indicated as follows: * $P < 0.05$; † $P < 0.025$; ‡ $P < 0.02$; § $P < 0.01$; ‖ $P < 0.005$; ¶ $P < 0.001$.

Poole, U.K.) and were of the purest commercially available grades.

Chemical, enzymic and other determinations. All enzymes were assayed in fresh liver homogenates. 5-ALA-S activity was measured by colorimetric determination [12] of the enzymatically produced [13] 5-aminolaevulinate as detailed previously [14]. Cytochrome P450 concentration was determined in whole homogenates by the method of McLean and Day [15]. Tryptophan pyrrolase (TP) activity was determined either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μM) haematin [16–18]. The activity of the apoenzyme (the haem-free predominant form of the enzyme in rat and human liver) was obtained by difference. The saturation of TP with its cofactor haem was expressed either as the haem saturation ratio (holoenzyme activity/apoenzyme activity) or as the percentage haem saturation (100 × holoenzyme activity/total enzyme activity). Statistical analysis of results was by Student's *t*-test.

RESULTS

Behaviour of CBZ in a laboratory screening test for drug exacerbation of the hepatic porphyrias

This screening test was developed in this laboratory [17] and shows that exacerbators of hepatic porphyrias potentiate the loss of the haem utilized by liver TP in starved rats caused by a sub-maximally acting dose of the porphyrogen DDC, whereas non-exacerbators do not. A similar screening test exists [19] which shows that exacerbators also potentiate the induction of 5-ALA-S activity by DDC. CBZ was among the exacerbators examined in this latter test [19]. As the results in Table 1 of the present work show, the loss of TP haem induced at 4 hr after administration of a 50 mg/kg dose of DDC, as illustrated by the 20% decrease in holo-(TP) activity and the 52% decrease in the haem saturation ratio of the enzyme, was potentiated further by co-administration of a small dose (1.5 mg/kg) of CBZ. CBZ alone exerted no significant effects. The results in Table 1 show also that the 104% increase in 5-ALA-S activity induced by the above porphyrogen alone was further potentiated (to 263%) by joint CBZ administration. For comparative purposes, cytochrome P450 levels were examined under these conditions and, as the data in Table 1 show, no loss of this major hepatic haemoprotein was observed after administration of CBZ, DDC or both.

Time-course of the effects of acute CBZ administration on rat liver 5-ALA-S TP activities, and cytochrome P450 concentration

These effects are shown in Fig. 1. The first significant increase in 5-ALA-S activity (39%) occurred at 1.5 hr after i.p. administration of CBZ (50 mg/kg). 5-ALA-S activity remained so elevated for 1.5 hr more, before starting to decline. It finally reached control (zero-time) values at 6–7 hr. As will be seen later in this section, the extent of this induction varied between experiments and could be as high as 100%. By contrast, CBZ increased significantly the holo-(TP) activity, by 24%, as early as 30 min after administration (Fig. 1). Enhancement of this form of the pyrrolase continued thereafter, reaching 114% at 4 hr before a final return to near-normal values at 7 hr. CBZ exerted no significant effect on the total TP activity. As a result, the haem saturation of the enzyme was increased by CBZ in a manner that paralleled the enhancement of the holoenzyme activity (a 26% increase at 0.5 hr and a 100% increase at 4 hr). The results in Fig. 1 also show that CBZ exerted no significant effect on cytochrome P450 concentration at any of the time intervals examined.

Prevention of the enhancement of rat liver 5-ALA-S activity after CBZ administration by blockade of the increase in the haem saturation of TP by allopurinol

The effects of pretreatment of rats with the TP inhibitor allopurinol on the CBZ-induced changes in TP and 5-ALA-S activities are shown in Table 2. Allopurinol prevented the CBZ-induced increases in the holo-(TP) activity and the haem saturation of

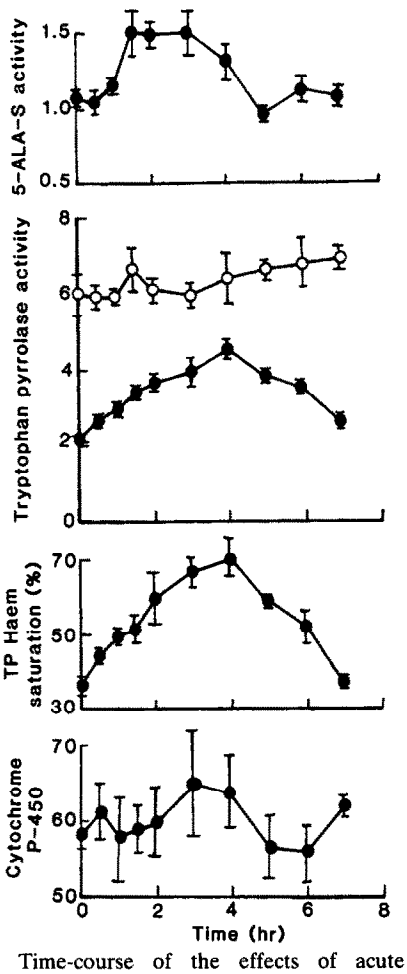

Fig. 1. Time-course of the effects of acute CBZ administration on rat liver 5-ALA-S and TP activities, and cytochrome P450 concentration. Rats were starved for 24 hr and received an i.p. injection of CBZ (50 mg/kg) at various times, before death. The parameters shown are expressed as in Tables 1 and 2. Activity of TP was measured as both holoenzyme (●) and total enzyme (○). Values are means ± SEM (bars) for each group of four rats.

the enzyme (expressed as the percentage haem saturation), and the associated enhancement of 5-ALA-S activity. Allopurinol alone exerted small, but significant, decreases in activities of these two enzymes. By contrast, there were no significant changes in cytochrome P450 concentration after CBZ, allopurinol or both.

Lack of effect of CBZ on the rate of degradation of rat liver 5-ALA-S

The half-life of 5-ALA-S was determined by monitoring the decline in enzyme activity (at 0, 1 and 2 hr) following treatment of rats with the protein synthesis inhibitor cycloheximide, which was itself administered at 3 hr after CBZ or saline. The results in Table 3 show that the half-life of the enzyme in saline-pretreated control rats was 1.31 hr and CBZ caused very little change (1.39 hr).

DISCUSSION

CBZ is considered unsafe for patients with prophyria [9] and its behaviour in two laboratory-screening tests for drug exacerbation of the hepatic porphyrias (see Refs 17 and 19 and Table 1) is typical of exacerbators of the human disease. However, the mechanism(s) by which CBZ and other exacerbators potentiate the DDC-induced enhancement of 5-ALA-S activity and the associated loss of the haem utilized by TP remains to be elucidated, though a number of possible explanations have been proposed [20–22].

As well as exacerbating porphyrias [9], CBZ induces disturbances in porphyrin metabolism in non-porphyric patients and control subjects [5–8]. CBZ influences the activities of a number of enzymes of the haem biosynthetic pathway in both mammalian and avian systems [7, 10], notably that of the rate-limiting enzyme, 5-ALA-S. Induction of this enzyme is a major biochemical event in the production of porphyria and it is now well established that this enzyme is the site of feedback regulation of haem biosynthesis exerted by the end product haem (for references, see Ref. 17). The likely molecular

Table 2. Effects of pretreatment of rats with allopurinol on the CBZ-induced changes in liver 5-ALA-S and TP activities, and cytochrome P450 concentration

Parameter	Control	CBZ	Allopurinol	Allopurinol + CBZ
5-ALA-S	1.05 ± 0.12	1.71 ± 0.19*	0.82 ± 0.05	0.82 ± 0.12
TP				
Holoenzyme	2.2 ± 0.07	7.3 ± 0.31§	1.6 ± 0.14‡	2.3 ± 0.17
Total enzyme	5.8 ± 0.76	9.9 ± 0.85†	3.7 ± 0.17*	4.5 ± 0.16
Haem saturation (%)	38 ± 4	74 ± 4§	43 ± 3	51 ± 5
Cytochrome P450	55.5 ± 1.8	55.5 ± 3.1	57.4 ± 5.5	57.4 ± 1.6

Rats were starved for 24 hr and received, 3 hr before death, an i.p. injection of either CBZ (50 mg/kg) or an equal volume (2 mL/kg) of the DMF:saline vehicle. The animals also received, at 0.5 hr before the above treatments, a similar injection of either allopurinol (20 mg/kg) or an equal volume (2 mL/kg) of saline. Expressions and other details are as described in Materials and Methods and the legend to Table 1, except that the haem saturation of TP is expressed here as a percentage (100 × holoenzyme/total enzyme). Values are means ± SEM for each group of four rats. The values in columns 2, 3 and 4 have been compared with those in column 1, and the significance of the differences is indicated as follows: * $P < 0.05$; † $P < 0.02$; ‡ $P < 0.01$; § $P < 0.001$.

Table 3. Effects of CBZ on the rate of degradation of rat liver 5-ALA-S

Pretreatment	5-ALA-S activity (nmol of 5-ALA formed/min per g wet wt of liver) (Hr after cycloheximide) 0	1	2
Saline	0.58 ± 0.03	0.34 ± 0.02	0.17 ± 0.01
CBZ	1.17 ± 0.11	0.49 ± 0.03	0.27 ± 0.04

Rats were starved for 24 hr before being killed. The animals received an i.p. injection of either CBZ (50 mg/kg) or an equal volume (2 mL/kg) of the DMF:saline vehicle. Three hours later, the animals then received a similar injection of cycloheximide (10 mg/kg) and were killed at 0, 1 and 2 hr after this protein synthesis inhibitor. 5-ALA-S activity was then determined. Values are means ± SEM for each group of four rats. The half-life of the enzyme was calculated after plotting the decline in activity on a semi-logarithmic paper as a function of time after cycloheximide administration, and was found to be 1.31 hr and 1.39 hr for saline- and CBZ-pretreated rats, respectively.

mechanisms involved have been discussed recently (see Ref. 23 and references cited therein). Two mechanisms have been suggested to explain the induction of 5-ALA-S by CBZ. In cultured chick embryo liver cells, induction is thought [10] to be caused by derepression after inhibition of haem biosynthesis secondarily to a decrease in uroporphyrinogen decarboxylase activity, whereas in man and rat, it was suggested [7] that 5-ALA-S induction is due to an increased demand for haem necessitated by the increased formation of cytochrome P450. However, in the experiments in rats [7], it was shown that 5-ALA-S induction by chronic CBZ administration precedes, rather than follows, the increase in cytochrome P450 concentration, thus arguing against this latter suggestion. Further evidence against this suggestion is provided by the results in Fig. 1 and Tables 1 and 2 of the present work, which show that there were no significant changes in cytochrome P450 concentration under a variety of conditions associated with enhancement of 5-ALA-S activity by acute CBZ administration. These results therefore exclude the possibility that enhancement of 5-ALA-S activity by CBZ is due to an increased demand for haem necessitated by increased production of the major hepatic haemoprotein cytochrome P450.

Enhancement of 5-ALA-S activity by CBZ could be due to an increased synthesis, a decreased degradation or both. Increased synthesis is more likely in view of the results in Table 3, which show that the drug does not influence the rate of degradation of the enzyme.

As the results in Fig. 1 show, the CBZ-induced enhancement of 5-ALA-S activity was preceded by an increase in the haem saturation of TP. This minor cytosolic haemoprotein is a sensitive, if indirect, marker of small changes in the hepatic concentration of the free or "readily exchangeable" pool of haem thought to regulate the synthesis of 5-ALA-S by the above-mentioned feedback-control mechanism (for review, see Ref. 24). That the above increase in the haem saturation of TP may be involved in the CBZ induction of 5-ALA-S activity is suggested by the finding (Table 2) that prevention of the former effect by allopurinol prevents the increase in 5-ALA-S activity. Allopurinol is a TP inhibitor that acts by preventing the conjugation of the apoenzyme with its cofactor haem [11] and, under the conditions of the experiments in Table 2, prevention of the CBZ induction of 5-ALA-S activity is best explained by allopurinol causing repression of synthesis of this latter enzyme via the haem made available after prevention of its utilization by TP. Therefore, it may be suggested that the enhancement of 5-ALA-S activity by CBZ is secondary to increased haem utilization by pre-existing apo-(TP). The mechanism(s) by which CBZ increases this utilization, however, requires investigation.

In conclusion, it is not unreasonable to suggest that acute CBZ administration may enhance rat liver 5-ALA-S activity by a derepression mechanism involving increased utilization of haem by TP, and that these effects could explain its exacerbation of the hepatic porphyrias.

Acknowledgement—We thank the Wellcome Foundation for a generous gift of allopurinol.

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