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# Stimulation of the Pathway of Porphyrin Synthesis in the Liver of Rats and Mice by Griseofulvin, 3,5-Diethoxycarbonyl-1,4-dihydrocollidine and Related Drugs: Evidence for Two Basically Different Mechanisms

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Griseofulvin and isogriseofulvin cause, like 3,5-diethoxycarbonyl-1,4-dihydrocollidine, a fall in the activity of the hepatic enzyme porphyrin–metal chelatase and accumulation of protoporphyrin in the liver. Analogues of either griseofulvin or 3,5-diethoxycarbonyl-1,4-dihydrocollidine which do not decrease the chelatase activity are not porphyrogenic on their own, but can potentiate the porphyria caused by 3,5-diethoxycarbonyl-1,4-dihydrocollidine. This suggests the existence of two basically different mechanisms by which drugs stimulate the pathway of porphyrin synthesis in the liver.

Drugs such as 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin cause a marked stimulation of 5-aminolaevulinate synthetase and accumulation of protoporphyrin in the liver of rats and mice (reviewed by Tschudy & Bonkowsky, 1972). Recent work (De Matteis & Gibbs, 1972; De Matteis *et al.*, 1973) has shown that 3,5-diethoxycarbonyl-1,4-dihydrocollidine causes a fall in the activity of the liver porphyrin–metal chelatase (the mitochondrial enzyme which converts protoporphyrin into haem) before any increase in the activity of 5-aminolaevulinate synthetase and in liver porphyrin concentration may be demonstrated. An inhibition of liver haem synthesis might be expected to lead, not only to the large accumulation of protoporphyrin which is seen in this type of porphyria (Onisawa & Labbe, 1963; Lockhead *et al.*, 1967), but also to a decrease in the concentration of haem available for negative feedback control of 5-aminolaevulinate synthetase, and might therefore be implicated in the marked stimulation of this latter enzyme.

The purpose of this communication is to record observations obtained with griseofulvin (7-chloro-2′,-4,6-trimethoxy-6′-methylgris-2′-ene-3,4′-dione) and with drugs chemically related to either griseofulvin or to 3,5-diethoxycarbonyl-1,4-dihydrocollidine which strongly support this interpretation. Griseofulvin causes, like 3,5-diethoxycarbonyl-1,4-dihydrocollidine, a fall in the activity of the mitochondrial chelatase and accumulation of protoporphyrin in the liver; it also markedly stimulates 5-aminolaevulinate synthetase. Analogues of either griseofulvin or 3,5-diethoxycarbonyl-1,4-dihydrocollidine which do not decrease the chelatase stimulate the synthetase only slightly; they are not porphyrogenic on their own, but can, when given together with 3,5-diethoxycarbonyl-1,4-dihydrocollidine, potentiate the porphyria caused by this latter drug.

## *Materials and methods*

Strain, body weight and age of male rats and mice were as described by De Matteis *et al.* (1973). The methods for assay of enzymes and determination of porphobilinogen and total porphyrins in liver homogenates and protein in mitochondrial fraction have also been given (De Matteis & Gibbs, 1972; De Matteis *et al.*, 1973). Griseofulvin and its analogues were incorporated in powdered diet 41B (Bruce & Parkes, 1956) at a concentration of 1% (w/w). Food consumption was measured and found to be similar in control and treated groups. Heparinized blood from rats and mice (at least three mice for each blood sample) was assayed for griseofulvin content by the method of Bedford *et al.* (1959).

3,5-Diethoxycarbonylcollidine was obtained by oxidation of 3,5-diethoxycarbonyl-1,4-dihydrocollidine as described by Singer & McElvain (1943) and purified by the method of Loev & Snader (1965) as modified by Racz & Marks (1972), except that the resulting oil was not distilled. The identity of the oxidation product was confirmed by i.r. and n.m.r. spectroscopy: its u.v. spectrum and extinction coefficient were in good agreement with those reported by Marks *et al.* (1965).

In some experiments rats were starved for 24h, then dosed orally or by intraperitoneal injection with drugs (suspended or dissolved in arachis oil) and killed 4, 15 or 24h later.

## *Results and discussion*

Analogues of both griseofulvin and 3,5-diethoxycarbonyl-1,4-dihydrocollidine have been described which are not porphyrogenic (De Matteis, 1966; Marks *et al.*, 1965). Evidence is presented below which suggests that these inactive analogues do not cause porphyria because they do not inhibit the chelatase

Table 1. *Effect of the administration of griseofulvin, isogriseofulvin or the 2′-β-hydroxyethyl thioether analogue of griseofulvin to either mice or rats on the 5-aminolaevulinate synthetase activity and total porphyrin content of the liver homogenate and on the chelatase activity of the isolated mitochondria*

Male rats and mice were given free access to powdered diet 41B for 3 days, then to diets containing, where appropriate, one of the drugs at a concentration of 1%. Results given refer to means ± S.E.M. of the numbers of observations in parentheses. With mice, each observation was obtained on the pooled livers of at least three animals. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, when compared with corresponding control values; † $P<0.001$, when compared with corresponding values obtained in mice given griseofulvin for 3 days.

| Species | Treatment | Length of treatment (days) | 5-Aminolaevulinate synthetase (nmol/min per g wet wt. of liver) | Total porphyrin (nmol/g wet wt. of liver) | Porphyrin–metal chelatase activity (nmol of $Co^{2+}$ mesoporphyrin formed/min per mg of mitochondrial protein) |
|---|---|---|---|---|---|
| Mouse | None (control) | — | 1.33 ± 0.16 (14) | <0.06 | 1.90 ± 0.05 (14) |
| | Griseofulvin | 0.7 | 2.20 ± 0.18 (3)** | 0.12 ± 0.04 (3) | 1.64 ± 0.10 (3)* |
| | Griseofulvin | 3 | 8.87 ± 0.38 (6)*** | 70.1 ± 8.8 (6) | 0.45 ± 0.05 (6)*** |
| | Isogriseofulvin | 0.7 | 2.83 ± 0.33 (4)** | 3.11 ± 1.3 (4) | 0.96 ± 0.01 (4)*** |
| | 2′-β-Hydroxyethyl thioether analogue of griseofulvin | 3 | 2.86 ± 0.27 (3)**† | <0.06 | 2.17 ± 0.09 (3)† |
| Rat | None (control) | — | 0.77 ± 0.22 (8) | <0.6 | 0.89 ± 0.05 (8) |
| | Griseofulvin | 10 | 1.82 ± 0.18 (4)** | 0.16 ± 0.01 (4) | 0.60 ± 0.03 (4)** |

and not because they do not reach the liver, or do not persist as they are metabolized too rapidly.

After administration of griseofulvin and two of its analogues there was a good correlation between the accumulation of porphyrins in liver and the fall in activity of the mitochondrial chelatase (Table 1). In mice, isogriseofulvin (7-chloro-4,4′,6-trimethoxy-6′-methylgris-3′-ene-2′,3-dione) was more active than griseofulvin in both respects, whereas the 2′-β-hydroxyethyl thioether analogue of griseofulvin was inactive. In addition, griseofulvin decreased the chelatase activity much more effectively in the mouse, where it was very porphyrogenic, than in the rat, where it caused only a marginal accumulation of porphyrins. A stimulation of 5-aminolaevulinate synthetase was observed also in the absence of an effect on the chelatase, but when the chelatase was markedly decreased the stimulation of the synthetase was far greater (compare rats and mice given griseofulvin, or mice treated for 3 days with either griseofulvin or its 2′-β-hydroxyethyl thioether analogue).

The blood concentration of griseofulvin was measured in both rats and mice after 3 days of feeding on a griseofulvin-containing diet, i.e. at a time when there was a very marked porphyria in the mouse, but only a very slight elevation of liver porphyrins in the rat. Values obtained, expressed as μg of griseofulvin/ml of blood, were as follows (average ± S.E.M. of four observations): rats, 7.8 ± 1.2, mice, 3.5 ± 0.24, with $P$ for difference between the two species <0.02. Therefore the lack of response of the rat cannot be due to impaired absorption of the drug or to rapid metabolic disposal. The mouse apparently metabolizes griseofulvin at a faster rate than does the rat (Chang *et al.*, 1973) and this may account for the lower blood concentrations of the drug found in the mouse. If a metabolite of griseofulvin rather than the parent compound was involved in the effect on the chelatase, a higher rate of production of this active metabolite in the mouse (as compared with the rat) might explain the difference in response between the two species.

The accumulation of porphyrins in the liver and the stimulation of hepatic 5-aminolaevulinate synthetase correlates well with the fall in the chelatase activity also after administration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine to either rats or mice (De Matteis *et al.*, 1973). It is now found that the non-porphyrogenic 3,5-diethoxycarbonylcollidine, when given intraperitoneally to starved rats in doses ranging from 100 to 750 mg/kg 4h before they were killed, does not lower the chelatase activity and stimulates 5-aminolaevulinate synthetase only two-fold. Doses greater than 500 mg/kg were found to cause sedation and lowering of body temperature, indicating that the drug was absorbed effectively and was not inactivated or disposed of too quickly. Also, a dose of 400 mg/kg caused in both rats and mice an increase in liver size and cytochrome *P*-450 content, indicating that this non-porphyrogenic analogue, even though it failed to lower the chelatase activity or to increase liver porphyrins, was nevertheless capable of reaching the liver and of producing several effects on it. The same conclusion can be drawn for the non-porphyrogenic 2′-β-hydroxyethyl thioether analogue of griseofulvin, which also caused within

24h of feeding enlargement of the liver and increase in microsomal cytochrome *P*-450. The increase in cytochrome *P*-450 and haem caused by these non-porphyrogenic analogues is a property possessed by phenobarbitone and many other non-porphyrogenic lipid-soluble drugs, and contrasts with the early loss of liver haemoprotein and haem observed after dosing with porphyrogenic compounds (Wada *et al.*, 1968; Abbritti & De Matteis, 1971–72; De Matteis & Gibbs, 1972; Badawy & Evans, 1973).

When starved rats were given either the 2′-β-hydroxyethyl thioether analogue of griseofulvin (200mg/kg) or 3,5-diethoxycarbonylcollidine (400mg/kg) orally, and at the same time 3,5-diethoxycarbonyl-1,4-dihydrocollidine (100mg/kg) by intraperitoneal injection, the porphyric response caused by the latter drug was markedly enhanced. The amount of porphyrins found in the liver 15h after dosing was at least twice as great as after 3,5-diethoxycarbonyl-1,4-dihydrocollidine was given on its own, and porphobilinogen also became demonstrable in the liver in large amounts. Therefore the non-porphyrogenic analogues of 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin are capable of stimulating the production of intermediates of the pathway in the liver, but can only do this when the rat has been 'primed' with 3,5-diethoxycarbonyl-1,4-dihydrocollidine. This is similar to previous findings obtained with several lipid-soluble drugs, including phenobarbitone, which are not porphyrogenic on their own, but can all potentiate the stimulation of 5-aminolaevulinate synthetase and the porphyria caused by porphyrogenic drugs (De Matteis & Gibbs, 1972; De Matteis, 1973; Bock *et al.*, 1973; Padmanaban *et al.*, 1973).

The most likely interpretation for these findings is that two basically different mechanisms are involved in the stimulation of 5-aminolaevulinate synthetase by drugs (see also De Matteis, 1971, 1973; De Matteis & Gibbs, 1972): one (mechanism A) involves a non-specific 'lipid-soluble' effect of some kind, and the other (mechanism B) a more specific interference with the feedback control of haem. Mechanism A requires the drug to be lipid-soluble (De Matteis, 1971) and mechanism B the presence of specific chemical constitutions which endow certain drugs with the ability to promote loss of liver haem (either by inhibiting haem synthesis or by increasing its degradation). Marked stimulation of 5-aminolaevulinate synthetase is found when both mechanisms A and B operate at the same time. This is so after administration of a porphyrogenic drug which is lipid-soluble and also promotes loss of liver haem.

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Abbritti, G. & De Matteis, F. (1971–72) *Chem.-Biol. Interactions* **4**, 281–286
Badawy, A. A.-B. & Evans, M. (1973) *Biochem. J.* **136**, 885–892
Bedford, C., Child, K. J. & Tomich, E. G. (1959) *Nature (London)* **184**, 364–365
Bock, K. W., Weiner, R. & Fröhling, W. (1973) *Enzyme* **16**, 295–301
Bruce, N. L. R. & Parkes, A. S. (1956) *J. Anim. Tech. Ass.* **7**, 54
Chang, R. L., Symchowicz, S. & Lin, C. (1973) *Biochem. Pharmacol.* **22**, 1389–1392
De Matteis, F. (1966) *Biochem. J.* **98**, 23c–25c
De Matteis, F. (1971) *S. Afr. J. Lab. Clin. Med.* **17**, 126–133
De Matteis, F. (1973) *Enzyme* **16**, 266–275
De Matteis, F. & Gibbs, A. H. (1972) *Biochem. J.* **126**, 1149–1160
De Matteis, F., Abbritti, G. & Gibbs, A. H. (1973) *Biochem. J.* **134**, 717–727
Lockhead, A. C., Dagg, J. H. & Goldberg, A. (1967) *Brit. J. Dermatol.* **79**, 96–102
Loev, B. & Snader, K. M. (1965) *J. Org. Chem.* **30**, 1914–1916
Marks, G. S., Hunter, E. G., Terner, U. K. & Schneck, D. (1965) *Biochem. Pharmacol.* **14**, 1077–1084
Onisawa, J. & Labbe, R. F. (1963) *J. Biol. Chem.* **238**, 724–727
Padmanaban, G., Rao, M. R. S. & Malathi, K. (1973) *Biochem. J.* **134**, 847–857
Racz, W. J. & Marks, G. S. (1972) *Biochem. Pharmacol.* **21**, 143–151
Singer, A. & McElvain, S. M. (1943) *Org. Synth. Collect. Vol.* **2**, 214–216
Tschudy, D. P. & Bonkowsky, H. L. (1972) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **31**, 147–159
Wada, O., Yano, Y., Urata, G. & Nakao, K. (1968) *Biochem. Pharmacol.* **17**, 595–603