The Effects of Acetate, Metal Cations, Phenobarbitone, Porphyrins and Substrates of Glycine Acyltransferase on the Utilization of Haem by Rat Liver Apo-(Tryptophan Pyrrolase)

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(Received 17 November 1976)

1. The utilization of haem by rat liver apo-(tryptophan pyrrolase) under basal conditions and after enhancement of the enzyme activity by various mechanisms was studied under the influence of treatments affecting various aspects of liver haem metabolism. 2. These treatments were: benzoate and *p*-aminobenzoate as substrates of glycine acyltransferase, acetate as an inhibitor of 5-aminolaevulinate synthase activity, enhancement of 5-aminolaevulinate dehydratase by aluminium, destruction of haem and inhibition of ferrochelatase by porphyrins, increased haem utilization by phenobarbitone and enhancement of haem oxygenase activity by metal cations. 3. The results show that the haem saturation of the apoenzyme is sensitive to all these treatments. 4. The possible usefulness of tryptophan pyrrolase in studying the regulation of liver haem is suggested.

Rat liver tryptophan pyrrolase [L-tryptophan-oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11] is haem-dependent. The saturation of the apoenzyme with its haem activator was shown (Badawy & Evans, 1973) to be sensitive to treatments known to cause destruction, inhibition of synthesis (at the ferrochelatase step), increased utilization and enhanced synthesis (at and beyond the 5-aminolaevulinate synthase step) of liver haem. It was also shown (Badawy & Evans, 1975*b*) that the enzyme can be activated by the administration of haematin, and it was suggested that the pyrrolase activity is regulated by a cofactor-type mechanism.

To gain further insight into this mechanism, experiments were performed to test the saturation of the apoenzyme with haem, under basal conditions and after enhancement of the activity by various mechanisms, after treatments affecting several steps of the haem-biosynthetic and degradative pathways. The present paper describes and discusses the results of these experiments. It is suggested that, because of its sensitivity to these treatments and its advantages over other haem proteins, tryptophan pyrrolase may be useful in studying the regulation of haem metabolism in the liver.

Materials and Methods

Chemicals

2-Allyl-2-isopropylacetamide was a gift from Roche Products Ltd., Welwyn Garden City, Herts., U.K. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine (from

Kodak Ltd., Kirkby, Liverpool, U.K.) was recrystallized from ethanol. *p*-Aminobenzoic acid, 5-aminolaevulinate hydrochloride, cortisol 21-acetate, griseofulvin {7-chloro-2',4,6-trimethoxy-6'- β -methylspiro-[benzofuran-2(3H),1'[2]cyclohexene]-3,4'-dione} and L-tryptophan were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. The analogues of the three above-mentioned porphyrins, 2-propyl-2-isopropylacetamide, 3,5-diethoxycarbonylcollidine and the 2'- β -hydroxyethyl thioether analogue of griseofulvin were gifts from Dr. F. De Matteis, MRC Toxicology Unit, Carshalton, Surrey, U.K. Ethanol (99.7%, v/v) and all other chemicals (from BDH Chemicals Ltd., Poole, Dorset, U.K.) were of AnalaR grade, except for dimethylformamide, FeCl₂, haemin (haematin hydrochloride), LiCl, HgCl₂, phenobarbitone sodium, K₂CrO₄, sodium benzoate and sodium salicylate, which were of reagent grade.

Animals and treatments

Male Wistar rats (150–200g) were allowed free access to cube diet 41B (Oxoid Ltd., Basingstoke, Hants., U.K.) and water. Some rats were starved for up to 72h before being killed. Phenobarbitone sodium was administered in drinking water (1 mg/ml) *ad libitum*, whereas *p*-aminobenzoic acid or sodium benzoate (1g/kg body wt. each) was given orally; control rats received equal volumes (10 ml/kg) of water by the same route. 2-Allyl-2-isopropylacetamide or its analogue, 2-propyl-2-isopropylacetamide (400 mg/kg each), was dissolved in dimethyl-

formamide and was injected into the loose subcutaneous tissues of the neck; each rat received 0.1 ml/100 g body wt. Solutions of metal cations in 0.9% (w/v) NaCl, in the doses shown in the Results section, were injected into the subcutaneous area of the abdomen; control rats received equal volumes of 0.9% NaCl. Most animals given metal cations were killed at 24 h after the injection. Additional rats received, 1 h before death, an intraperitoneal injection of $\text{Al}_2(\text{SO}_4)_3$ (150 mg/kg), lead acetate (2 mg/kg) or simultaneous injections of both salts; control rats were given 0.9% NaCl.

The rest of the chemicals were given intraperitoneally. Sodium acetate (1.9 g/kg) and sodium salicylate (400 mg/kg) were dissolved in 0.9% NaCl; each rat received respectively 0.4 or 0.2 ml/100 g body wt. Ethanol (5 ml/kg) was given as a 25% (v/v) solution in 0.9% NaCl; each rat received 2 ml/100 g body wt. 5-Aminolaevulinate hydrochloride was dissolved in 0.9% NaCl and was given in three or four doses (15 mg/kg each) at 1 h intervals and the animals were killed at 1 h after the last injection. Tryptophan (500 mg) was dissolved in the minimum amount of 4 M-NaOH and was diluted to 20 ml with 0.9% NaCl after the pH had been adjusted to 7.4 with 1 M-HCl; each rat received 2 ml/100 g body wt. Cortisol acetate (20 mg/kg), haematin hydrochloride (35 mg/kg), 3,5-diethoxycarbonyl-1,4-dihydrocollidine or 3,5-diethoxycarbonylcollidine (200 mg/kg each) and griseofulvin or its thioester analogue (150 mg/kg each) were dissolved in dimethylformamide; each rat received 0.1 ml/100 g body wt.

Determination of tryptophan pyrrolase activity

The activity of the enzyme was determined in liver

homogenates by measuring the formation of kynurenine from L-tryptophan (Feigelson & Greengard, 1961) in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin ($2 \mu\text{M}$) as detailed previously (Badawy & Evans, 1975b). The apoenzyme activity, obtained by difference, was then used to calculate the ratio of holoenzyme/apoenzyme activity. This ratio, which is referred to as the haem-saturation ratio, indicates the extent of the haem saturation of the apoenzyme (see also Badawy & Evans, 1973, 1975b). The haem-saturation ratio of the basal enzyme and the enzyme induced by cortisol (or agents acting by the hormonal-induction mechanism) is less than 1, whereas that observed after pyrrolase enhancement by the substrate- (tryptophan, salicylate or ethanol) or the cofactor-type mechanism (haematin or 5-aminolaevulinate) is increased to above 1. It is also shown in the Results section that some agents may increase the total pyrrolase activity but do not produce a relatively similar rise in that of the holoenzyme. These agents therefore cause a decrease in the haem-saturation ratio.

Results

Effects of substrates of glycine acyltransferase on rat liver tryptophan pyrrolase activity

The effects of two such substrates, benzoate and *p*-aminobenzoate, are shown in Table 1. At 4 h after administration to fed rats, both compounds significantly ($P < 0.005$) decreased the holoenzyme activity by 36–40%, but exerted no significant effects on that of the total enzyme ($P > 0.10$). The haem-saturation

Table 1. *Effects of benzoate and p-aminobenzoate on rat liver tryptophan pyrrolase activity after various treatments in vivo*
Fed rats received an oral intubation of water (10 ml/kg), sodium benzoate or *p*-aminobenzoic acid (1 g/kg body wt. each) at 4 h before being killed. At 1 h after each of the above pretreatments, the animals were given an injection of cortisol (20 mg/kg), tryptophan (500 mg/kg), salicylate (400 mg/kg), ethanol (5 ml/kg) or haematin (35 mg/kg) or three injections (15 mg/kg body wt. each) of 5-aminolaevulinate hydrochloride at 1 h intervals. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin ($2 \mu\text{M}$). The apoenzyme activity, obtained by difference, was used to calculate the haem-saturation ratio (i.e. the ratio of holoenzyme/apoenzyme activity). Values are means $\pm$ S.E.M. for each group of four rats.

Pretreatment ...	Kynurenine formed ($\mu\text{mol/h}$ per g wet wt. of liver)					
	Water		Benzoate		<i>p</i> -Aminobenzoate	
	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
None	3.0 $\pm$ 0.2	6.6 $\pm$ 0.6	1.8 $\pm$ 0.1	5.9 $\pm$ 0.4	1.9 $\pm$ 0.1	6.7 $\pm$ 0.4
Cortisol	9.1 $\pm$ 0.7	19.1 $\pm$ 1.9	5.4 $\pm$ 0.5	19.1 $\pm$ 1.1	4.8 $\pm$ 0.4	14.1 $\pm$ 1.2
Tryptophan	13.5 $\pm$ 0.7	16.5 $\pm$ 0.9	9.6 $\pm$ 0.6	18.6 $\pm$ 0.6	9.1 $\pm$ 0.4	17.5 $\pm$ 1.1
Salicylate	7.7 $\pm$ 0.8	11.7 $\pm$ 0.7	5.3 $\pm$ 0.3	12.0 $\pm$ 0.5	2.9 $\pm$ 0.1	10.2 $\pm$ 0.6
Ethanol	7.7 $\pm$ 0.6	10.5 $\pm$ 0.8	4.2 $\pm$ 0.2	11.4 $\pm$ 0.9	3.6 $\pm$ 0.3	10.6 $\pm$ 0.6
5-Aminolaevulinate	5.4 $\pm$ 0.6	6.0 $\pm$ 0.9	6.1 $\pm$ 0.2	7.4 $\pm$ 0.4	5.0 $\pm$ 0.4	6.0 $\pm$ 0.2
Haematin	6.6 $\pm$ 0.3	7.3 $\pm$ 0.6	7.5 $\pm$ 0.6	7.6 $\pm$ 0.5	7.2 $\pm$ 0.6	7.2 $\pm$ 0.6

ratio was also decreased from a control value (in the water-pretreated rats) of 0.83 to 0.40–0.49. In experiments not shown here, similar results were obtained at 4 or 24 h after the administration of benzoate or *p*-aminobenzoate to 24 h-starved rats, but not at 24 h after their injection into fed animals.

The results in Table 1 also show that the rise in the holoenzyme activity produced by the administration of cortisol, tryptophan, salicylate or ethanol was significantly ($P = 0.05$ – 0.001) inhibited by pretreatment of rats with benzoate or *p*-aminobenzoate. The haem-saturation ratio in these rats was also decreased by both compounds. The 5-aminolaevulinate- or haematin-induced rise in the holoenzyme activity or in the haem-saturation ratio was, however, unaltered by benzoate or *p*-aminobenzoate.

Effects of acetate on rat liver tryptophan pyrrolase activity

At 24 h after administration of sodium acetate to 24 h-starved rats, the holotryptophan pyrrolase activity was decreased by 31% ($P < 0.05$), whereas that of the total enzyme was not. The haem-saturation ratio was also decreased from 0.71 to 0.41 (Table 2). Pretreatment of rats with acetate also inhibited the rise in the holoenzyme activity (by 13–58%; $P = 0.02$ – 0.005) and decreased the haem-saturation ratio in rats treated with cortisol, tryptophan or ethanol, but has no effect in those given 5-aminolaevulinate or haematin.

Effects of aluminium and lead on rat liver tryptophan pyrrolase activity

The 1 h effects of the above cations are shown in Table 3. $Al_2(SO_4)_3$ doubled the holoenzyme activity ($P < 0.01$), whereas lead acetate exerted a slight inhibitory effect (17%; $P < 0.05$). Simultaneous

administration of the two salts led to a reversal of the aluminium-induced rise in the holoenzyme activity and in the haem-saturation ratio.

Effects of chemical porphyrins and their analogues on rat liver tryptophan pyrrolase activity

The 4 h effects of the above compounds in fed rats are shown in Table 4. The porphyrins 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin all increased the total pyrrolase activity, but caused a simultaneous decrease in the haem saturation of the newly formed apoenzyme. As a result, the haem-saturation ratio was decreased (from a control value of 0.63 in untreated rats) to 0.19–0.35. The analogues of the latter two porphyrins, on the other hand, increased both total enzyme and holoenzyme activities by relatively similar extents, whereas that of 2-allyl-2-isopropylacetamide was not very active in increasing the total enzyme activity, and failed to decrease the holoenzyme activity or the haem-saturation ratio.

The effects of pretreatment of rats with griseofulvin on the basal pyrrolase activity and on that increased by various treatments are shown in Table 5. The effects of griseofulvin on the basal activities and the haem-saturation ratio are as described in Table 4. Griseofulvin significantly decreased the elevated holoenzyme activity (by 46–68%; $P < 0.001$) in rats given cortisol, tryptophan, salicylate or ethanol, but did not alter the effects of 5-aminolaevulinate or haematin.

Effects of phenobarbitone on rat liver tryptophan pyrrolase activity

The above effects are shown in Table 6. The activity of the basal holoenzyme in fed (untreated, dimethylformamide- or 0.9% NaCl-treated) rats was signifi-

Table 2. *Effects of acetate on rat liver tryptophan pyrrolase activity*

Rats were given an intraperitoneal injection of either sodium acetate (1.9 g/kg) or an equal volume (4 ml/kg) of 0.9% NaCl and were starved for 24 h before being killed. At 20 h after the above pretreatments, some rats received a single injection of cortisol, tryptophan, ethanol or haematin or four injections of 5-aminolaevulinate at 1 h intervals. Doses of these compounds were as described in Table 1. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. Values are means $\pm$ S.E.M. for each group of four rats.

Pretreatment ...	Kynurenine formed (μ mol/h per g wet wt. of liver)			
	0.9% NaCl		Sodium acetate	
	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
None	3.5 $\pm$ 0.4	8.4 $\pm$ 0.6	2.4 $\pm$ 0.1	8.2 $\pm$ 0.6
Cortisol	6.5 $\pm$ 0.6	14.1 $\pm$ 0.7	2.7 $\pm$ 0.3	13.3 $\pm$ 1.7
Tryptophan	7.6 $\pm$ 0.2	11.2 $\pm$ 0.9	6.6 $\pm$ 0.2	10.7 $\pm$ 0.3
Ethanol	4.8 $\pm$ 0.5	7.6 $\pm$ 0.4	2.4 $\pm$ 0.1	8.1 $\pm$ 0.7
Haematin	4.0 $\pm$ 0.2	4.1 $\pm$ 0.2	5.0 $\pm$ 0.5	5.1 $\pm$ 0.5
5-Aminolaevulinate	4.8 $\pm$ 0.5	5.3 $\pm$ 0.6	4.5 $\pm$ 0.2	4.7 $\pm$ 0.2

Table 3. *Effects of $Al_2(SO_4)_3$ and lead acetate on rat liver tryptophan pyrrolase activity*

Fed rats received an intraperitoneal injection of 0.9% NaCl, $Al_2(SO_4)_3$ (150mg/kg) or lead acetate (2mg/kg) or two simultaneous injections of the latter two salts and were killed 1 h later. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. Values are means $\pm$ S.E.M. for each group of four rats.

Treatment	Kynurenine formed (μ mol/h per g wet wt. of liver)	
	Holoenzyme activity	Total enzyme activity
0.9% NaCl	1.2 $\pm$ 0.1	3.2 $\pm$ 0.3
$Al_2(SO_4)_3$	2.4 $\pm$ 0.3	3.0 $\pm$ 0.2
Lead acetate	1.0 $\pm$ 0.02	3.3 $\pm$ 0.1
$Al_2(SO_4)_3$ + lead acetate	1.4 $\pm$ 0.1	3.3 $\pm$ 0.1

cantly ($P=0.02-0.001$) increased by 73–100% at 24h after administration of the drug in drinking water. In experiments not shown here, it was found that phenobarbitone caused increases of 48 and 150% ($P<0.001$) in the holoenzyme activity respectively at 2 and 3 days after administration to starved rats. The results in Table 6 also show that the rise in the holoenzyme activity produced by cortisol, tryptophan or 5-aminolaevulinate, but not that caused by haematin, is further enhanced by phenobarbitone, by 29–41% ($P=0.01-0.005$).

Effects of metal cations on rat liver tryptophan pyrrolase activity

The effects of a single injection of various metal cations on the pyrrolase activities are shown in Table 7. In 24h-starved rats, the holoenzyme activity was significantly ($P=0.05-0.001$) decreased by 15–50% by most cations, as compared with the value in

Table 4. *Effects of chemical porphyrins and their analogues on rat liver tryptophan pyrrolase activity*

Fed rats received subcutaneous injections of 2-allyl-2-isopropylacetamide or 2-propyl-2-isopropylacetamide (400mg/kg each) or intraperitoneal injections of 3,5-diethoxycarbonyl-1,4-dihydrocollidine, 3,5-diethoxycarbonylcollidine (200mg/kg each) or griseofulvin or its thioether analogue (150mg/kg each) 4h before being killed. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. Values are means $\pm$ S.E.M. for each group of four rats.

Treatment	Kynurenine formed (μ mol/h per g wet wt. of liver)		Haem-saturation ratio
	Holoenzyme activity	Total enzyme activity	
None	1.5 $\pm$ 0.1	3.9 $\pm$ 0.3	0.63
2-Allyl-2-isopropylacetamide	1.8 $\pm$ 0.1	10.2 $\pm$ 0.9	0.21
2-Propyl-2-isopropylacetamide	2.0 $\pm$ 0.1	4.4 $\pm$ 0.1	0.83
3,5-Diethoxycarbonyl-1,4-dihydrocollidine	2.6 $\pm$ 0.2	10.1 $\pm$ 0.4	0.35
3,5-Diethoxycarbonylcollidine	5.1 $\pm$ 0.1	10.5 $\pm$ 0.7	0.94
Griseofulvin	2.0 $\pm$ 0.2	12.4 $\pm$ 0.6	0.19
Griseofulvin thioether analogue	5.2 $\pm$ 0.1	10.7 $\pm$ 0.8	0.95

Table 5. *Effects of griseofulvin on the enhancement of rat liver tryptophan pyrrolase activity by various treatments in vivo*

Fed rats were given an intraperitoneal injection of either griseofulvin (150mg/kg) or an equal volume (1ml/kg) of dimethylformamide 4h before being killed. At 1h after these two pretreatments, the animals received an injection of cortisol, tryptophan, salicylate, ethanol or haematin or three injections of 5-aminolaevulinate at 1h intervals. Doses of these compounds were as described in Table 1. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. Values are means $\pm$ S.E.M. for each group of four rats. The corresponding activities in untreated rats (expressed as below) were 1.5 $\pm$ 0.1 and 3.9 $\pm$ 0.3, thus having a haem-saturation ratio of 0.63.

Pretreatment	Kynurenine formed (μ mol/h per g wet wt. of liver)			
	Dimethylformamide		Griseofulvin	
	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
None	1.4 $\pm$ 0.1	3.6 $\pm$ 0.1	2.0 $\pm$ 0.2	12.4 $\pm$ 0.6
Cortisol	4.6 $\pm$ 0.1	9.9 $\pm$ 0.9	2.5 $\pm$ 0.1	8.1 $\pm$ 0.8
Tryptophan	10.4 $\pm$ 0.5	13.2 $\pm$ 1.2	3.5 $\pm$ 0.2	13.6 $\pm$ 1.0
Salicylate	4.8 $\pm$ 0.3	6.3 $\pm$ 0.5	1.9 $\pm$ 0.1	6.0 $\pm$ 0.4
Ethanol	5.6 $\pm$ 0.4	8.1 $\pm$ 0.3	1.8 $\pm$ 0.1	10.8 $\pm$ 0.3
Haematin	4.3 $\pm$ 0.4	4.4 $\pm$ 0.3	5.9 $\pm$ 0.2	6.4 $\pm$ 0.2
5-Aminolaevulinate	3.6 $\pm$ 0.1	5.7 $\pm$ 0.2	3.6 $\pm$ 0.4	5.3 $\pm$ 0.3

Table 6. *Effects of phenobarbitone on the enhancement of rat liver tryptophan pyrrolase activity by various treatments in vivo*

Fed rats were allowed free access to a solution (1 mg/ml) of phenobarbitone in drinking water for 24 h before being killed. At 4 h before death, the animals were given injections of 0.9% NaCl, dimethylformamide (1 ml/kg), cortisol, tryptophan or haematin or four injections of 5-aminolaevulinate at 1 h intervals. Doses of these compounds were as described in Table 1. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. Values are means $\pm$ S.E.M. for each group of four rats.

Treatment	Kynurenine formed (μ mol/h per g wet wt. of liver)			
	Untreated rats		Phenobarbitone-treated rats	
	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
None	1.2 $\pm$ 0.1	2.8 $\pm$ 0.1	2.3 $\pm$ 0.1	3.0 $\pm$ 0.1
0.9% NaCl	1.4 $\pm$ 0.1	4.6 $\pm$ 0.4	2.8 $\pm$ 0.3	4.9 $\pm$ 0.4
Dimethylformamide	1.5 $\pm$ 0.1	4.5 $\pm$ 0.3	2.6 $\pm$ 0.3	3.9 $\pm$ 0.3
Cortisol	4.6 $\pm$ 0.3	10.4 $\pm$ 0.8	6.5 $\pm$ 0.4	9.8 $\pm$ 0.7
Tryptophan	7.2 $\pm$ 0.5	11.4 $\pm$ 0.5	9.3 $\pm$ 0.2	10.7 $\pm$ 0.6
Haematin	6.4 $\pm$ 0.7	7.1 $\pm$ 0.5	6.9 $\pm$ 0.8	6.9 $\pm$ 0.8
5-Aminolaevulinate	4.3 $\pm$ 0.3	6.3 $\pm$ 0.5	6.0 $\pm$ 0.1	6.2 $\pm$ 0.1

Table 7. *Effects of metal cations on rat liver tryptophan pyrrolase activity*

Rats received a single subcutaneous injection of 0.9% NaCl or of metal cations and were starved for 24 h before being killed. Doses of the metal salts were 25 μ mol/100 g body wt. except the following: CdCl₂ (2.5 μ mol/kg); CuCl₂ (12.5 μ mol/kg); zinc acetate (100 μ mol/kg). The metal salts were dissolved in 0.9% NaCl, except lead acetate, which was dissolved in water; it was given intraperitoneally. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. The apoenzyme activity, obtained by difference, was then used to calculate the haem-saturation ratio. Values are means $\pm$ S.E.M. for each group of four rats.

Treatment	Tryptophan pyrrolase activity (μ mol of kynurenine formed/h per g wet wt. of liver)		Haem- saturation ratio
	Holoenzyme activity	Total enzyme activity	
None	2.7 $\pm$ 0.2	6.2 $\pm$ 0.6	0.77
0.9% NaCl	2.0 $\pm$ 0.1	5.8 $\pm$ 0.2	0.53
FeCl ₂	1.4 $\pm$ 0.1	8.0 $\pm$ 0.4	0.21
NiCl ₂	1.3 $\pm$ 0.1	9.7 $\pm$ 0.4	0.15
FeCl ₃	1.6 $\pm$ 0.2	8.7 $\pm$ 0.5	0.22
CdCl ₂	1.6 $\pm$ 0.1	8.6 $\pm$ 0.5	0.23
MnCl ₂	1.0 $\pm$ 0.0	8.4 $\pm$ 0.6	0.14
CuCl ₂	1.4 $\pm$ 0.2	6.7 $\pm$ 0.2	0.26
LiCl	1.7 $\pm$ 0.2	8.0 $\pm$ 0.8	0.27
CoCl ₂	1.2 $\pm$ 0.1	6.9 $\pm$ 0.5	0.21
K ₂ CrO ₄	1.7 $\pm$ 0.1	7.2 $\pm$ 0.6	0.30
(CH ₃ CO ₂) ₂ Zn	1.8 $\pm$ 0.1	4.5 $\pm$ 0.2	0.67
(CH ₃ CO ₂) ₂ Hg	2.7 $\pm$ 0.3	15.3 $\pm$ 1.2	0.21
Water	2.1 $\pm$ 0.2	4.9 $\pm$ 0.5	0.75
(CH ₃ CO ₂) ₂ Pb	1.3 $\pm$ 0.1	6.4 $\pm$ 0.3	0.25

0.9%-NaCl-treated rats. Exceptions were the absence of effect by zinc acetate and LiCl, the weak inhibition by FeCl₃ and K₂CrO₄ and the enhancement (by 35%) by mercuric acetate. All the cations tested, except Zn²⁺, decreased the haem-saturation ratio, by decreasing the holoenzyme activity, by enhancing that of the total enzyme or by exerting both effects.

The effects of pretreatment of rats with CoCl₂ on the enhancement of the holoenzyme activity by various treatments are shown in Table 8. CoCl₂ caused a significant ($P = 0.02$ – 0.001) inhibition (32–67%) of the effects of these treatments and also decreased the haem-saturation ratio, except in rats treated with ethanol.

Discussion

As in the Results section, the Discussion has been divided into subsections, but first the mechanisms of enhancement of pyrrolase activity by the various agents used to increase the holoenzyme are summarized.

Cortisol enhances the synthesis of the apoenzyme, whereas tryptophan causes stabilization of pre-existing apo-(tryptophan pyrrolase) in the presence of the normal rate of synthesis (Schimke *et al.*, 1965; Knox, 1966; Schimke, 1969). Salicylate or ethanol acts by a substrate (tryptophan)-type mechanism by increasing the availability of circulating tryptophan to the liver, whereas haematin or 5-aminolaevulinate acts by a cofactor-type mechanism (Badawy & Smith, 1971, 1972; Badawy & Evans, 1975a,b, 1976). There is evidence that the substrate mechanism may also involve increased haem synthesis (Badawy & Evans, 1975b, and references therein).

Table 8. *Effects of CoCl₂ on the enhancement of rat liver tryptophan pyrrolase activity by various treatments in vivo*
Rats were given a subcutaneous injection of CoCl₂ (0.462 mmol/kg) or an equal volume of an equimolar dose of NaCl (27 mg/kg) and were starved for 24 h before being killed. At 4 h before death, the animals were given an injection of 0.9% NaCl, dimethylformamide, cortisol, tryptophan, salicylate, ethanol or haematin or four injections of 5-aminolaevulinic acid at 1 h intervals. Doses of these compounds were as described in Table 1. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. Values are means $\pm$ S.E.M. for each group of four rats.

Pretreatment ... Treatment	Kynurenine formed (μ mol/h per g wet wt. of liver)			
	NaCl		CoCl ₂	
	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
None	2.1 $\pm$ 0.1	5.1 $\pm$ 0.1	1.1 $\pm$ 0.1	5.2 $\pm$ 0.1
0.9% NaCl	2.2 $\pm$ 0.1	5.4 $\pm$ 0.2	1.5 $\pm$ 0.2	5.9 $\pm$ 0.5
Dimethylformamide	2.2 $\pm$ 0.2	5.4 $\pm$ 0.2	1.3 $\pm$ 0.1	6.9 $\pm$ 0.5
Cortisol	6.0 $\pm$ 0.3	12.3 $\pm$ 0.7	3.0 $\pm$ 0.1	10.2 $\pm$ 0.6
Tryptophan	10.1 $\pm$ 0.4	13.7 $\pm$ 0.8	3.9 $\pm$ 0.2	8.7 $\pm$ 0.6
Salicylate	6.6 $\pm$ 0.3	9.9 $\pm$ 1.0	2.2 $\pm$ 0.2	10.3 $\pm$ 0.9
Ethanol	5.0 $\pm$ 0.3	5.8 $\pm$ 0.3	1.7 $\pm$ 0.2	7.8 $\pm$ 0.1
Haematin	7.4 $\pm$ 0.9	7.7 $\pm$ 0.8	3.8 $\pm$ 0.4	7.5 $\pm$ 0.4
5-Aminolaevulinic acid	5.7 $\pm$ 0.1	6.6 $\pm$ 0.3	2.0 $\pm$ 0.3	4.3 $\pm$ 0.3

Substrates of glycine acyltransferase

Two such substrates, benzoate and *p*-amino-benzoate, have been used by Piper *et al.* (1973) to test the possibility that, by enhancing hippurate formation, they could divert glycine away from the 5-aminolaevulinic acid synthase step of the haem-biosynthetic pathway. The above authors have shown that the two compounds can reverse the experimental porphyria produced in rats by 3,5-diethoxycarbonyl-1,4-dihydrocollidine. The results in Table 1 also suggest that substrates of glycine acyltransferase can decrease the saturation of apo-(tryptophan pyrrolase) with its haem activator, probably by the above mechanism. Since the effects of these substrates occur at 4 h, it may be suggested that the pyrrolase can utilize a haem pool with a rapid turnover rate. Moreover, the absence of effect on the pyrrolase enhancement by 5-aminolaevulinic acid suggests that benzoate or *p*-aminobenzoate acts at the glycine acyltransferase step and not at any step in the haem-biosynthetic pathway beyond that of 5-aminolaevulinic acid synthase.

Acetate as an inhibitor of 5-aminolaevulinic acid synthase

Acetate has been shown (Piper & Tephly, 1974) to reverse the experimental porphyria produced in rats by 3,5-diethoxycarbonyl-1,4-dihydrocollidine. The mechanism of action of acetate, which is different from that of substrates of glycine acyltransferase, has been suggested by the above authors to involve the inhibition of 5-aminolaevulinic acid synthase activity, as is suggested by the results of experiments both *in vitro* and after administration. The above mechanism may also explain the inhibition by acetate (Table 2) of holo-(tryptophan pyrrolase) activity

and the decrease in the haem-saturation ratio both under basal conditions and after hormonal induction and substrate activation of the enzyme. The inability of acetate to prevent the pyrrolase activation by haematin or 5-aminolaevulinic acid (Table 2) suggests that it does not affect the degradation of exogenous or newly synthesized haem, nor does it inhibit steps in the haem-biosynthetic pathway beyond that catalysed by 5-aminolaevulinic acid synthase.

Enhancement of 5-aminolaevulinic acid dehydratase activity by Al³⁺ and its prevention by Pb²⁺

It has been shown (Meredith *et al.*, 1974) that the activity of the above enzyme in rat liver is enhanced by Al³⁺ and that Pb²⁺ is capable of both preventing this effect and inhibiting the basal enzyme activity. The Al³⁺-induced twofold increase in the holo-(tryptophan pyrrolase) activity and its prevention by Pb²⁺ (Table 3) are consistent with the effects of the above cations on the dehydratase. Although the latter enzyme is generally believed not to be rate-limiting for haem biosynthesis, the present results with Al³⁺ suggest that dehydratase activation can increase the saturation of endogenous apo-(tryptophan pyrrolase) with its haem activator under conditions presumably involving increased haem synthesis.

Inhibition of ferrochelatase and destruction of liver haem by porphyrins

It has been suggested (De Matteis, 1972) that the porphyrins 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin produce porphyria in animals by removing the negative-feedback mechanism by causing an initial decline in liver haem concentration. This latter effect

is achieved by either increased destruction of haem to green pigments or inhibition of haem synthesis at the ferrochelatase step. At 4h after administration of these three porphyrins, it was shown (Badawy & Evans, 1973) that the haem-saturation ratio of the pyrrolase is decreased. The results in Table 4 confirm this finding and also show that the non-porphyrinogenic analogues of the latter two porphyrins do not decrease the haem-saturation ratio. These findings therefore support the above suggestion (De Matteis, 1972), and are also consistent with the finding (De Matteis & Gibbs, 1975) that these analogues do not inhibit the ferrochelatase activity. The results in Table 5 also show that ferrochelatase inhibition by griseofulvin decreases the haem saturation of apo-(tryptophan pyrrolase) after hormonal induction and substrate activation. It is not clear, however, why griseofulvin is as effective as the other two porphyrins in decreasing the haem-saturation ratio, in view of the finding (De Matteis & Gibbs, 1975) that it is a weak inhibitor of rat liver chelatase activity. It is possible that this weak effect in the rat may be sufficient to inhibit the synthesis of a certain haem pool that is utilizable by apo-(tryptophan pyrrolase). The absence of effect by griseofulvin on the pyrrolase enhancement by 5-aminolaevulinate (Table 5) has previously (Badawy & Evans, 1973) been explained by griseofulvin inhibiting the synthesis, from exogenous 5-aminolaevulinate, of a certain haem pool(s) that is not utilizable for rapid saturation of the pyrrolase apoenzyme. These two anomalies require further investigation.

Enhancement of haem utilization by phenobarbitone

It was suggested (see De Matteis, 1972) that phenobarbitone increases the utilization of liver haem by cytochrome *P*-450. Increased utilization of haem by apo-(tryptophan pyrrolase) occurs both under basal conditions (Badawy & Evans, 1973) and after induction by cortisol and activation by tryptophan or 5-aminolaevulinate (Table 6). Although the mechanism by which phenobarbitone enhances haem utilization by liver haem proteins is not clearly understood (see Badawy & Evans, 1973), it is possible that it may be due to the ability of the drug moderately to enhance the activity of 5-aminolaevulinate synthase, probably by decreasing its rate of degradation (Satyanarayana Rao *et al.*, 1972). It is not, however, clear why phenobarbitone enhances the saturation of the pyrrolase with haem caused by 5-aminolaevulinate.

Enhancement of haem oxygenase activity by metal cations

CoCl_2 was shown to cause a decrease in the concentration of rat liver microsomal haem and its constituents cytochrome *P*-450 and cytochrome *b*₅ (Tephly *et al.*, 1973) and to decrease the synthesis of

catalase haem (Yasukochi *et al.*, 1974). The cobalt-induced degradation of liver haem, which is dissociable from changes in cytochrome *P*-450 (Maines & Kappas, 1975), involves the induction of haem oxygenase activity (Maines & Kappas, 1976). The complexity of the actions of Co^{2+} on haem metabolism is illustrated by the findings (De Matteis & Gibbs, 1976) that it causes an early inhibition of haem synthesis both at and beyond the 5-aminolaevulinate synthase step, and a late enhancement of haem oxygenase activity. The late (24h) effects of CoCl_2 on the saturation of apo-(tryptophan pyrrolase) with its haem activator (Table 8) are consistent with this metal cation decreasing the availability of haem by increasing its oxidation. The cobalt-induced inhibition of the pyrrolase activation by 5-aminolaevulinate or haematin suggests that both newly formed and exogenous haem undergo degradation in the presence of Co^{2+} .

Maines & Kappas (1976) have also reported that other metal cations enhance haem oxygenase activity. The results in Table 7 show that these cations, except Zn^{2+} , are also capable of decreasing the haem saturation of the pyrrolase apoenzyme. These results, however, do not provide evidence of a correlation between the effect on the pyrrolase and the extent by which these cations enhance haem oxidation or decrease haem concentration and/or cytochrome *P*-450.

Conclusions and comments

The present results and those previously reported (Badawy & Evans, 1973, 1975b) both establish an association between tryptophan pyrrolase and metabolism of porphyrin and haem, and suggest that the pyrrolase is regulated by its cofactor haem. The rapidity with which pyrrolase responds to changes in haem metabolism suggests that the enzyme utilizes a pool of free haem with a rapid turnover rate. The finding (Badawy & Smith, 1971) that very small amounts of haem are required to saturate the apoenzyme fully, together with the sensitivity of the pyrrolase to changes in haem metabolism, render it a suitable and sensitive marker for assessing liver haem concentration. Although other haem proteins such as cytochrome *P*-450 and catalase have been used in this context, the pyrrolase has the following additional advantages: (1) its short half-life; (2) its rapid response to various stressful and physiological stimuli; (3) the ease with which the activities of the holoenzyme and apoenzyme can be enhanced (or decreased) and measured both simultaneously and independently.

I am very grateful to the Wellcome Trustees for support, Dr. F. De Matteis for helpful discussions and a generous gift of analogues of porphyrins, Dr. M. Evans for his interest and encouragement, Mr. C. J. Morgan and

Miss Jill C. Davis for skilful assistance, Mr. A. Dacey for animal maintenance and Roche Products Ltd. for a generous gift of 2-allyl-2-isopropylacetamide.

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