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The Effects of Chemical Porphyrogens and Drugs on the Activity of Rat Liver Tryptophan Pyrrolase

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1. Drugs such as phenobarbitone and phenylbutazone, which increase the concentration of microsomal haem and cytochrome *P*-450, also increase the saturation of rat liver apo-(tryptophan pyrrolase) with its haem activator, as does the haem precursor 5-aminolaevulinate. 2. At 4h after the administration of the porphyrogens 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin, the total pyrrolase activity is increased whereas the haem saturation of the apoenzyme is decreased. This decreased saturation is prevented by pretreatment of the animals with the inhibitor of drug-metabolizing enzymes, SKF 525-A. 3. Pretreatment of rats with the above porphyrogens inhibits the rise in holo-(tryptophan pyrrolase) activity produced by subsequent administration of cortisol, tryptophan and 5-aminolaevulinate with two single exceptions, the possible reasons for which are discussed. 4. At 24h after the administration, in starved rats, of a single daily injection of the above porphyrogens for 1 or 2 days, the holoenzyme activity is significantly increased. 5. It is suggested that the saturation of rat liver apo-(tryptophan pyrrolase) with its haem activator can be modified by treatments known to cause destruction, inhibition of synthesis, increased utilization and enhanced synthesis of liver haem. The possible involvement of the latter phenomenon in the aetiology of mental disorders in some patients with porphyria is discussed.

The incidence of severe mental disturbances in some patients with porphyria (Hare, 1953) and during acute crises is not understood. It has been reported (Price, 1961) that patients with porphyria exhibit an increased urinary excretion of hepatic tryptophan metabolites of the kynurenine pathway. This pathway is governed by the first and rate-limiting haem-dependent enzyme tryptophan pyrrolase (L-tryptophan–O_2 oxidoreductase, EC 1.13.1.12) and the pattern of metabolite excretion in urine reported by Price (1961) is consistent with an enhanced tryptophan pyrrolase activity leading to a functional vitamin B_6 deficiency that manifests itself in the abnormal excretion of some metabolites involved in the reactions catalysed by pyridoxal phosphate-requiring enzymes along the pathway. The relation between porphyria and liver tryptophan metabolism is further suggested by the finding (Druyan & Kelly, 1972) that the administration of the haem and porphyrin precursor 5-aminolaevulinate increases the saturation, in rat liver, of endogenous apo-(tryptophan pyrrolase) with its haem activator.

An enhanced tryptophan pyrrolase activity may decrease the availability of circulating tryptophan for uptake by the brain and the consequent synthesis in this tissue of the putative neurotransmitter 5-hydroxytryptamine. We have suggested (Badawy & Evans, 1972, 1973) that the inverse relation between liver tryptophan pyrrolase activity and brain 5-hydroxytryptamine concentration, and the involvement of the latter in mood disorders (Curzon, 1969), may contribute to the incidence of mental disturbances in patients with porphyria. As a first step in studying this problem, we have examined the effects of chemical porphyrogens and drugs on the activity of rat liver tryptophan pyrrolase. The results presented in this paper provide evidence suggesting that the saturation of liver apo-(tryptophan pyrrolase) with its haem activator is influenced by conditions leading to destruction, inhibition of synthesis, increased utilization and enhanced synthesis of liver haem.

Materials and Methods

Chemicals

2-Allyl-2-isopropylacetamide was a gift from Roche Products Ltd., Welwyn Garden City, Herts., U.K. 5-Aminolaevulinate hydrochloride, cortisol 21-acetate, dimethylformamide, griseofulvin (7-chloro-2′,4,6-trimethoxy-6′-β-methylspiro[benzofuran-2(3H),1′[2]cyclohexene]-3,4′-dione), haemin (haematin hydrochloride) and L-tryptophan were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine (from Kodak Ltd., Kirkby, Liverpool, U.K.) was recrystallized from ethanol. Compound SKF 525-A (2-diethylaminoethyl 3,3-diphenylpropylacetate) was a gift from Smith, Kline and

French Ltd., Welwyn Garden City, Herts., U.K. Phenylbutazone (4-*n*-butyl-1,2-diphenyl-3,5-dioxopyrazolidine) was a gift from Geigy Pharmaceuticals, Macclesfield, Cheshire, U.K. All other chemicals (from BDH Chemicals Ltd., Poole, Dorset, U.K.) were of AnalaR grade except for phenobarbitone (sodium 5-ethyl-5-phenylbarbiturate), which was of reagent grade.

Animals and treatments

Male Wistar rats (150–200g), maintained on cube diet MRC no. 41B, were killed between 1200 and 1500h by stunning and cervical dislocation. Some rats were starved for periods of up to 48h before being killed. Most chemicals were given intraperitoneally at various times before death. 2-Allyl-2-isopropylacetamide (400mg/kg body wt., dissolved in dimethylformamide) was injected into the loose subcutaneous tissue of the neck; each rat received 0.1ml/100g body wt. Phenobarbitone sodium was given once intraperitoneally (100mg/kg in 0.9% NaCl) or freely in drinking water (1mg/ml). By the latter method of administration of phenobarbitone, each rat received an average of 100mg of the drug/24h per kg body wt. Cortisol acetate (20mg/kg), 3,5-diethoxycarbonyl-1,4-dihydrocollidine (150mg/kg) and griseofulvin (100mg/kg) were dissolved in dimethylformamide; each rat received 0.1ml/100g body wt. The corresponding control rats received an equal quantity of the solvent which gave results identical with another control group of rats that had received 0.9% (w/v) NaCl. 5-Aminolaevulinate hydrochloride (dissolved in 0.9% NaCl) was given in four hourly doses (15mg/kg body wt. each) and the animals were killed at 1h after the last injection. Compound SKF 525-A (45mg/kg, in 0.9% NaCl) was injected 45min before the administration of some compounds and the animals were killed 4h after the latter. Phenylbutazone (150mg) and tryptophan (200mg) were dissolved in the minimum amounts of 2M- and 1M-NaOH respectively and diluted to 10ml with 0.9% NaCl after the pH had been adjusted to 7.3 with 1M-HCl; each rat received 1ml/100g body wt. The corresponding control rats received an equal volume of 0.9% NaCl.

Preparation of homogenates

The liver was removed within 30s of the death of the animal and was homogenized for 1min at 1100rev./min in 7vol. of 140mM-KCl–2.5mM-NaOH at 0°C in a glass homogenizer with a loose-fitting Teflon pestle. The homogenates were used within 10min of preparation.

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*a*) either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added haematin. The apoenzyme activity (calculated by difference) was then used to measure the holoenzyme/apoenzyme ratio. Samples (15ml) of the homogenates were added to a solution containing 5ml of 0.03M-L-tryptophan, 15ml of 0.2M-sodium phosphate buffer, pH7.0, and 25ml of water at 0°C. Where necessary, haematin hydrochloride was dissolved in 0.1M-NaOH and 0.1ml was included in the overall mixture to give a concentration (2μM) that was optimum for enzyme activation. Samples (3ml) of the mixture were incubated with shaking at 37°C in stoppered 25ml conical flasks in an atmosphere of O_2 for appropriate time-intervals (15min each) up to 90min. The reaction was stopped by the addition of 2ml of 0.9M-trichloroacetic acid; the flasks and contents were shaken for a further 2min and then filtered on Whatman no. 1 filter paper. To a measured portion of the filtrate (2.5ml) was added 1.5ml of 0.6M-NaOH and the kynurenine present was determined by measuring the E_{365} with a Unicam SP.500 spectrophotometer and by using $\epsilon = 4540$ litre$\cdot$mol$^{-1}\cdot$cm^{-1}. Tryptophan pyrrolase activity was calculated from the increase in the E_{365} with time during the linear phase. The latter was preceded by a lag phase that persisted for 30–45min with the basal enzyme and the enzyme induced after the administration of cortisol and other substances that mainly increased the apoenzyme activity. The enzyme activated by the administration of tryptophan, 5-aminolaevulinate and other treatments that increased the haem saturation of the apoenzyme had the lag phase either abolished or shortened to 15min. For an extinction range (at 365nm) of 0.1–0.8, the line representing the linear phase exactly covered three to four points 15min apart.

Results

Effects of 5-aminolaevulinate, phenobarbitone and phenylbutazone on rat liver tryptophan pyrrolase activity

Repeated injections of the haem precursor 5-aminolaevulinate strongly increased the saturation of rat liver apotryptophan pyrrolase with its haem activator (Table 1). The holoenzyme/apoenzyme ratio, which is indicative of the degree of haem saturation of the apoenzyme, was increased from a control (0.9% NaCl) value of 0.89 to 13.03 after 5-aminolaevulinate administration. The ratio was equally increased (3.20–3.22) at 24h after administration of either phenobarbitone in drinking water to fed, or phenylbutazone (once intraperitoneally) to starved, rats. Starvation of rats for 48h increased the pyrrolase activities by 79–110%. Although the administration of phenobarbitone to starved rats gave results

Table 1. *Effects of phenobarbitone, 5-aminolaevulinate and phenylbutazone on liver tryptophan pyrrolase activity in fed and starved rats*

Phenobarbitone was administered in drinking water (1 mg/ml), whereas phenylbutazone was given intraperitoneally (150mg/kg body wt.) and the animals were killed at 24h after either treatment. In starved rats, either drug was given half-way through the 48h starvation period. 5-Aminolaevulinate hydrochloride was injected in four 1-hourly doses (15mg/kg body wt. each) in fed rats, which were killed at 1h after the last injection. 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 (2μM). The apoenzyme activity was calculated by difference. The results are given as the means±S.E.M. of each group of four rats except that the nil group in the experiments on fed rats had a mean from 30 animals.

Treatment	Kynurenine formed (μmol/h per g wet wt. of liver)		Holoenzyme/apoenzyme ratio
	Holoenzyme activity	Total enzyme activity	
Fed rats			
Nil	1.56±0.10	3.38±0.20	0.86
Phenobarbitone	2.61±0.35	3.42±0.56	3.22
0.9% NaCl	1.82±0.21	3.86±0.39	0.89
5-Aminolaevulinate	4.56±0.35	4.91±0.50	13.03
Starved rats			
0.9% NaCl	3.25±0.31	8.11±0.29	0.67
Phenylbutazone	4.71±0.44	6.18±0.67	3.20
Phenobarbitone	2.31±0.16	3.88±0.25	1.47

Table 2. *Effects of cortisol, tryptophan, 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin on rat liver tryptophan pyrrolase activity*

Each rat received an injection of 0.9% (w/v) NaCl, cortisol acetate (20mg/kg), tryptophan (200mg/kg), 2-allyl-2-isopropylacetamide (400mg/kg), 3,5-diethoxycarbonyl-1,4-dihydrocollidine (150mg/kg) or griseofulvin (100mg/kg body wt.) 4h before being killed. The administration of dimethylformamide (0.1 ml/100g body wt.) gave results identical with the 0.9% NaCl-treated controls. 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 (2μM). The apoenzyme activity was calculated by difference. Values are means±S.E.M. of each group of four rats.

Injection	Kynurenine formed (μmol/h per g wet wt. of liver)		Holoenzyme/apoenzyme ratio
	Holoenzyme activity	Total enzyme activity	
0.9% NaCl	1.82±0.21	3.86±0.39	0.89
Dimethylformamide	1.74±0.20	3.72±0.41	0.88
Cortisol	6.57±0.43	16.94±1.12	0.63
Tryptophan	6.15±0.42	9.30±0.83	1.95
2-Allyl-2-isopropylacetamide	2.16±0.12	10.75±0.66	0.25
3,5-Diethoxycarbonyl-1,4-dihydrocollidine	2.10±0.12	8.31±0.62	0.34
Griseofulvin	3.69±0.57	16.13±0.94	0.30

essentially similar to those observed in fed rats, the phenobarbitone-induced rise in the holoenzyme/apoenzyme ratio in the latter was higher than that produced in the former rats. In separate experiments not reported here, the pyrrolase activities were not altered at 4 or 24h after intraperitoneal administration of phenobarbitone (100mg/kg) to fed or starved rats, or of phenylbutazone (150mg/kg) to fed rats.

Effects of porphyrogens on rat liver tryptophan pyrrolase activity

The effects on the enzyme activities of the porphyrogens 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin, were compared, 4h after administration, with those of cortisol (hormonal-type inducer) and tryptophan (substrate- or cofactor-type activator) (Table 2).

Table 3. *Effects of pretreatment of rats with 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin on the induction of rat liver tryptophan pyrrolase activity by cortisol*

The enzyme activity was determined, at 4h after administration of cortisol acetate (20mg/kg body wt.), as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2μM). The apoenzyme activity was calculated by difference. 2-Allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin was administered in the dose listed in Table 2 at 1h before cortisol. The results are given as the means±S.E.M. of each group of four rats.

Pretreatment	Kynurenine formed (μmol/h per g wet wt. of liver)		Holoenzyme/apoenzyme ratio
	Holoenzyme activity	Total enzyme activity	
Nil	6.57±0.43	16.94±1.12	0.63
2-Allyl-2-isopropylacetamide	3.99±0.60	14.64±0.70	0.37
3,5-Diethoxycarbonyl-1,4-dihydrocollidine	3.20±0.45	12.62±2.54	0.34
Griseofulvin	3.17±0.41	14.38±1.18	0.28

Table 4. *Effects of pretreatment of rats with 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin on the enhancement of rat liver tryptophan pyrrolase activity by tryptophan*

The enzyme activity was determined, at 4h after administration of tryptophan (200mg/kg body wt.), as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2μM). The apoenzyme activity was calculated by difference. 2-Allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin was administered in the dose listed in Table 2 at 1h before tryptophan. The results are given as the means±S.E.M. of each group of four rats.

Pretreatment	Kynurenine formed (μmol/h per g wet wt. of liver)		Holoenzyme/apoenzyme ratio
	Holoenzyme activity	Total enzyme activity	
Nil	6.15±0.42	9.30±0.83	1.95
2-Allyl-2-isopropylacetamide	6.98±0.95	15.36±1.50	0.83
3,5-Diethoxycarbonyl-1,4-dihydrocollidine	3.30±0.61	10.81±0.74	0.44
Griseofulvin	3.81±0.56	15.25±2.34	0.33

Cortisol induces apo-(tryptophan pyrrolase) synthesis and increases the holoenzyme activity in proportion to the rise in the total activity so that the holoenzyme/apoenzyme ratio is essentially similar to that observed in untreated rats (less than 1). Substrate- or cofactor-type enhancement of the pyrrolase activity by tryptophan, on the other hand, produces a large increase in the holoenzyme activity, little synthesis of new apoenzyme and a rise in the holoenzyme/apoenzyme ratio of 1.95. With the above three porphyrogens, the total pyrrolase activity was increased by 121–334% ($P<0.001$), whereas that of the holoenzyme was not significantly altered by either 2-allyl-2-isopropylacetamide or 3,5-diethoxycarbonyl-1,4-dihydrocollidine ($P>0.1$), and although griseofulvin moderately increased the holoenzyme activity, this differed significantly from the control (dimethylformamide) ($P<0.025$) as well as the cortisol ($P<0.01$) values. The holoenzyme/apoenzyme ratio was therefore decreased from a control value of 0.88 to 0.25–0.34 after the administration of the above porphyrogens.

2-Allyl-2-isopropylacetamide has been reported to enhance the pyrrolase activity by a cofactor-type mechanism in rats that had been either fed (Feigelson & Greengard, 1961*b*) or starved for 20h (Wetterberg *et al.*, 1970) or 40h (Marver *et al.*, 1966) previously. The rats used by the above authors were of either sex, of Sprague–Dawley or Long–Evans strains, and received 400mg of 2-allyl-2-isopropylacetamide/kg body wt. either subcutaneously or intraperitoneally. The present results show that this porphyrogen does not cause a cofactor-type enhancement of the enzyme activity in fed rats (Table 2) nor in those starved for 24 or 44h previously. Starvation for 24 or 44h

Table 5. *Effects of pretreatment of rats with 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin on the increased haem saturation of rat liver apo-(tryptophan pyrrolase) by repeated injections of 5-aminolaevulinate*

The enzyme activity was determined at 1 h after the last of four 1-hourly injections of 5-aminolaevulinate hydrochloride (15 mg/kg body wt. each), as described in the Materials and Methods section, in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2 μM). The apoenzyme activity was calculated by difference. 2-Allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin was administered in the dose listed in Table 2 at 1 h before the first 5-aminolaevulinate injection. The results are given as the means ± S.E.M. of each group of four rats.

Pretreatment	Kynurenine formed (μmol/h per g wet wt. of liver)		Holoenzyme/apoenzyme ratio
	Holoenzyme activity	Total enzyme activity	
Nil	4.56 ± 0.35	4.91 ± 0.50	13.03
2-Allyl-2-isopropylacetamide	2.80 ± 0.36	11.53 ± 0.83	0.32
3,5-Diethoxycarbonyl-1,4-dihydrocollidine	3.11 ± 0.45	9.66 ± 1.00	0.47
Griseofulvin	4.82 ± 0.34	6.63 ± 0.68	2.66

Table 6. *Effect of pretreatment of rats with compound SKF 525-A on the decreased haem saturation of rat liver apo-(tryptophan pyrrolase) caused by 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin*

Rats were given compound SKF 525-A (45 mg/kg body wt.) at 45 min before receiving dimethylformamide, 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin in the doses listed in Table 2, and were killed 4 h after the latter treatments. 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 μM). The apoenzyme activity was calculated by difference. The results are given as the means ± S.E.M. of each group of four rats.

Treatment after SKF 525-A	Kynurenine formed (μmol/h per g wet wt. of liver)		Holoenzyme/apoenzyme ratio
	Holoenzyme activity	Total enzyme activity	
Dimethylformamide	2.43 ± 0.41	6.02 ± 0.63	0.68
2-Allyl-2-isopropylacetamide	5.38 ± 0.82	11.54 ± 1.96	0.87
3,5-Diethoxycarbonyl-1,4-dihydrocollidine	4.82 ± 0.55	8.67 ± 1.11	1.25
Griseofulvin	6.53 ± 0.75	11.95 ± 1.12	1.20

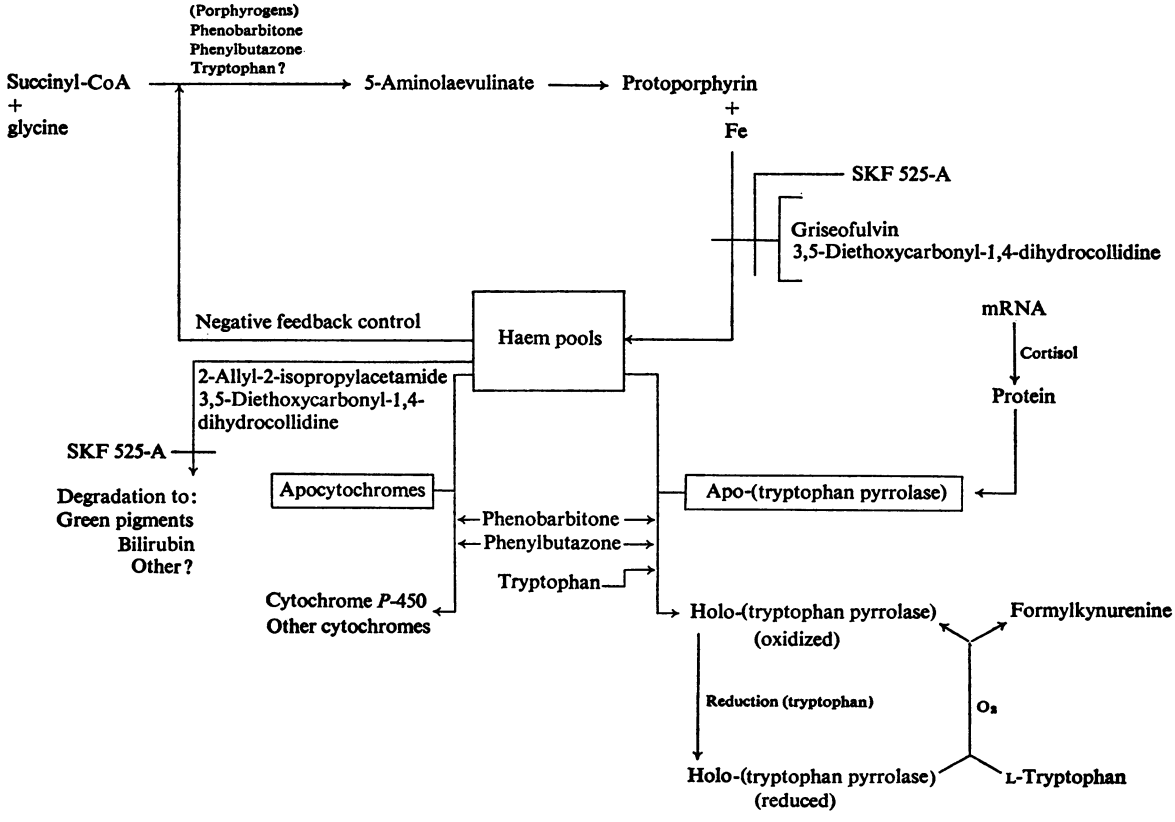
doubled the holoenzyme activity when either dimethylformamide or 2-allyl-2-isopropylacetamide were injected 4 h before death. The total pyrrolase activity was increased by 131–143% after dimethylformamide and by 203–227% after 2-allyl-2-isopropylacetamide administration respectively for the two periods of starvation.

The effects produced by pretreatment of the animals with porphyrogens on the enhancement of the pyrrolase activities by cortisol, tryptophan and 5-aminolaevulinate were examined. All three porphyrogens significantly inhibited ($P = 0.02$–0.005) the rise in the holoenzyme activity observed after cortisol administration (Table 3). A similar effect was observed when 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin was administered before tryptophan ($P = 0.02$–0.01) (Table 4). Pretreatment of rats with 2-allyl-2-isopropylacetamide did not affect the rise in the holoenzyme activity produced by subsequent tryptophan administration ($P > 0.1$). The increased haem saturation of apo-(tryptophan pyrrolase) caused by repeated injections of 5-aminolaevulinate was significantly inhibited ($P = 0.05$–0.02) by pretreatment of the animals with 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (Table 5) but not by that with griseofulvin ($P > 0.1$).

Table 7. *Effects of single or repeated injections of 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin on liver tryptophan pyrrolase activity in 48h-starved rats*

The animals were starved for 48h before being killed and received injections of dimethylformamide, 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin in the doses listed in Table 2 either at zero time (the start of starvation) or at zero time and at 24h. 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 (2μM). The results are given as the means±S.E.M. of each group of four rats.

	Kynurenine formed (μmol/h per g wet wt. of liver)			
	Single injection		Two injections	
Injection	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
Dimethylformamide	1.69±0.14	4.30±0.40	1.70±0.18	4.84±0.58
2-Allyl-2-isopropylacetamide	2.97±0.42	4.05±0.61	3.87±0.28	7.08±0.60
3,5-Diethoxycarbonyl-1,4-dihydrocollidine	2.91±0.26	5.12±0.64	3.40±0.24	5.98±0.73
Griseofulvin	3.24±0.36	5.00±0.09	2.90±0.09	4.56±0.51



Scheme 1. *Summarizing diagram of the points at which drugs that influence the metabolism of haem act to alter the conjugation of apo-(tryptophan pyrrolase) with its haem activator*

Pretreatment of rats with compound SKF 525-A (Table 6) resulted in all three porphyrogens subsequently enhancing the holo-(tryptophan pyrrolase) activity in proportion to the rise in that of the total enzyme. SKF 525-A, given before dimethylformamide, significantly increased the total enzyme ($P<0.05$) but not the holoenzyme ($P>0.1$) activity by comparison with dimethylformamide alone (see Table 2). The holoenzyme activity, increased after the administration of porphyrogens into rats pretreated with SKF 525-A, significantly differed from that due to the porphyrogens alone ($P = 0.05$–0.005) (see Table 2 for comparison).

An increased saturation of apo-(tryptophan pyrrolase) with its haem activator was observed at 24h after the administration of porphyrogens to starved but not to fed rats (Table 7). At 24h after a daily injection of 2-allyl-2-isopropylacetamide, 3,5-diethoxycarbonyl-1,4-dihydrocollidine or griseofulvin for 1 or 2 days in 48h-starved rats, the holoenzyme activity was significantly enhanced ($P = 0.02$–0.001). The total pyrrolase activity, on the other hand, was not significantly altered ($P>0.1$) except in rats given two injections of 2-allyl-2-isopropylacetamide ($P< 0.05$).

The results presented in this paper are summarized in a diagram (Scheme 1) showing the points at which drugs and compounds that influence the metabolism of haem act to alter the conjugation of apo-(tryptophan pyrrolase) with its haem activator.

Discussion

A number of chemically unrelated compounds stimulate the hepatic formation of porphyrins in experimental animals (De Matteis, 1967) and enhance the activity of mitochondrial 5-aminolaevulinate synthetase, which is the rate-limiting enzyme in the biosynthetic pathway of porphyrins and haem (Granick, 1966). One of these compounds, 2-allyl-2-isopropylacetamide, has been reported to produce a haem-mediated cofactor-type enhancement of liver tryptophan pyrrolase activity within a few hours of administration to fed (Feigelson & Greengard, 1961*b*) or starved (Marver *et al.*, 1966; Wetterberg *et al.*, 1970) rats. We could not demonstrate this effect in starved or fed rats, and in the latter, the above and two other porphyrogens (3,5-diethoxycarbonyl-1,4-dihydrocollidine and griseofulvin) all increased the total activity of the enzyme (Table 2), but produced neither a relatively similar (hormonal-type) nor a larger (substrate- or cofactor-type) rise in that of the holoenzyme. The resultant decrease by the above porphyrogens of the haem saturation of the apoenzyme is, however, consistent with the reported (De Matteis, 1972) decline in the concentration of liver haem and microsomal cytochrome *P*-450, another haem-requiring enzyme.

The marked increase in 5-aminolaevulinate synthetase activity and in the accumulation of porphyrins observed, in rat liver, at a later time after administration of the above porphyrogens has been contrasted (De Matteis, 1972) with the moderate stimulation of the synthetase by drugs such as phenobarbitone and phenylbutazone that act mainly by increasing the concentration of liver microsomal haem and cytochrome *P*-450. The present results (Table 1) suggest that apo-(tryptophan pyrrolase) exhibits an increased haem saturation after the administration of phenobarbitone and phenylbutazone. A similar, though stronger, effect is observed after repeated injections of 5-aminolaevulinate, thus confirming a previous finding (Druyan & Kelly, 1972). It is generally believed that the phenobarbitone-induced rise in the concentration of the liver microsomal cytochrome *P*-450 is due to enhanced apoenzyme synthesis (for a review, see Remmer, 1972). The possibility, however, remains that the drug may also increase the utilization of liver haem by cytochrome *P*-450 (De Matteis, 1972) as a result of moderately enhancing 5-aminolaevulinate synthetase activity probably by decreasing its rate of degradation (Satyanarayana Rao *et al.*, 1972). The increased haem saturation of apo-(tryptophan pyrrolase) caused by phenobarbitone and phenylbutazone (Table 1) is not associated with elevated apoenzyme concentrations and may therefore represent a pure effect on haem utilization. The effect of phenylbutazone requires starvation, whereas that by phenobarbitone does not. Further experiments on the effect of starvation are required to clarify this difference between the two drugs.

The porphyrogen-induced increase in the total pyrrolase activity (Table 2) may be due to a stress- or corticosteroid-mediated mechanism, whereas the concomitant decrease in the haem saturation of the apoenzyme may be caused by the loss of liver haem. With 2-allyl-2-isopropylacetamide, the haem loss may be produced by increased destruction in the microsomes (De Matteis, 1971) or in the cytosol or in both (De Matteis, 1973), whereas griseofulvin acts by inhibiting the mitochondrial ferrochelatase activity (De Matteis, 1972) thus leading to inhibition of haem synthesis. Ferrochelatase inhibition (De Matteis & Gibbs, 1972) and less significantly destruction of liver haem (De Matteis, 1972) may both account for the effect of 3,5-diethoxycarbonyl-1,4-dihydrocollidine. That the destruction of liver haem and the inhibition of its synthesis are involved in the decreased haem saturation of apotryptophan pyrrolase is further suggested by the finding (Table 6) that compound SKF 525-A, an inhibitor of drug-metabolizing enzymes (Fouts & Brodie, 1955), which protects against the haem loss caused by 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (De Matteis, 1972), restores the saturation of apo-(tryptophan pyrrolase) with its haem activator, thus leading

to a typical hormonal-type enhancement of the enzyme activity by these two porphyrogens. A similar effect by SKF 525-A in rats subsequently given griseofulvin (Table 6) also suggests that the inhibition of haem synthesis by this porphyrogen may be prevented by inhibition of drug metabolism.

The decreased haem saturation of apo-(tryptophan pyrrolase) after administration of porphyrogens was observed in normal rats (Table 2) and in those subsequently given cortisol, tryptophan and 5-aminolaevulinate (Tables 3, 4 and 5). Two exceptions were apparent. The lack of effect by 2-allyl-2-isopropylacetamide on the tryptophan-induced rise in the holoenzyme activity (Table 4) may be explained by newly synthesized haem being required for the tryptophan effect. This is suggested by the reported increase in the microsomal haem concentration (Greengard & Feigelson, 1961) and in the activity of 5-aminolaevulinate synthetase (Marver *et al.*, 1966) by tryptophan. The second exception involves the lack of inhibition by griseofulvin pretreatment of the 5-aminolaevulinate-induced rise in holo-(tryptophan pyrrolase) activity (Table 5). A possible explanation of this finding is that of griseofulvin inhibiting the synthesis of a certain haem pool(s) that is not utilized for rapid saturation of the pyrrolase apoenzyme.

The findings discussed so far suggest that the saturation of apo-(tryptophan pyrrolase) with its haem activator is influenced by conditions known to cause destruction, inhibition of synthesis and increased utilization of liver haem. Enhanced haem synthesis and accumulation of porphyrins in the liver at a later stage of treatment with porphyrogens could also affect the pyrrolase by increasing its saturation with haem (Table 7). An overall enhancement of the enzyme activity, presumably cofactor in nature, would be expected to occur after prolonged administration of porphyrogens, as has been reported (Calandra *et al.*, 1972) with 3,5-diethoxycarbonyl-1,4-dihydrocollidine in pigs. These animals, as well as porphyric patients (Price, 1961), show an increased urinary excretion of hepatic tryptophan metabolites that is consistent with an enhanced tryptophan pyrrolase activity leading to a functional vitamin B_6 deficiency. The inverse relation between liver tryptophan pyrrolase activity and brain 5-hydroxytryptamine concentration and the involvement of the latter in mood disorders (Curzon, 1969) may contribute to mental disturbances in patients with porphyria. It is not clear, however, why only 25% of all cases of porphyria present with severe mental disorders (Hare, 1953) or whether all porphyrics exhibit an enhanced rate of removal of tryptophan by the liver. It is possible that certain porphyrins which inhibit the pyrrolase activity by competing with the haem activator (Greengard & Feigelson, 1962) may prevent the enhancement of tryptophan pyrrolase activity in certain types of porphyrias. Although most studies in this area have been limited to animals, it may be of interest to examine changes in tryptophan metabolism in patients with porphyria in relation to mood disorders.

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