Effects of the haem precursor 5-aminolaevulinate on tryptophan metabolism and disposition in the rat

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5-Aminolaevulinate administration to rats inhibits cerebral 5-hydroxytryptamine synthesis by decreasing tryptophan availability to the brain secondarily to activation of hepatic tryptophan pyrrolase. The results show that tryptophan metabolism and disposition can be influenced by changes in liver haem concentration, and are discussed briefly in relation to mood disorders in the hepatic porphyrias.

INTRODUCTION

Administration of the haem precursor 5-aminolaevulinate has previously been shown to increase the haem saturation of the liver cytosolic haemoprotein tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) in both fed (Wetterberg *et al.*, 1969; Druyan & Kelly, 1972; Badawy & Evans, 1975a) and starved (Badawy *et al.*, 1981b) rats. Tryptophan pyrrolase is the first and rate-limiting enzyme of the quantitatively most important (hepatic kynurenine–nicotinic acid) pathway of tryptophan degradation (Young *et al.*, 1978; Young, 1981), and there is considerable evidence that pyrrolase activity is a major peripheral determinant of tryptophan availability to the brain (see, e.g., Badawy *et al.*, 1981a) and that the cerebral concentration of this amino acid is, in turn, an important factor in 5-hydroxytryptamine synthesis (Fernstrom & Wurtman, 1971; Carlsson & Lindqvist, 1978; Curzon, 1979).

The above haem saturation of tryptophan pyrrolase, as examined in rat liver homogenates or any other suitable cytosol-containing broken-cell preparation, is currently the only simple, if indirect, means of assessing small changes in hepatic haem concentration (for review and discussion, see Badawy, 1979; Welch & Badawy, 1980). Though endorsing this concept, Salter & Pogson (1986) have suggested that, because tryptophan pyrrolase appears to be fully saturated with haem in intact isolated rat liver cells, it is unlikely that changes in intracellular 'free' haem concentration *in vivo* will be effective in altering the flux of tryptophan through the kynurenine pathway, and hence, by inference, could not influence tryptophan metabolism and disposition through other routes. However, it is already known (Litman & Correia, 1985) that depleting rat liver haem during the course of production of experimental hepatic porphyria by 3,5-diethoxycarbonyl-2,6-dimethyl-4-ethyl-1,4-dihydrocollidine, which decreases the activity of holo-(tryptophan pyrrolase) as assayed in homogenates, increases brain tryptophan and 5-hydroxyindole concentrations and that both these hepatic and cerebral changes can be reversed by provision of exogenous haem through administered haematin. In the present work, we provide evidence that the opposite is also true, namely that increasing hepatic haem concentration by administration to rats of the precursor 5-aminolaevulinate, which

increases holo-(tryptophan pyrrolase) activity in homogenates, decreases cerebral tryptophan and 5-hydroxyindole concentrations by decreasing tryptophan availability to the brain.

MATERIALS AND METHODS

Chemicals

Allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) was a gift from the Wellcome Foundation (London, U.K.), whereas all other chemicals were purchased from BDH Chemicals and Sigma Chemical Co. (both of Poole, Dorset, U.K.) and were of the purest commercially available grades.

Animals and treatments

Locally bred male Wistar rats (150–170 g) were maintained on cube diet 41B (Oxoid) and water under standard conditions. 5-Aminolaevulinate hydrochloride was dissolved in 0.9% (w/v) NaCl (saline) and was injected intraperitoneally in four doses at 1 h intervals (15 mg/kg body wt. each) and the animals were killed at 1 h after the last injection. This regimen has previously been shown (Druyan & Kelly, 1972; Badawy & Evans, 1975a) to increase the haem saturation of liver tryptophan pyrrolase. Control rats received four equivalent saline injections (1 ml/kg body wt. each) by the same route. Both saline- and 5-aminolaevulinate-treated rats were also pretreated, 0.5 h previously, with an intraperitoneal injection of either allopurinol (20 mg/kg) or an equal volume (4 ml/kg) of saline. The allopurinol solution for injection was prepared as described by Badawy & Evans (1973a). Animals were killed by decapitation between 13:30 and 14:00 h.

Chemical, enzymic and other determinations

Tryptophan pyrrolase activity was determined (Badawy & Evans, 1975a; Badawy, 1981) in fresh homogenates from livers perfused *in situ* with ice-cold saline either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μ M) haematin. The extent of saturation of pyrrolase with haem was expressed as the percentage haem saturation (100 $\times$ holoenzyme activity/total enzyme activity). Despite a criticism (Stowell & Mørland, 1983) of the above method,

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we found (Badawy *et al.*, 1983) that the haem saturation of tryptophan pyrrolase is not artifactually elevated if the enzyme is assayed in homogenates of either fresh unperfused, or fresh or frozen perfused, liver. Concentrations of liver, free (ultrafiltrable) serum, total (acid-soluble) serum and brain tryptophan, brain 5-hydroxytryptamine and its major metabolite 5-hydroxyindol-3-ylacetic acid and serum albumin, corticosterone, glucose and non-esterified fatty acids were all determined by standard procedures (for references, see Badawy *et al.*, 1981a, 1984). Statistical analysis of results was performed by Student's *t* test.

RESULTS AND DISCUSSION

As shown in Table 1, treatment of rats with the haem precursor 5-aminolaevulinate did not influence the total activity of hepatic tryptophan pyrrolase, but caused a 161 % increase in that of the holoenzyme. The basal apoenzyme therefore became almost fully saturated with haem, and this was also reflected in the proportionate (162 %) increase in the percentage haem saturation. Salter & Pogson (1986) suggested that dilution of the liver during preparation of homogenates could cause an artifactual decrease in the haem saturation of tryptophan pyrrolase, as compared with the situation in intact hepatocytes. This problem requires investigation through comparative studies, but its implications are unlikely to influence interpretation of the present results, which demonstrate an increase, rather than a decrease, in the haem saturation of the enzyme.

The results in Table 1 also show that, under the above conditions, liver tryptophan concentration was decreased by 28 %, and similar decreases (32–33 %) in those of free serum and total serum tryptophan also occurred. These

latter proportionate decreases therefore were not associated with altered tryptophan binding to serum proteins when this latter parameter was expressed as the percentage free serum tryptophan; this is consistent with the absence of any significant changes in concentrations of the physiological binder (albumin) or displacers (non-esterified fatty acids) of circulating tryptophan. Thus in saline- and 5-aminolaevulinate-treated rats, the concentrations of serum albumin and non-esterified fatty acids were 44.9 ± 1.8 and 40.8 ± 1.2 g/l respectively and 0.44 ± 0.03 and 0.41 ± 0.03 mM respectively (means $\pm$ S.E.M. for each group of six rats). Also in experiments not given in Table 1, it was found that 5-aminolaevulinate administration did not significantly influence serum glucose or corticosterone concentration. Thus serum glucose and corticosterone concentrations in saline- and 5-aminolaevulinate-treated rats were 139 ± 1 and 142 ± 2 mg/dl respectively and 26.0 ± 1.2 and 24.9 ± 2.8 (nM) respectively (means $\pm$ S.E.M. for each group of six rats). The absence of change in serum corticosterone concentration, together with the nature of the above activation of the pyrrolase, exclude hormonal induction as the mechanism of the pyrrolase enhancement by this haem precursor.

The results in Table 1 also show that the decrease in availability of circulating tryptophan caused by 5-aminolaevulinate was associated with decreases in concentrations of brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid of 38 %, 35 % and 22 % respectively. These results are therefore consistent with 5-aminolaevulinate inhibiting rat cerebral 5-hydroxytryptamine synthesis and possibly also turnover by decreasing tryptophan availability to the brain secondarily to activation of hepatic tryptophan pyrrolase. Similar changes in tryptophan disposition and its cerebral

Table 1. Effects of 5-aminolaevulinate administration on tryptophan metabolism and disposition in rats

Rats received four intraperitoneal injections at 1 h intervals, of either 5-aminolaevulinate hydrochloride (15 mg/kg each) or an equal volume (1 ml/kg each) of 0.9 % (w/v) NaCl (saline) and were killed 1 h after the last injection. The animals were also pretreated, 0.5 h earlier, with a similar injection of either allopurinol (20 mg/kg) or an equal volume (4 ml/kg) of saline. The various parameters listed were determined as described in the Materials and methods section and are expressed as follows: liver tryptophan pyrrolase activity (μ mol of kynurenine formed/h per g wet wt.); pyrrolase haem saturation percentage ($100 \times$ holoenzyme activity/total enzyme activity); all other parameters except the percentage free serum tryptophan (μ g/ml of serum or per g wet wt. of tissue). Values are means $\pm$ S.E.M. for each group of four (pyrrolase activities and haem saturation) or six rats (all other expressions). The results in columns 2 and 3 were compared with those in column 1, whereas those in column 4 were compared with those in column 3, and the significance of the differences is indicated as follows: **P* < 0.05; ***P* < 0.005; ****P* < 0.001. Abbreviations used: 5-ALA, 5-aminolaevulinate; Trp, tryptophan; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindol-3-ylacetic acid.

Parameter	Pretreatment...	Saline		Allopurinol	
	Treatment...	Saline (1)	5-ALA (2)	Saline (3)	5-ALA (4)
Pyrrolase:					
Holoenzyme		1.30 ± 0.02	$3.40 \pm 0.16^{***}$	1.30 ± 0.08	1.30 ± 0.07
Total enzyme		3.50 ± 0.09	3.50 ± 0.22	3.70 ± 0.27	3.50 ± 0.27
Haem saturation (%)		37 ± 1	$97 \pm 2^{***}$	35 ± 2	37 ± 2
Liver Trp		7.18 ± 0.41	$5.18 \pm 0.15^{**}$	6.92 ± 0.24	7.08 ± 0.42
Free serum Trp		1.14 ± 0.05	$0.78 \pm 0.09^{**}$	1.22 ± 0.05	1.14 ± 0.04
Total serum Trp		14.32 ± 0.49	$9.60 \pm 0.39^{***}$	15.46 ± 1.29	14.53 ± 0.88
Free serum Trp (%)		7.96 ± 0.55	8.12 ± 0.59	7.89 ± 0.97	7.85 ± 0.71
Brain Trp		2.21 ± 0.17	$1.37 \pm 0.09^{**}$	2.03 ± 0.14	2.06 ± 0.07
Brain 5-HT		0.66 ± 0.03	$0.43 \pm 0.01^{***}$	0.66 ± 0.03	0.73 ± 0.03
Brain 5-HIAA		0.32 ± 0.02	$0.25 \pm 0.02^*$	0.33 ± 0.02	0.32 ± 0.01

metabolism are also observed when liver tryptophan pyrrolase activity is enhanced by hormonal induction by cortisol (Green *et al.*, 1975) or corticosterone during withdrawal of drugs of dependence (Badawy *et al.*, 1981a).

Further evidence for pyrrolase involvement in the 5-aminolaevulinate-induced changes in tryptophan metabolism and disposition is provided by the finding (Table 1) that all the effects of the haem precursor are prevented by pre-treatment of rats with allopurinol, a pyrrolase inhibitor known to inactivate the enzyme by preventing its conjugation with haem (Badawy & Evans, 1973a). Allopurinol given to control (saline-treated) rats did not cause any significant changes at the experimental time interval (4.5 h) at which parameters of tryptophan metabolism and disposition were examined (Table 1); this should be contrasted with the ability of this drug to inhibit the basal pyrrolase activity (Badawy & Evans, 1973a) and to increase brain tryptophan concentration (Badawy & Evans, 1975b) at earlier time intervals.

The present findings, together with those mentioned in the Introduction (Litman & Correia, 1985), therefore demonstrate that changes in hepatic haem concentration in either direction can influence tryptophan metabolism and disposition in the rat *in vivo* by exerting the appropriate effect on liver tryptophan pyrrolase activity. Although Litman & Correia (1985) did not measure liver tryptophan concentration, the decrease observed in the present work with 5-aminolaevulinate (Table 1) together with the associated changes in tryptophan disposition imply that tryptophan flux through the kynurenine pathway was accelerated under these conditions. Both these conclusions are in apparent contrast with those reached by Salter & Pogson (1986) from experiments with intact isolated liver cells. This latter system clearly differs from the intact rat *in vivo* in aspects related to haem homeostasis, particularly the influence of tryptophan on haem availability (Smith *et al.*, 1980; Smith & Pogson, 1981; Salter & Pogson, 1985, 1986). Because of these differences and the need to understand more clearly the nature of the haem homeostatic control process in such cells, it is premature to extrapolate data obtained from such an isolated, if intact, cell system to the whole animal *in vivo*, in relation to haem metabolism and its influence on that of tryptophan.

The present findings may also have implications in relation to mood disturbances in the hepatic porphyrias. This group of inherited disorders of porphyrin metabolism is associated with a variety of neurological and psychological disturbances, including depression (Beattie & Goldberg, 1972–1974), but the possible aetiological factor(s) involved remains to be determined (Kappas *et al.*, 1983, and references cited therein). As regards depression, we (Badawy & Evans, 1973b) suggested that, in view of the role of disturbed cerebral 5-hydroxytryptamine function in this illness (for reviews, see van Praag, 1978, 1982), patients with hepatic porphyrias may have a defective brain 5-hydroxytryptamine synthesis brought about by a possible activation of liver tryptophan pyrrolase by haem produced after induction of the limiting biosynthetic enzyme 5-aminolaevulinate syn-

thase. The results in Table 1 of the present work are consistent with this suggestion, and it is noteworthy in this context that depression occurs in those varieties of the hepatic porphyrias (all, except porphyria cutanea tarda) associated with increased excretion of the haem precursor 5-aminolaevulinate (Beattie & Goldberg, 1972–1974; Kappas *et al.*, 1983). It therefore remains to be seen whether, in such patients, depression could be correlated with changes in tryptophan metabolism and disposition, as suggested in the present work.

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REFERENCES

- Badawy, A. A.-B. (1979) *Biochem. Soc. Trans.* **7**, 575–583
- Badawy, A. A.-B. (1981) *J. Pharmacol. Methods* **6**, 77–85
- Badawy, A. A.-B. & Evans, M. (1973a) *Biochem. J.* **133**, 585–591
- Badawy, A. A.-B. & Evans, M. (1973b) *Biochem. J.* **136**, 885–892
- Badawy, A. A.-B. & Evans, M. (1975a) *Biochem. J.* **150**, 511–520
- Badawy, A. A.-B. & Evans, M. (1975b) *Lancet* **ii**, 869
- Badawy, A. A.-B., Punjani, N. F. & Evans, M. (1981a) *Biochem. J.* **196**, 161–170
- Badawy, A. A.-B., Welch, A. N. & Morgan, C. J. (1981b) *Biochem. J.* **198**, 309–314
- Badawy, A. A.-B., Morgan, C. J. & Davis, N. R. (1983) *Biochem. J.* **215**, 709–710
- Badawy, A. A.-B., Morgan, C. J., Davis, N. R. & Dacey, A. (1984) *Biochem. J.* **217**, 863–864
- Beattie, A. & Goldberg, A. (1972–1974) *Medicine* **12**, 774–782
- Carlsson, A. & Lindqvist, M. (1978) *Naunyn-Schiedeborg's Arch. Pharmacol.* **303**, 157–164
- Curzon, G. (1979) *J. Neural Transm. Suppl.* **15**, 81–92
- Druyan, R. & Kelly, A. (1972) *Biochem. J.* **129**, 1095–1099
- Fernstrom, J. D. & Wurtman, R. J. (1971) *Science* **173**, 149–152
- Green, A. R., Woods, H. F., Knott, P. J. & Curzon, G. (1975) *Nature (London)* **255**, 170
- Kappas, A., Sassa, S. & Anderson, K. E. (1983) in *The Metabolic Basis of Inherited Disease*, 5th Edn. (Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. & Brown, M. S., eds.), pp. 1301–1384, McGraw-Hill Book Co., New York
- Litman, D. A. & Correia, M. A. (1985) *J. Pharmacol. Exp. Ther.* **232**, 337–345
- Salter, M. & Pogson, C. I. (1985) *Biochem. J.* **229**, 499–504
- Salter, M. & Pogson, C. I. (1986) *Biochem. J.* **240**, 259–263
- Smith, S. A. & Pogson, C. I. (1981) *Biochem. J.* **200**, 605–609
- Smith, S. A., Carr, F. P. A. & Pogson, C. I. (1980) *Biochem. J.* **192**, 673–686
- Stowell, L. & Mørland, J. (1983) *Biochem. J.* **209**, 831–836
- van Praag, H. M. (1978) *Handb. Psychopharmacol.* **13**, 187–297
- van Praag, H. M. (1982) *Lancet* **ii**, 1259–1264
- Welch, A. N. & Badawy, A. A.-B. (1980) *Biochem. J.* **192**, 403–410
- Wetterberg, L., Yuwiler, A. & Geller, E. (1969) *Life Sci.* **8**, 1047–1049
- Young, S. N. (1981) *Br. J. Pharmacol.* **74**, 695–700
- Young, S. N., St. Arnaud-McKenzie, D. & Sourkes, T. L. (1978) *Biochem. Pharmacol.* **27**, 763–767