Tryptophan pyrrolase in haem regulation

Experiments with administered haematin and the relationship between the haem saturation of tryptophan pyrrolase and the activity of 5-aminolaevulinate synthase in rat liver

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1. Administration of haematin to rats decreases 5-aminolaevulinate synthase activity in whole liver homogenates. 2. An inverse relationship between this decrease and the increase in saturation of apo-(tryptophan pyrrolase) with haem is observed during the initial phase of treatment with haematin. 3. Significant changes in both functions are caused by a 1 mg/kg dose of haematin, whereas the maximum effects are achieved by the 5 mg/kg dose. 4. Prevention by allopurinol of the conjugation of exogenously administered haematin with apo-(tryptophan pyrrolase) renders this haem available for further repression of 5-aminolaevulinate synthase. 5. The various aspects of the relationship between synthase activity and the haem saturation of tryptophan pyrrolase are discussed.

5-Aminolaevulinate synthase (EC 2.3.1.37), the rate-limiting enzyme of the haem-biosynthetic pathway, is the point at which haem is thought to regulate its own synthesis by a feedback mechanism(s) in avian and mammalian (including human) liver. This concept has arisen from the findings that certain chemicals (porphyrogens) enhance markedly the activity of this enzyme by causing a rapid depletion of liver haem, and that administered haematin decreases synthase activity and is effective in the treatment of acute hepatic porphyrias (Granick, 1966; Hayashi *et al.*, 1972; Granick *et al.*, 1975; Dhar *et al.*, 1975; De Matteis, 1975, 1978). The mechanism(s) by which haem exerts this control is not fully understood. Current theories suggest an inhibition of transport of newly synthesized 5-aminolaevulinate synthase from cytosol to mitochondria in rat liver (Hayashi *et al.*, 1972), repression of synthase synthesis (Granick *et al.*, 1975) or inhibition of synthase activity (De Matteis, 1978; Tait, 1978, and references cited therein). Whichever mechanism(s) is involved, it is now thought that the above control is exerted by a small, readily exchangeable and rapidly turning over pool(s) of haem, that is most probably cytosolic and whose approximate concentration under normal

conditions may be 0.05–0.1 μM (Granick *et al.*, 1975; Badawy, 1978). It is because of the small size of this pool that the above depletion of liver haem has been demonstrated by indirect methods involving the determination of activity or concentration of, or haem utilization by, the hepatic haemoproteins catalase (EC 1.11.1.6), cytochrome *P*-450 and tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) (for a review see Badawy, 1979).

Whereas numerous studies of the effects of haematin or related substances on avian liver synthase activity have been performed, relatively less work has been done with the mammalian enzyme after haematin administration. In particular, very little is known about the effects of various doses of haematin on the activity of the basal enzyme. Moreover, it would be useful to monitor changes in liver haem concentration under these conditions. An opportunity of doing so is provided by the finding (Badawy & Evans, 1975) that administered haematin increases the saturation of rat liver apo-(tryptophan pyrrolase) with haem *in vivo*. That pyrrolase may be the only hepatic haemoprotein suitable for this purpose is suggested by the findings that it is the only haemoprotein to conjugate haem newly synthesized from exogenous 5-aminolaevulinate (Druyan & Kelly, 1972), and that, although labelled exogenous haem is incorporated into the major hepatic haemoprotein cytochrome *P*-450, both

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concentration and specific radioactivity of this labelled haemoprotein are decreased under these conditions (Bissell & Hammaker, 1976a). We have therefore performed a detailed investigation of the effects of administration of various doses of haematin on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase, the results of which form the subject of the present paper.

Materials and methods

Chemicals

Allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) and 2-allyl-2-isopropylacetamide were gifts from the Wellcome Foundation, London NW1 2BP, U.K., and Roche Products, Welwyn Garden City, Herts., U.K., respectively. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine (from Kodak, Kirkby, Liverpool, U.K.) was recrystallized from ethanol. All other chemicals were from BDH Chemicals or Sigma (London) Chemical Co. (both of Poole, Dorset, U.K.), and were of the purest commercially available grades.

Animals and treatments

Male Wistar rats (100–150 g body wt.) were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water, and were starved for 48 h (unless otherwise stated) before being killed by stunning and cervical dislocation between 13:00 and 15:30 h. All compounds were administered intraperitoneally, except 2-allyl-2-isopropylacetamide (400 mg/kg, in 1 ml of dimethylformamide), which was injected into the loose subcutaneous tissues of the neck. Control rats for this treatment received an equal volume of the solvent by the same route. Dimethylformamide (1 ml/kg) was also the solvent for 3,5-diethoxycarbonyl-1,4-dihydrocollidine (150 mg/kg), griseofulvin (100 mg/kg) and various doses (0.5–10 mg/kg) of haematin hydrochloride. Allopurinol (20 mg/kg) was administered as a solution in 0.9% NaCl (4 ml/kg) prepared as described by Badawy & Evans (1973). Control rats received equal volumes of the appropriate solvent(s).

Chemical, enzymic and other determinations

Tryptophan pyrrolase activity was determined in liver homogenates (Badawy & Evans, 1975) either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added ($2\mu\text{M}$) haematin. The apoenzyme activity was calculated by difference. The saturation of tryptophan pyrrolase with haem was expressed as the percentage haem saturation ($100 \times \text{holoenzyme activity} / \text{total enzyme activity}$).

5-Aminolaevulinate synthase activity was deter-

mined in liver homogenates by a modification (Badawy & Morgan, 1980) of the procedures describing the enzymic production of 5-aminolaevulinate (Yoda *et al.*, 1974) and its colorimetric determination (De Matteis, 1971).

Statistical analysis of results was performed by using Student's *t* test.

Results and discussion

Saturation of rat liver tryptophan pyrrolase with haem after haematin administration

We have previously reported (Badawy & Evans, 1975) that administration to fed rats of a 35 mg/kg dose of haematin hydrochloride caused an almost complete saturation of endogenous apo-(tryptophan pyrrolase) with haem at 2 h and produced a maximum increase in the haem saturation of the newly synthesized apoenzyme at 6 h. In the present work we have examined the effects of administration of various doses of haematin at 2 h in fed rats and in those starved for 24 or 48 h (Fig. 1). None of the doses administered caused any significant changes in the total pyrrolase activity ($P > 0.10$). By contrast, maximum activation of the holoenzyme (110–120%; $P < 0.001$) and full (100%; $P < 0.001$) saturation of the apoenzyme with haem was observed with the 5 mg/kg dose of haematin. It is noteworthy that this dose has been reported by Hayashi *et al.* (1972) to cause the maximum decrease in rat liver mitochondrial 5-aminolaevulinate synthase activity at 1 h after intravenous administration, and by Dhar *et al.* (1975) to be a safe and effective daily dose in the treatment of acute hepatic porphyrias. The results in Fig. 1 also show that the 1 mg/kg dose of haematin caused a significant increase in holo-(tryptophan pyrrolase) activity (44%; $P < 0.02$) only in 48 h-starved rats.

The time course of the effects of administration of the 5 mg/kg dose of haematin on pyrrolase activity in 48 h-starved rats is shown in Fig. 2. There were no major changes in the total enzyme activity during the first 4 h. Thereafter, the total activity was increased to a maximum (104%; $P < 0.005$) at 6 h, and it finally returned to the basal value at 12 h. By contrast, the holoenzyme activity was significantly enhanced by 67% ($P < 0.01$) as early as 0.5 h after haematin administration. As with the total activity, the maximum increase in that of the holoenzyme (269%; $P < 0.001$) was observed at 6 h, and the basal value was reached at 12 h. The haem saturation of the pyrrolase was increased (from a control value of 47% at zero time) to values of 76, 88 and 100% at 0.5, 1 and 2 h respectively. The enzyme remained fully saturated with haem for 2 h more, and this was then followed by a gradual return to the basal saturation value at 12 h. In experiments not given in Fig. 2, it was found that pyrrolase activities were not

Fig. 1. Effects of administration of various doses of haematin hydrochloride on the saturation of liver tryptophan pyrrolase with haem at 2 h in fed, 24 h- and 48 h-starved rats

Fed (a), 24 h-starved (b) and 48 h-starved (c) rats received an intraperitoneal injection of various doses of haematin hydrochloride or an equal volume (1 ml/kg) of the solvent dimethylformamide and were killed 2 h later. The enzyme activity was determined as described in the Materials and methods sections either in the absence (holoenzyme activity, ●) or in the presence (total enzyme activity, ○) of added ($2\mu\text{M}$) haematin. Values are means $\pm$ S.E.M. for each group of four rats.

Fig. 2. Time course of the effects of administration of haematin hydrochloride (5 mg/kg) on liver tryptophan pyrrolase activity of 48 h-starved rats

Haematin hydrochloride (5 mg/kg) was injected at zero time and the enzyme activity was determined, at various times, as described in the Materials and methods section either in the absence (holoenzyme activity, ●) or in the presence (total enzyme activity, ○) of added ($2\mu\text{M}$) haematin. Values are means $\pm$ S.E.M. for each group of four rats.

different from the basal values during the period 24–72 h after administration of haematin (5 mg/kg).

The effects of administration of various doses of haematin on pyrrolase activity were also examined at 6 h in 48 h-starved rats. Maximum enhancement of the holoenzyme activity (110%; $P < 0.001$) and full (100%; $P < 0.001$) saturation of the enzyme with haem were produced by the 5 mg/kg dose of haematin. The total pyrrolase activity was not significantly altered by any of the doses administered. It was also found that the holoenzyme activity was not altered at 6 h after administration of the 0.5 mg/kg dose of haematin, but that it was increased by 27, 84, 96 and 98% ($P = 0.05$ – 0.001) by doses of 1, 2, 3 and 4 mg/kg respectively.

The time course of the effects of administration of the 1 mg/kg dose of haematin on pyrrolase activity is shown in Fig. 3. In the 48 h-starved rats used, the holoenzyme and total pyrrolase activities were increased to a maximum (146 and 66% respectively; $P < 0.001$) at 3 h, and basal values were reached at about 6 h.

It may therefore be concluded from these results that administered haematin is rapidly taken up by the liver, where it becomes available for saturation of apo-(tryptophan pyrrolase). The correlation of this increase in saturation with the dose of haematin administered (up to 5 mg/kg) provides a means of at

Fig. 3. Time course of the effects of administration of haematin hydrochloride (1 mg/kg) on liver tryptophan pyrrolase activity of 48 h-starved rats

Experimental details are as described in Fig. 2, except that the dose of haematin hydrochloride was 1 mg/kg. Values are means $\pm$ S.E.M. for each group of four rats. Symbols: $\bullet$, holoenzyme activity; $\circ$, total enzyme activity.

least qualitative assessment of the hepatic concentration of exogenously administered haem. It is noteworthy that the increase in the haem saturation of tryptophan pyrrolase reported in the present work is a more rapid phenomenon than the reported (Bissell & Hammaker, 1976a,b) decrease in concentration and radioactivity of cytochrome *P*-450 caused by administration of haem. This suggests that the pyrrolase saturation with haem observed here is caused by administered haematin itself and not by the possible release of haem from cytochrome *P*-450, which the above authors have suggested. It is also clear from the present results that the total pyrrolase activity was not altered when various doses of haematin were compared (Fig. 1 and the text), but was increased in experiments involving the time course of the effects of the 1 and 5 mg/kg doses of the pigment (see Figs. 2 and 3). This may be explained by the fact that, whereas the control (zero-dose) values in the former instances were obtained in rats given the solvent dimethylformamide, the control (zero-time) values in Figs. 2 and 3 were determined in untreated animals. It is therefore possible that the stress of the injection could have caused the observed increase in the total pyrrolase activity under these latter conditions. The finding that the total enzyme activity was increased early after administration of the 1 mg/kg dose of haematin (Fig. 3), whereas there was a delay in the increase with the 5 mg/kg dose (Fig. 2), cannot be explained at present. It is, however, possible that animal variations may be responsible.

Fig. 4. Time course of the effects of administration of haematin hydrochloride (5 mg/kg) on liver 5-aminolaevulinate synthase activity of 48 h-starved rats

Experimental details are as described in Fig. 2. 5-Aminolaevulinate synthase activity was determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for each group of four rats.

Effects of administration of haematin on rat liver 5-aminolaevulinate synthase activity and correlation with the haem saturation of tryptophan pyrrolase

The time course of the effects of administration of the 5 mg/kg dose of haematin on 5-aminolaevulinate synthase activity in liver homogenates of 48 h-starved rats is shown in Fig. 4. A 27% decrease ($P < 0.005$) in synthase activity was observed at 0.5 h. The extent of this decrease was unaltered (27–31%; $P = 0.025$ – 0.005) for 2.5 h more, but became stronger (63%; $P < 0.001$) at 4 h. The maximum decrease in synthase activity (81%; $P < 0.001$) occurred at the same time interval (6 h) at which tryptophan pyrrolase exhibited the highest holoenzyme activity (see Fig. 2). The above maximum decrease in synthase activity was then followed by alternate phases of partial recovery and no change, until the basal value was reached at 72 h.

Hayashi *et al.* (1972) reported that haematin administration does not alter the total activity of 5-aminolaevulinate synthase in rat liver homogenates, but that it decreases the activity found in the mitochondrial fraction and increases that found in the cytosol. Although we have not examined the effects of haematin on the activity of both forms of the enzyme, the results in Fig. 4 and elsewhere in the present paper clearly demonstrate the haematin-induced decrease in synthase activity in whole-liver homogenates, and are thus incompatible with the suggestion (Hayashi *et al.*, 1972) that administered haematin acts by inhibiting the transport of newly synthesized 5-aminolaevulinate synthase from cytosol to mitochondria. Schacter *et al.* (1976) also reported that haematin administration to rats decreases synthase activity in liver homogenates. These latter authors, however, also reported that

haematin caused cyclic oscillations in synthase activity, the mechanism of which was suggested to involve alternate repression by haematin of synthase synthesis and induction of this synthesis secondarily to the repression. In the present work (Fig. 4) we have not encountered such oscillatory behaviour of the enzyme, although we observed alternate phases of partial recovery and no change. It is possible that the different doses and forms of haematin used and the routes of its administration may explain these different findings.

The effects of administration of various doses of haematin were also examined at 2 or 6 h in 48 h-starved rats (Fig. 5). The saturation of trypto-

phan pyrrolase with haem is included for comparison. At 2 or 6 h, pyrrolase saturation with haem and the decrease in synthase activity exhibited a reciprocal relationship, and neither function was significantly altered by the 0.5 mg/kg dose of haematin. The 5 mg/kg dose, which caused full (100%) saturation of pyrrolase with haem, also caused the largest decrease in synthase activity (45% at 2 h and 84% at 6 h; $P = 0.01-0.001$). It is noteworthy that, although full saturation of pyrrolase with haem was observed at 2 or 6 h after administration of the 5 mg/kg dose of haematin, the decrease in synthase activity at 2 h was not stronger with larger doses. That synthase activity could be decreased to a larger extent at 2 h is suggested by the findings given in Table 1 (see below). It may therefore be suggested that, not only does the pyrrolase saturation with haem mirror the effect of administered haematin on synthase activity, but also the extra haem expected to be present in livers of rats treated with doses of haematin larger than 5 mg/kg is not available for synthase repression. This latter point further suggests that, although exogenous haematin enters the regulatory haem pool, it does so only to a certain extent and by a mechanism in which tryptophan pyrrolase also appears to be affected, involved or both. Further work on these aspects and on the kinetics of the entry of exogenous haematin into the regulatory pool is therefore required.

A reciprocal relationship between pyrrolase saturation with haem and the decrease in synthase activity was also observed during the time course of the effects of administration of the 1 mg/kg dose of haematin in 48 h-starved rats (Fig. 6). The first significant decrease in synthase activity (29%; $P < 0.05$) occurred at 1 h. The decrease reached a maximum (76%; $P < 0.001$) at 3 h, after which the enzyme activity recovered gradually, reaching the basal value at 24 h.

It should be noted from the results in Figs. 4 and 6 that, although pyrrolase saturation with haem is no longer enhanced at 24 or 6 h after administration of doses of haematin of 5 and 1 mg/kg respectively, synthase activity is still decreased beyond these time intervals. This may be explained by the possibility that, although the prolonged decrease in synthase activity may be caused by the slow release of administered haematin from its binding sites on carrier proteins in plasma (Sears & Huser, 1966), the haem released at intervals after pyrrolase saturation with haem has returned to normal may not be available for further saturation of this enzyme. Alternatively, the prolonged decrease in synthase activity may be caused by the ability of administered haematin to release haem from apo-(cytochrome *P-450*) (see Bissell & Hammaker, 1976*a,b*) without this haem being available for

Fig. 5. Effects of administration of various doses of haematin hydrochloride on liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase at 2 and 6 h in 48 h-starved rats

Rats received an injection of various doses of haematin hydrochloride or an equal volume (1 ml/kg) of dimethylformamide and were killed 2 h (a) or 6 h (b) later. 5-Aminolaevulinate synthase activity (● or ○) and the haem saturation of tryptophan pyrrolase (▲ or △) were determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for each group of four rats.

Fig. 6 Time course of the effects of administration of haematin hydrochloride (1 mg/kg) on liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase in 48 h-starved rats

Experimental details are as described in Fig. 3, from the results of which the haem saturation of tryptophan pyrrolase is included here. This saturation and the activity of 5-aminolaevulinate synthase were determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for each group of four rats. Symbols: $\bullet$, 5-aminolaevulinate synthase activity; $\blacktriangle$, haem saturation of tryptophan pyrrolase.

pyrrolase saturation. Further work is clearly required to examine these possibilities.

Haematin administration has also been reported (Hayashi *et al.*, 1972) to inhibit the 2-allyl-2-isopropylacetamide-induced increase in 5-aminolaevulinate synthase activity. In the present work we found that administration of haematin (5 mg/kg) 2 h after pretreatment of 48 h-starved rats with 2-allyl-2-isopropylacetamide (400 mg/kg) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (150 mg/kg) completely abolished the increases in synthase activity observed at 6 h after administration of these two porphyrins (results not shown); synthase activity under these conditions was similar to that observed in rats treated with haematin alone, which was 40% below the control value ($P < 0.01$). The above two porphyrins alone decreased the haem saturation of tryptophan pyrrolase by 25–41% ($P = 0.01$ – 0.001) and lowered the haematin-induced increase in this saturation by 44–52% ($P < 0.001$). Of the two porphyrins, 2-allyl-2-isopropylacetamide was the more active. By contrast, griseofulvin (100 mg/kg) failed to influence pyrrolase saturation with haem in starved control or haematin-treated rats, and did not enhance synthase activity in the former animals. This inactivity of griseofulvin in the rat is consistent with this species

being less sensitive to the porphyrinogenic and metal chelatase-inhibitory effects of this drug (De Matteis & Gibbs, 1975).

Relationship between the haem saturation of tryptophan pyrrolase and 5-aminolaevulinate synthase activity

There are four aspects of this relationship. Firstly, synthase activity is enhanced when pyrrolase saturation with haem is decreased by treatments that cause an increased destruction or degradation or a decreased synthesis of haem. This can be satisfactorily explained by the removal of the regulatory haem causing a positive-feedback control of synthase activity (see also De Matteis, 1975). These treatments are with porphyrins (alone or jointly with their analogues or with certain drugs), metal cations, Pb^{2+} plus phenobarbitone, and selenium deficiency plus administered phenobarbitone (De Matteis, 1973; Maines & Kappas, 1976; Maxwell & Meyer, 1976; Badawy, 1977a; Correia & Burk, 1978). Secondly, synthase activity is decreased when pyrrolase saturation with haem is increased after administration of haematin (e.g. see the present work) or endotoxin (Bissell & Hammaker, 1977), and this may be explained by the increased liver haem concentration caused by these treatments

exerting a negative-feedback control of synthase activity. Tryptophan, which also increases the saturation of tryptophan pyrrolase with haem (see, e.g., Knox, 1966), has been reported (Marver *et al.*, 1966) to enhance synthase activity in rat liver. However, we could not confirm this finding, and have actually observed a decrease in synthase activity (A. A.-B. Badawy & A. N. Welch, unpublished work).

The two aspects discussed so far suggest that pyrrolase saturation with haem could simply be considered a passive expression of the availability of the regulatory haem pool. However, it is not known whether synthase activity is also increased when pyrrolase saturation with haem is decreased, but by a mechanism other than decreased synthesis or increased destruction or degradation of haem. Under these latter conditions, haem is simply not available or has been converted into other substances that do not influence synthase activity. An opportunity of examining this third aspect is provided by the ability of allopurinol to prevent the conjugation of liver apo-(tryptophan pyrrolase) with haem in the fed rat (Badawy & Evans, 1973). Under these conditions, haem should still be available for synthase regulation. The effects of allopurinol on the conjugation of apo-(tryptophan pyrrolase) with haem and on synthase activity were therefore examined in fed and 48 h-starved rats in either the absence or the presence of joint haematin administration (Table 1). In fed rats, allopurinol prevented the conjugation of apo-(tryptophan pyrrolase) with exogenously added haematin, as suggested by the 57% ($P < 0.001$)

decrease in the total pyrrolase activity, but did not affect the basal haem pool that is present in the holoenzyme. This latter finding is consistent with the inability of allopurinol to alter synthase activity in fed rats. The almost-complete saturation of apo-(tryptophan pyrrolase) with haem observed at 2 h after administration of haematin (5 mg/kg) to fed rats was associated with a 27% decrease ($P < 0.02$) in synthase activity. The magnitude of this decrease is similar to that observed in 48 h-starved rats (see Fig. 4). Pretreatment of fed rats with allopurinol prevented the haematin-induced increase in the haem saturation of tryptophan pyrrolase, but, instead of abolishing the decrease in synthase activity, it potentiated it by 28% ($P < 0.02$). Similarly, in 48 h-starved rats the haematin-induced increase in pyrrolase saturation with haem was abolished by allopurinol, whereas the haematin-induced decrease in synthase activity (40%; $P < 0.05$) was further potentiated by the drug by 42% ($P < 0.005$). Thus, although these results suggest that, when holo-(tryptophan pyrrolase) loses haem by this mechanism (prevention of conjugation), this haem becomes free to cause a further decrease in synthase activity, this should be contrasted with synthase enhancement when pyrrolase loses haem by other mechanisms that involve increased destruction or inhibition of synthesis. This suggests that the relationship between pyrrolase saturation with haem and synthase activity is not always a simple inverse one, but that this saturation determines the availability of haem for synthase regulation, and the manner with which this saturation is altered deter-

Table 1. *Effects of administration of allopurinol, haematin or both on liver tryptophan pyrrolase and 5-aminolaevulinate synthase activities in fed and 48 h-starved rats*

Fed and 48 h-starved rats received an injection of either haematin hydrochloride (5 mg/kg) or an equal volume (1 ml/kg) of dimethylformamide at 2 h before being killed. The animals also received, 20 min before the above treatments, an injection of either allopurinol (20 mg/kg) or an equal volume (4 ml/kg) of 0.9% NaCl. Pyrrolase and synthase activities were determined as described in the Materials and methods section. Values are means $\pm$ s.e.m. for each group of four rats. In both fed and starved rats the values obtained with allopurinol or haematin are compared with those of the control groups, whereas those observed in rats treated with allopurinol plus haematin are compared with those in animals given haematin alone. Additionally, the values in the control starved rats are compared with those in the corresponding fed group. The significance of differences is indicated as follows: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.005$; † $P < 0.001$.

Nutrition	Treatment	Kynurenine formed (μ mol/h per g wet wt. of liver)		5-Aminolaevulinate formed (nmol/min per g wet wt. of liver)
		Holoenzyme activity	Total enzyme activity	
Fed	Control	2.0 $\pm$ 0.07	4.4 $\pm$ 0.12	0.78 $\pm$ 0.05
	Allopurinol	1.9 $\pm$ 0.07	1.9 $\pm$ 0.07†	0.76 $\pm$ 0.03
	Haematin	4.2 $\pm$ 0.17†	4.3 $\pm$ 0.20	0.57 $\pm$ 0.04**
	Allopurinol + haematin	1.9 $\pm$ 0.14†	1.9 $\pm$ 0.09†	0.41 $\pm$ 0.02**
Starved	Control	4.2 $\pm$ 0.21†	11.7 $\pm$ 0.26†	1.59 $\pm$ 0.24**
	Allopurinol	2.8 $\pm$ 0.13***	4.9 $\pm$ 0.42†	0.81 $\pm$ 0.05**
	Haematin	12.0 $\pm$ 0.32†	12.2 $\pm$ 0.40	0.95 $\pm$ 0.07*
	Allopurinol + haematin	3.9 $\pm$ 0.30†	3.9 $\pm$ 0.30†	0.47 $\pm$ 0.06***

mines the direction of change in synthase activity.

Fourthly, synthase activity could be altered in the absence of any change in the haem saturation of tryptophan pyrrolase. This is only observed in starvation (Table 1). Starvation increased both holoenzyme and total pyrrolase activities by 2.1- to 2.6-fold ($P < 0.001$), by a hormonal-type mechanism not associated with an altered extent of saturation of the apoenzyme with haem (see also Badawy, 1979), but sensitive to reversal by actinomycin D and prevention by adrenalectomy (Badawy, 1977b; Morgan & Badawy, 1980). Starvation also doubled synthase activity (Table 1), thus confirming the finding by Bock *et al.* (1971). The results in Table 1 also show that allopurinol reversed the starvation-induced increases in synthase and pyrrolase activities, and this confirms the findings made by Morgan & Badawy (1980), who suggested that synthase enhancement by starvation may involve the increase in holo-(tryptophan pyrrolase) activity utilizing the regulatory haem pool, thus decreasing its availability for synthase regulation. Further work is required to examine this possibility. It should, however, be pointed out that increased utilization of haem by liver haemoproteins has been suggested (De Matteis, 1975) as a means of increasing synthase activity. The above possibility that tryptophan pyrrolase may play a role in the starvation-induced enhancement of synthase activity clearly suggests that haem utilization by, as opposed to haem saturation of, tryptophan pyrrolase may be involved in synthase regulation.

Concluding remarks

The present results have established a close association between the haem saturation of tryptophan pyrrolase and the decrease in 5-aminolaevulinic synthase activity during the early stages of treatment with haematin. The several aspects of the relationship between the above saturation and synthase activity discussed suggest that further work may be useful in understanding the relationship between these two enzymes.

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