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Tryptophan and tryptophan pyrrolase in haem regulation

The role of lipolysis and direct displacement of serum-protein-bound tryptophan in the opposite effects of administration of endotoxin, morphine, palmitate, salicylate and theophylline on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase

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1. The increase in the haem saturation of rat liver tryptophan pyrrolase caused by tryptophan administration was previously shown to be associated with a decrease in 5-aminolaevulinate synthase activity. 2. It is now shown that similar reciprocal effects are caused by palmitate and salicylate, both of which increase tryptophan availability to the liver by direct displacement of the serum-protein-bound amino acid. 3. The reciprocal effects on the former two parameters caused by endotoxin and morphine are associated with an increase in liver tryptophan concentration produced by a lipolysis-dependent, non-esterified fatty acid-mediated, displacement of the serum-protein-bound amino acid. 4. All these changes and those caused by another lipolytic agent, theophylline, are prevented by the β-adrenoceptor-blocking agent propranolol and by the opiate-receptor antagonist naloxone, whose anti-lipolytic nature is demonstrated. 5. High correlation coefficients have been obtained for one or more pairs of the following parameters: serum non-esterified fatty acid concentration, free serum tryptophan concentration, liver tryptophan concentration, liver 5-aminolaevulinate synthase activity, liver holo-(tryptophan pyrrolase) activity and the haem saturation of liver tryptophan pyrrolase. 6. It is suggested that liver tryptophan concentration may play an important role in the regulation of 5-aminolaevulinate synthase synthesis, and that the latter may be subject to control by changes in lipid metabolism and may be influenced by pharmacological agents that affect tryptophan disposition. 7. Preliminary evidence suggests that tryptophan may be bound in the liver and that such a possible binding may control its availability for its hepatic functions.

An increase in rat liver haem concentration, such as that caused by administration of haematin or 5-aminolaevulinate, is associated with an increase in the haem saturation of tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11), a repression of synthesis of 5-aminolaevulinate synthase (EC 2.3.1.37) and an enhancement of activity of haem oxygenase (EC 1.14.99.3) (Wetterberg *et al.*, 1969; Druyan & Kelly, 1972; Badawy & Evans, 1973*b*, 1975*b*; Schacter *et al.*, 1976; Bissell & Hammaker, 1976*a*,*b*; Welch & Badawy, 1980; Badawy *et al.*, 1981). Another agent capable of increasing liver haem concentration for saturation of tryptophan pyrrolase and repression of 5-aminolaevulinate synthase synthesis is tryptophan, whose mechanism of action has been suggested (Badawy *et al.*, 1981) to involve an enhanced conversion of 5-aminolaevulinate into haem. Bissell & Hammaker (1976*a*,*b*, 1977) reported that the increase in the haem saturation of rat liver tryptophan pyrrolase, the concomitant decrease in 5-aminolaevulinate synthase activity and the subsequent enhancement of that of haem oxygenase observed after administration of endotoxin (lipopolysaccharide) are caused by the ability of this agent to dissociate the haem moiety of cytochrome *P*-450, thus rendering it available to exert the above effects. A similar mechanism has been suggested by Gurantz & Correia (1981) to explain the ability of administered morphine to enhance the haem saturation of tryptophan pyrrolase (see Badawy & Evans, 1975*a*, for this effect) and the activity of haem oxygenase. Gurantz & Correia (1981) did not, however, demonstrate a decrease in 5-aminolaevulinate synthase activity after morphine administration.

An alternative explanation of the ability of

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endotoxin and morphine to increase hepatic haem concentration and, thereby, cause the above changes in haem-utilizing and metabolizing enzymes is that of these two agents acting via tryptophan, whose availability to the liver is expected to be increased as a result of possible displacement from serum-protein-binding sites by non-esterified fatty acids secondarily to the lipolytic actions of the above two agents. That morphine enhances lipolysis has been demonstrated by Wong *et al.* (1977), whereas there is evidence (Wright, 1981) strongly suggesting that endotoxin may be lipolytic, because of its ability to enhance the release of catecholamines by reflex activation of the sympathetic nervous system secondarily to vasodilation. In the present paper, we provide evidence supporting the above suggestion and demonstrate the anti-lipolytic nature of the opiate-receptor antagonist naloxone. We also show that another lipolytic agent, theophylline, which is known (Kim & Kikuchi, 1974) to decrease 5-aminolaevulinate synthase activity, increases the haem saturation of tryptophan pyrrolase, and that similar reciprocal effects are caused by palmitate and salicylate, which displace directly serum-protein-bound tryptophan in the rat (see Badawy & Smith, 1972; Badawy & Evans, 1975*a*).

Materials and methods

Chemicals

Morphine sulphate and the hydrochloride of naloxone (17-allyl-4,5α-epoxy-3,14-dihydroxynormorphinan-6-one), which was in ampoules, each containing 0.4 mg/ml, were purchased from Macarthys Ltd. (Trecenydd Industrial Estate, Caerphilly, Mid Glamorgan, Wales, U.K.) and Winthrop Laboratories (Surbiton, Surrey, U.K.) respectively. The β-adrenoceptor-blocking agent propranolol [1-isopropylamino-3-(1-naphthyloxy)propan-2-ol] was a gift from Imperial Chemical Industries (Alderley Park, Macclesfield, Cheshire, U.K.), whereas all other chemicals (of the purest commercially available grades) were obtained from BDH Chemicals or Sigma Chemical Co. (both of Poole, Dorset, U.K.).

Animals and treatments

Locally bred male Wistar rats (150–170 g) were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water and were starved for 24 h before being killed (between 13:00 and 15:00 h) either by stunning and cervical dislocation (for the determination of 5-aminolaevulinate synthase and tryptophan pyrrolase activities in fresh-liver homogenates) or by decapitation (for the determination of concentrations of serum non-esterified fatty acids and tryptophan and of the latter in samples of liver obtained by a locally manufactured freeze-clamp).

All chemicals were intraperitoneally injected. Palmitic acid (100 mg/kg body wt.) was dissolved in dimethylformamide (1 ml/kg body wt.), whereas all other chemicals were dissolved in 0.9% (w/v) NaCl and were given in volumes of 2–20 ml/kg body wt. and in the following doses: endotoxin (2 mg/kg body wt.), morphine sulphate (45 mg/kg body wt.), naloxone hydrochloride (1 or 5 mg/kg body wt.), propranolol (10 mg/kg body wt.), sodium salicylate (50 or 400 mg/kg body wt.), theophylline (100 mg/kg body wt.), tryptophan (50 or 400 mg/kg body wt.). Control rats received an appropriate volume(s) of 0.9% NaCl. The tryptophan solution for injection was prepared as described by Badawy & Evans (1975*b*).

Chemical, enzymic and other determinations

Tryptophan pyrrolase activity was determined in liver homogenates either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (2 μM) haematin (Badawy & Evans, 1975*b*; see also the fuller description by Badawy, 1981*b*). The haem saturation of tryptophan pyrrolase was expressed as the percentage haem saturation (100 × holoenzyme activity/total enzyme activity). 5-Aminolaevulinate synthase activity was determined as described by Badawy & Morgan (1980). When rats were treated with tryptophan, synthase activity was determined by the above procedure, but with the modification thereof described by Badawy *et al.* (1981). The concentrations of free (ultrafiltrable) serum, total (acid-soluble) serum and liver tryptophan were determined as described by Badawy & Evans (1976*a*), whereas that of serum non-esterified fatty acids was determined by the method of Mikac-Dević *et al.* (1973). Statistical analysis of results was performed by using Student's *t* test.

Results and discussion

Reciprocal effects of administration of endotoxin, morphine, palmitate, salicylate, theophylline and tryptophan on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase and prevention of the effects of endotoxin, morphine and theophylline by propranolol

The results in Table 1 show that liver 5-aminolaevulinate synthase activity of 24 h-starved rats was significantly decreased by 22–58% ($P = 0.01$–0.001) at 4 h after administration of the above first six compounds. The decreases in synthase activity caused by palmitate and salicylate are reported here for the first time, whereas those by theophylline, endotoxin and tryptophan confirm previous findings (Kim & Kikuchi, 1974; Bissell & Hammaker, 1977; Badawy *et al.*, 1981). The morphine effect on synthase activity is also reported here for the first

Table 1. *Effects of endotoxin, morphine, theophylline, palmitate, salicylate and tryptophan on rat liver 5-aminolaevulinate synthase activity and prevention of the effects of lipolytic agents by propranolol*

Rats were starved for 24h and received an intraperitoneal injection of endotoxin (2mg/kg body wt.), morphine sulphate (45mg/kg body wt.), palmitic acid (100mg/kg body wt.), sodium salicylate (400mg/kg body wt.), theophylline (100mg/kg body wt.), tryptophan (400mg/kg body wt.) or 0.9% (w/v) NaCl (2ml/kg body wt.) at 4h before death. The animals also received, 0.5h before the above treatments, another injection of either propranolol (10mg/kg body wt.) or an equal volume (2ml/kg body wt.) of 0.9% NaCl. 5-Aminolaevulinate synthase activity was determined as described in the Materials and methods section. Each group of compounds was tested in relation to its own control. Values are means ± S.E.M. for each group of four rats. The synthase activity values obtained after administration of various compounds have been compared with those observed in 0.9% NaCl-treated controls in each individual column, and the significances of the differences are indicated as follows: $^{*}P < 0.01$; $^{**}P < 0.005$; $^{***}P < 0.001$.

Treatment	Pretreatment	5-Aminolaevulinate synthase activity (nmol of 5-aminolaevulinate formed/min per g wet wt. of liver)	
	...	0.9% NaCl	Propranolol
0.9% NaCl		0.42 ± 0.04	0.44 ± 0.05
Endotoxin		0.24 ± 0.01**	0.41 ± 0.03
Morphine		0.20 ± 0.02**	0.50 ± 0.03
0.9% NaCl		0.44 ± 0.05	0.51 ± 0.05
Theophylline		0.23 ± 0.01*	0.49 ± 0.05
0.9% NaCl		0.51 ± 0.01	0.49 ± 0.02
Palmitate		0.39 ± 0.03*	0.38 ± 0.01**
Salicylate		0.39 ± 0.02**	0.30 ± 0.01***
Tryptophan		0.40 ± 0.01***	0.39 ± 0.01**

time, but is at variance with the finding (Gurantz & Correia, 1981) that a similar dose of the drug did not influence the activity of the enzyme during the first 6h. No explanation of these differences in results can as yet be offered. The results in Table 1 of the present work also show that the β-adrenoceptor-blocking agent propranolol was capable of preventing the decreases in synthase activity caused by endotoxin, morphine and theophylline, but not those exerted by palmitate, salicylate and tryptophan. The failure of propranolol to alter synthase activity in starved control rats confirms a previous finding (Blum *et al.*, 1973).

Because the above experiments were performed over a period of several weeks, and in order to minimize possible variations in control 5-aminolaevulinate synthase activity, the above compounds were examined in groups, each of which had its own 0.9% NaCl-treated control treatment. Theophylline was examined alone, because its use in this work was decided towards the end of the project. It is nevertheless clear from the control data in Table 1 that synthase activity of various control groups did not vary significantly ($P > 0.05$).

When 24h-starved rats were examined for liver tryptophan pyrrolase activity, it was found (Table 2) that the haem saturation of the enzyme (expressed as the percentage haem saturation) was significantly increased by endotoxin, morphine, palmitate, salicylate, theophylline and tryptophan, by 70–160% ($P < 0.001$), because of the relatively larger increase in the holoenzyme activity (in comparison with that in the total enzyme), and that pretreatment with propranolol prevented the increases in pyrrolase activities and the haem saturation of the enzyme caused by endotoxin and morphine, but not those produced by palmitate, salicylate and tryptophan. Propranolol also prevented the increase in the haem saturation of the pyrrolase caused by theophylline, but it also caused additional effects. Thus propranolol caused a 99% increase in the total pyrrolase activity of theophylline-treated rats without altering the value of the holoenzyme activity in such animals. These results suggest that propranolol causes, in theophylline-treated rats, a hormonal-type induction of pyrrolase activity (characterized by proportionate increases in both the holoenzyme and total enzyme activities without changing the haem saturation of the enzyme), probably by enhancing the release of corticosterone. This effect was confirmed when these experiments were repeated, but the mechanism requires investigation. The ability of theophylline administered alone to increase the haem saturation of tryptophan pyrrolase is reported here for the first time, whereas the similar effect caused by the above five other compounds has previously been demonstrated in fed and/or starved rats (Badawy & Evans, 1973*a*, 1975*a*,*b*, 1976*b*; Badawy, 1977; Bissell & Hammaker, 1977; Badawy *et al.*, 1981; Gurantz & Correia, 1981), as has the

Table 2. *Effects of endotoxin, morphine, theophylline, palmitate, salicylate and tryptophan on rat liver tryptophan pyrrolase activity and prevention of the effects of lipolytic agents by propranolol*

Details of design, doses and comparisons of results are as described in Table 1, except that liver tryptophan pyrrolase activity (expressed in μmol of kynurenine formed/h per g wet wt.) and the saturation of the enzyme with haem (expressed as the percentage haem saturation) were determined as described in the Materials and methods section. Values are means ± S.E.M. for each group of four rats. The significance of the differences is indicated as follows: †$P<0.05$; *$P<0.01$; **$P<0.005$; ***$P<0.001$.

	Tryptophan pyrrolase					
	Pretreatment ... 0.9% NaCl			Propranolol		
Treatment	Holoenzyme activity	Total enzyme activity	Saturation with haem (%)	Holoenzyme activity	Total enzyme activity	Saturation with haem (%)
0.9% NaCl	2.1 ± 0.15	7.0 ± 0.10	30 ± 2	2.2 ± 0.06	7.1 ± 0.30	31 ± 1
Endotoxin	6.3 ± 0.22***	8.3 ± 0.25**	76 ± 1***	2.3 ± 0.20	8.4 ± 0.81	21 ± 1†
Morphine	5.3 ± 0.29***	6.8 ± 0.30	78 ± 2***	2.0 ± 0.17	6.7 ± 0.20	30 ± 2
Palmitate	5.8 ± 0.51***	10.7 ± 0.60***	54 ± 2***	5.7 ± 0.44***	10.3 ± 0.70*	53 ± 1***
Salicylate	6.1 ± 0.27***	8.9 ± 0.40**	68 ± 2***	5.8 ± 0.21***	8.6 ± 0.20*	67 ± 1***
Tryptophan	10.5 ± 0.65***	16.1 ± 1.40***	65 ± 2***	9.4 ± 0.51***	15.3 ± 0.80***	61 ± 2***
0.9% NaCl	3.1 ± 0.16	8.4 ± 0.22	37 ± 1	3.4 ± 0.14	8.3 ± 0.12	41 ± 1
Theophylline	6.9 ± 0.14***	10.9 ± 0.56*	63 ± 2***	7.1 ± 0.25***	16.5 ± 0.98***	43 ± 1

failure of propranolol alone to influence pyrrolase activity in starved control animals (Badawy, 1981*a*). This latter finding and that (see Table 1) showing that propranolol also failed to alter 5-aminolaevulinate synthase activity in 24h-starved rats provide further support to the suggestion (Morgan & Badawy, 1980) that synthase enhancement by starvation of rats may involve an increased utilization of (as distinct from increased saturation with) the regulatory-haem pool by the apo-(tryptophan pyrrolase) newly synthesized during starvation.

Lipolysis-dependent and direct displacement of serum-protein-bound tryptophan as the causes of increased availability of circulating tryptophan to the liver after administration of endotoxin, morphine, theophylline, palmitate and salicylate and demonstration of the antilipolytic nature of the opiate-receptor antagonist naloxone

The results described so far are compatible with the possibility that the reciprocal effects on 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase observed after administration of the above first five compounds may be caused by tryptophan secondarily to a possible increase in its availability to the liver. This increased availability has previously been demonstrated in the rat *in vivo* after administration of salicylate and palmitate (Badawy & Smith, 1972; Badawy & Evans, 1975*a*) and is caused by the direct displacement by these last two agents of the serum-protein-bound amino acid (for this latter effect *in vitro*, see McArthur & Dawkins, 1969; Curzon *et al.*, 1973), whereas that by the phosphodiesterase inhibitor theophylline is strongly suggested in view of its lipolytic nature (Butcher *et al.*, 1965), which leads to a non-esterified fatty acid-mediated displacement of bound tryptophan (see Badawy & Evans, 1976*b*). As far as we could ascertain, the possible elevation of serum non-esterified fatty acid concentration by morphine or endotoxin has not been reported. As shown in Table 3, serum non-esterified fatty acid concentration was increased at 1h after administration of endotoxin or morphine to 24h-starved rats by 30 and 40% respectively, and these increases were prevented by the β-adrenoceptor-blocking agent propranolol, which was capable of decreasing fatty acid concentration in serum of starved control rats (by 36%) to a value that was only moderately higher than that observed in untreated fed animals (this latter value, expressed as in Table 3, had a mean ± S.E.M. for four rats of 0.40 ± 0.01). In experiments not described here, it was found that serum non-esterified fatty acid concentration was increased by 70% at 1h after administration to 24h-starved rats of a 100mg/kg body wt. dose of theophylline, and that propranolol was also capable of blocking this increase. These results therefore demonstrate the ability of administered endotoxin and morphine (as well as theophylline) to increase serum non-esterified fatty acid concentration, and thus confirm their lipolytic nature.

The results in Table 3 also show that endotoxin and morphine increased liver and free serum tryptophan concentrations and the percentage free serum tryptophan, which is an expression of tryptophan binding to serum proteins, and that all these increases were prevented by the anti-lipolytic

Table 3. *Effects of endotoxin and morphine on the concentrations of rat serum non-esterified fatty acids and liver and serum tryptophan and on tryptophan binding to serum proteins and their prevention by propranolol and naloxone*

Rats were starved for 24h and received, 1h before death, an intraperitoneal injection of endotoxin (2mg/kg body wt.), morphine sulphate (45mg/kg body wt.) or an equal volume (2ml/kg body wt.) of 0.9% NaCl. The animals also received, at 0.5h before the above treatments, a similar injection of propranolol (10mg/kg body wt.), naloxone hydrochloride (5mg/kg body wt.) or 0.9% NaCl (2ml/kg body wt.) The concentrations of serum non-esterified fatty acids and of liver, free serum and total serum tryptophan and the percentage free serum tryptophan were determined as described in the Materials and methods section. Values are means ± S.E.M. for each group of five rats. The values in lines 2, 3, 4 and 7 are compared with those in line 1, those in lines 5 and 6 are compared with those in line 4, and those in lines 8 and 9 are compared with those in line 7. The significance of the differences is indicated as follows: †$P<0.05$; ††$P<0.02$; *$P<0.01$; **$P<0.005$; ***$P<0.001$.

Pretreatment	Treatment	Line number	Serum non-esterified fatty acid concn. (mM)	Tryptophan concentration (μg/ml or per g wet wt.) or %			
				Free serum tryptophan	Total serum tryptophan	Free serum tryptophan (%)	Liver tryptophan
0.9% NaCl	0.9% NaCl	1	0.77 ± 0.05	1.96 ± 0.06	31.37 ± 0.90	6.25 ± 0.13	8.00 ± 0.18
	Endotoxin	2	1.00 ± 0.04††	2.21 ± 0.09**	24.44 ± 1.05**	9.04 ± 0.05***	10.30 ± 0.40***
	Morphine	3	1.08 ± 0.04**	2.71 ± 0.23††	30.12 ± 0.83	9.00 ± 0.66**	11.80 ± 0.54***
Propranolol	0.9% NaCl	4	0.49 ± 0.04**	1.46 ± 0.18†	30.50 ± 0.71	4.79 ± 0.57†	7.35 ± 0.17†
	Endotoxin	5	0.47 ± 0.04	1.49 ± 0.06	25.87 ± 1.14*	5.76 ± 0.20	7.44 ± 0.27
	Morphine	6	0.36 ± 0.03†	1.70 ± 0.12	29.00 ± 1.69	5.86 ± 0.22	8.24 ± 0.39
Naloxone	0.9% NaCl	7	0.53 ± 0.04*	1.54 ± 0.07***	21.50 ± 1.63***	7.16 ± 0.58	7.08 ± 0.10*
	Endotoxin	8	0.49 ± 0.02	1.51 ± 0.13	20.44 ± 1.21	7.39 ± 0.47	6.94 ± 0.10
	Morphine	9	0.50 ± 0.05	1.41 ± 0.10	21.00 ± 0.85	6.71 ± 0.63	7.66 ± 0.45

and β-adrenoceptor-blocking agent propranolol. These results therefore strongly suggest that the endotoxin- and morphine-induced enhancement of lipolysis increases tryptophan availability to the liver secondarily to displacement of the serum-protein-bound amino acid. The ability of propranolol to alter tryptophan binding and disposition in starved control rats (Table 3) confirms previous findings (Badawy, 1981*a*).

The opiate-receptor antagonist naloxone has been reported (Gurantz & Correia, 1981) to prevent the morphine-induced enhancement of haem oxygenase activity and of cytochrome *P*-450 degradation, although the possible prevention of the associated increase in the haem saturation of tryptophan pyrrolase was not examined by those authors. Naloxone is also known (Wright, 1981) to antagonise some haematological effects of endotoxin that are caused by reflex activation of the sympathetic nervous system. It was therefore considered of interest in view of those earlier findings and in the general context of the present work to find out if naloxone is also capable of preventing the lipolytic effects of endotoxin and morphine and the consequent changes in tryptophan binding and disposition. That naloxone does so in 24h-starved rats is suggested by the results in Table 3, which also show that, in starved control animals, naloxone decreased the concentrations of serum non-esterified fatty acids, liver, free serum and total serum tryptophan by 31, 11, 21 and 31% respectively. As is the case with propranolol in starved control rats (Table 3), the naloxone-induced inhibition of lipolysis could explain the decrease in free serum and liver tryptophan concentrations. Naloxone, however, differed from propranolol in failing to decrease the percentage free serum tryptophan, because it also decreased total serum tryptophan concentration. The mechanism of this latter effect requires investigation.

In experiments not listed here, it was found that naloxone was also capable of preventing the enhancement of lipolysis by administration to 24h-starved rats of a 100mg/kg body wt. dose of theophylline, and that both basal and theophylline-induced lipolysis in fed rats were also inhibited by this opiate-receptor antagonist, even when it was given in a smaller dose (1mg/kg body wt.). These findings therefore demonstrate for the first time the anti-lipolytic nature of naloxone, which may have important implications for anaesthetic and surgical practices. Whether naloxone inhibits lipolysis by acting on opiate receptors, by influencing opioid-peptide release and/or actions or by another mechanism(s) requires investigation.

Prevention by naloxone of the reciprocal changes in rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase caused by administration of endotoxin, morphine and theophylline

If these reciprocal changes are caused by the lipolytic effects of endotoxin, morphine and theophylline, then the ability of naloxone to inhibit

lipolysis and thereby alter tryptophan disposition suggests that this opiate-receptor antagonist may also be capable of preventing the above reciprocal changes, as is propranolol. That naloxone indeed prevents these reciprocal changes is demonstrated by the results in Tables 4, 5 and 6 in 24h-starved rats treated with endotoxin, morphine and theophylline respectively. When administered alone, naloxone did not cause any significant changes in synthase and pyrrolase activities nor in the haem saturation of this latter enzyme in starved control animals. The results in Tables 4–6 therefore provide further support to the possibility that the above three agents influence haem metabolism by enhancing lipolysis.

Correlations between serum non-esterified fatty acid, free serum and liver tryptophan concentrations and liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities

The results described so far strongly suggest that tryptophan plays a major role in the reciprocal effects of lipolytic agents and direct displacers of bound tryptophan on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase. Further support to this suggestion was sought and obtained after determination of correlation coefficients between the various parameters mentioned above. Thus, as shown in Fig. 1, r values of 0.7824 to 0.9528 were obtained after comparing the following parameters: (1) serum non-esterified fatty acid and free serum tryptophan concentrations, thus confirming a previous finding in human plasma (Curzon *et al.*, 1974); (2) free serum and liver tryptophan concentrations; (3) free serum tryptophan concentration and liver 5-aminolaevulinate synthase activity; (4) liver tryptophan concentration and 5-aminolaevulinate synthase activity; (5) liver tryptophan concentration and the haem saturation of tryptophan pyrrolase; (6) the haem saturation of tryptophan pyrrolase and 5-aminolaevulinate synthase activity; (7) holo-(tryptophan pyrrolase) and 5-aminolaevulinate synthase activities.

Possible control of tryptophan availability in the liver by hepatic intracellular binding

As the results in Tables 1–3 show, lipolytic agents and direct displacers of serum-protein-bound tryptophan were as effective as administered tryptophan in influencing 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase, although the former two groups of compounds caused only moderate increases in liver tryptophan concentration (Badawy & Smith, 1972; Badawy & Evans, 1975*a*; Table 3 of the present work), in comparison with those produced by the administered amino acid (see, e.g., Badawy & Smith, 1972). These last authors presented evidence suggesting that tryptophan may be bound in the liver, and showed that a dose of sodium salicylate (200mg/kg body wt.), which did not cause any significant stimulation of pyrrolase activity in fed rats, rendered a similarly inactive dose of tryptophan (50mg/kg body wt.) capable of activating the enzyme. These findings therefore suggest that the availability of tryptophan in the liver may be controlled by the extent of its possible binding. To test this point in relation to the present work, the experiments whose results are shown in Table 7 were

Table 4. *Prevention by naloxone of the effects of endotoxin on rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities*

Rats were starved for 24h and received, 4h before death, an intraperitoneal injection of either endotoxin (2mg/kg body wt.) or an equal volume (2ml/kg body wt.) of 0.9% NaCl. The animals also received, at 0.5h before the above treatments, a similar injection of either naloxone hydrochloride (5mg/kg body wt.) or an equal volume (12.5ml/kg body wt.) of 0.9% NaCl. Liver synthase and pyrrolase activities were determined as described in the Materials and methods section. Values are means ± S.E.M. for each group of four rats. The values in columns (2) and (3) are compared with those in column (1), whereas those in column (4) are compared with those in column (3), and the significance of the differences is indicated as follows: $^{*}P<0.025$; $^{**}P<0.005$; $^{***}P<0.001$. Synthase activity is expressed in nmol of 5-aminolaevulinate formed/min per g wet wt. of liver, whereas pyrrolase activities are in μmol of kynurenine formed/h per g wet wt. of liver. The haem saturation of the latter enzyme is expressed as the percentage haem saturation (100 × holoenzyme activity/total enzyme activity).

	Pretreatment ...	0.9% NaCl		Naloxone	
	Treatment ...	0.9% NaCl	Endotoxin	0.9% NaCl	Endotoxin
Determination		(1)	(2)	(3)	(4)
Synthase activity		0.42 ± 0.06	0.22 ± 0.03*	0.47 ± 0.04	0.40 ± 0.02
Pyrrolase activity					
Holoenzyme		3.20 ± 0.20	5.90 ± 0.57**	3.60 ± 0.36	3.40 ± 0.18
Total enzyme		10.40 ± 0.57	10.20 ± 1.10	10.70 ± 0.35	11.10 ± 0.62
Pyrrolase saturation with haem (%)		31 ± 1	58 ± 4***	34 ± 1	31 ± 1

Table 5. *Prevention by naloxone of the effects of morphine on rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities*

Experimental details, design, comparisons and expressions of results are as described in Table 4, except that the rats received, 4h before death, an intraperitoneal injection of either morphine sulphate (45mg/kg body wt.) or an equal volume (2ml/kg body wt.) of 0.9% NaCl. The significance of the differences is indicated as follows: $*P<0.005$; $**P<0.001$.

Pretreatment ...	0.9% NaCl		Naloxone	
Treatment ...	0.9% NaCl	Morphine	0.9% NaCl	Morphine
Determination	(1)	(2)	(3)	(4)
Synthase activity	0.52 ± 0.03	0.28 ± 0.01**	0.50 ± 0.02	0.55 ± 0.05
Pyrrolase activity				
Holoenzyme	2.70 ± 0.17	5.00 ± 0.42*	2.70 ± 0.30	3.10 ± 0.28
Total enzyme	7.10 ± 0.53	9.00 ± 1.09	6.90 ± 0.63	9.00 ± 0.90
Pyrrolase saturation with haem (%)	38 ± 1	55 ± 3*	39 ± 2	34 ± 2

Table 6. *Prevention by naloxone of the effects of theophylline on rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities*

Experimental details, design, comparisons and expressions of results are as described in Table 4, except that the rats received, 4h before death, an intraperitoneal injection of either theophylline (100mg/kg body wt.) or an equal volume (2ml/kg body wt.) of 0.9% NaCl. The significance of the differences is indicated as follows: $*P<0.01$; $**P<0.001$.

Pretreatment ...	0.9% NaCl		Naloxone	
Treatment ...	0.9% NaCl	Theophylline	0.9% NaCl	Theophylline
Determination	(1)	(2)	(3)	(4)
Synthase activity	0.44 ± 0.05	0.23 ± 0.01*	0.45 ± 0.03	0.44 ± 0.02
Pyrrolase activity				
Holoenzyme	3.10 ± 0.16	6.90 ± 0.14**	3.20 ± 0.06	3.30 ± 0.21
Total enzyme	8.40 ± 0.22	10.90 ± 0.56*	8.70 ± 0.93	8.50 ± 0.23
Pyrrolase saturation with haem (%)	37 ± 1	63 ± 2**	37 ± 3	39 ± 1

performed. As shown, neither the activities of 5-aminolaevulinate synthase and tryptophan pyrrolase nor the haem saturation of this latter enzyme were significantly altered in 24h-starved rats after administration of a 50mg/kg body wt. dose of either salicylate or tryptophan. The ineffectiveness of this latter dose of tryptophan in starved rats has previously been reported (Badawy *et al.*, 1981), despite its ability to increase liver tryptophan concentration by over 200% (Badawy & Smith, 1972). By contrast, combined administration of salicylate plus tryptophan (Table 7) caused a 35% decrease in synthase activity and increased the holo-(tryptophan pyrrolase) activity and the haem saturation of this latter enzyme by 49 and 44% respectively. The magnitude of these changes resembles approximately that caused by administration of a 75mg/kg body wt. dose of tryptophan (Badawy *et al.*, 1981). These findings and those previously reported (Badawy & Smith, 1972) therefore suggest that salicylate increases tryptophan availability for production of these hepatic effects by displacing it from binding sites on serum and possbily also liver proteins. Much work is clearly required to establish such possible hepatic tryptophan binding.

General conclusions and comments

In addition to providing considerable support to the concept of the inverse relationship between the haem saturation of rat liver tryptophan pyrrolase and the activity of 5-aminolaevulinate synthase (see Welch & Badawy, 1980; Badawy *et al.*, 1981), the present findings have established the anti-lipolytic nature of the opiate-receptor antagonist naloxone and the ability of direct displacers of serum-protein-bound tryptophan to cause reciprocal changes in the above two parameters. The results also strongly suggest that endotoxin and morphine increase haem availability to tryptophan pyrrolase and 5-aminolaevulinate synthase by acting via tryptophan secondarily to stimulation of lipolysis. This latter hypothesis could serve as an alternative to that (Bissell & Hammaker, 1976*a*,*b*, 1977; Gurantz & Correia, 1981) postulating a dissociation by these two agents of the haem moiety of cytochrome *P*-450. This latter haemoprotein has not

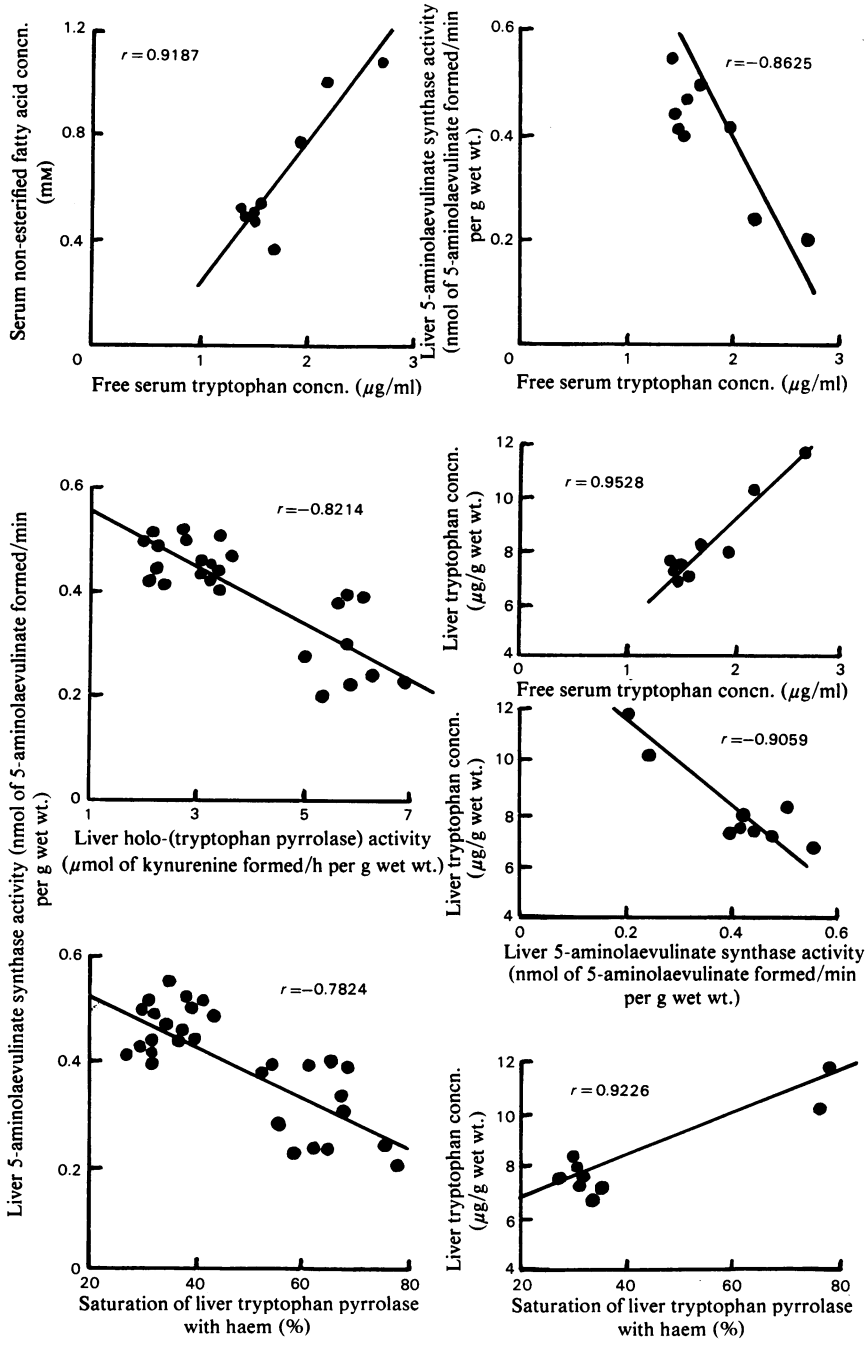

Fig. 1. *Correlations between various parameters of lipolysis, tryptophan disposition and the activities of liver 5-aminolaevulinate synthase and tryptophan pyrrolase*

Correlation coefficients for various pairs of the parameters examined have been determined from the various results obtained in the present work. The only data not included in these correlations were those on the holo-(tryptophan pyrrolase) activities in rats treated with either tryptophan or theophylline plus propranolol, because the large increases in the holoenzyme activity observed under these two conditions (see Table 2) were associated with large increases in the total enzyme activity which usually lead to simultaneous and often proportionate increases in that of the holoenzyme by a mechanism not necessarily involving altered saturation of the enzyme with haem.

Table 7. *Effects of salicylate, tryptophan or both on rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities*

Rats were starved for 24h and received, 0.5h before death, an intraperitoneal injection of either tryptophan (50mg/kg body wt.) or an equal volume (20ml/kg body wt.) of 0.9% NaCl. The animals also received, at 0.25h before the above treatments, a similar injection of either sodium salicylate (50mg/kg body wt.) or an equal volume (2ml/kg body wt.) of 0.9% NaCl. Synthase and pyrrolase activities and the haem saturation of the latter enzyme were determined as described in the Materials and methods section, and are expressed as in Table 4. Values are means ± S.E.M. for each group of four rats. The values obtained in rats treated with salicylate, tryptophan or both are compared with those observed in animals given 0.9% NaCl alone, and the significance of the differences is indicated as follows: $*P < 0.01$; $**P < 0.001$.

	Tryptophan pyrrolase			
Treatment	Holoenzyme activity	Total enzyme activity	Saturation with haem (%)	5-Aminolaevulinate synthase activity
0.9% NaCl	4.1 ± 0.09	8.6 ± 0.23	48 ± 1	0.49 ± 0.02
Salicylate	4.2 ± 0.19	8.0 ± 0.14	52 ± 3	0.44 ± 0.04
Tryptophan	4.0 ± 0.20	8.4 ± 0.32	48 ± 0	0.45 ± 0.03
Salicylate + tryptophan	6.1 ± 0.25**	8.8 ± 0.39	69 ± 3**	0.32 ± 0.04*

been examined in the present work, and it therefore remains to be seen if its haem moiety could also be influenced by the various manipulations used in the present experiments. Also because haem oxygenase activity was not examined in the present work, it would be of interest to find out if direct displacers of serum-protein-bound tryptophan and also tryptophan itself and theophylline are capable of enhancing it, and whether such an enhancement by lipolytic agents (including endotoxin and morphine) is sensitive to anti-lipolytic agents, Finally, the relationship between lipolysis, tryptophan disposition and 5-aminolaevulinate synthase activity demonstrated here raises the strong possibility that synthesis of this latter enzyme may be subject to control by liver tryptophan and by nutritional and other physiological factors influencing lipid metabolism and tryptophan disposition, and may be influenced by pharmacological agents acting on these latter two metabolic systems.

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