Biochem. J. (1980) **186**, 993–996
Printed in Great Britain

Tryptophan Pyrrolase in Haem Regulation.

THE MECHANISM OF THE PERMISSIVE EFFECT OF CORTISOL ON THE ENHANCEMENT OF 5-AMINOLAEVULINATE SYNTHASE ACTIVITY BY 2-ALLYL-2-ISOPROPYLACETAMIDE IN THE ADRENALECTOMIZED-RAT LIVER

Christopher J. MORGAN and Abdulla A.-B. BADAWY*
South Glamorgan Health Authority, Addiction Unit Research Laboratory, Whitchurch Hospital, Cardiff CF4 7XB, Wales, U.K.

(*Received 26 November 1979*)

The decreased ability of 2-allyl-2-isopropylacetamide to enhance liver 5-aminolaevulinate synthase activity in the adrenalectomized rat is not associated with a marked depletion of the already low amount of tryptophan pyrrolase haem. Cortisol permits the porphyrogen markedly to enhance synthase activity by rendering it capable of causing a stronger depletion of pyrrolase haem, presumably as a result of hormonal induction of pyrrolase synthesis.

The mechanism by which certain chemicals (porphyrogens) markedly enhance mammalian liver 5-aminolaevulinate synthase (EC 2.3.1.37) activity represents an interference with the negative feedback control of this enzyme involving the early depletion of a small and rapidly turning-over pool(s) of haem (De Matteis, 1975). Because of the small size of this regulatory and readily exchangeable pool (Granick *et al.*, 1975; Badawy, 1978), this depletion has been indirectly demonstrated by determination of the 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). Of these, pyrrolase is the most sensitive marker of delicate changes in liver haem concentration (Badawy, 1979). Moreover, evidence (Badawy & Morgan, 1980) suggests that pyrrolase haem may play an important role in the marked enhancement of 5-aminolaevulinate synthase activity by the porphyrogen 2-allyl-2-isopropylacetamide.

Marver *et al.* (1966) reported that administration of 2-allyl-2-isopropylacetamide to adrenalectomized rats fails to enhance markedly synthase activity, but that simultaneous administration of cortisol enables the porphyrogen to exert its effect to the extent observed in intact animals. Marver *et al.* (1966) described this effect of cortisol as permissive and suggested that it may be related to the action of the hormone on RNA synthesis. Since this porphyrogen markedly enhances synthase activity in intact rats by causing an early depletion of liver haem, it is reasonable to suggest that its failure to do so in adrenalectomized animals may be associated with an absence of haem depletion. Since tryptophan pyrrolase activity (and hence the amount of haem bound to the apoenzyme) is decreased after adrenalectomy (Wetterberg *et al.*, 1970), and in view of the importance of haem in enhancement of synthase activity by this porphyrogen, we have examined the hypothesis that the permissive effect of cortisol involves a pyrrolase–haem-mediated mechanism.

Materials and Methods

Male Wistar rats (150–180g) were used. Intact rats were bred locally, whereas adrenalectomized rats and their controls (sham-operated) were purchased from Hacking and Churchill, Wyton, Huntingdon, Cambs., U.K. Completeness of adrenalectomy was verified by visual inspection at the time of death. The rats were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and either drinking water (sham-operated) or 0.9% (w/v) NaCl (adrenalectomized rats) for 7–10 days after surgery. Most rats were then starved for 48h before death, but all had free access to their drinking fluids during this time.

Allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) and 2-allyl-2-isopropylacetamide were gifts from the Wellcome Foundation, London, U.K., and Roche Products, Welwyn Garden City, Herts., U.K. respectively. The sources of all other chemicals have been described by Badawy & Evans (1975). Allopurinol was injected intraperitoneally (20mg/kg) as a solution (4ml/kg) in 0.9% NaCl prepared as described by Badawy & Evans (1973*a*). Cortisol acetate (20mg/kg) and 2-allyl-2-isopropylaceta-

* To whom reprint requests should be addressed.

0306-3283/80/030993-04 $1.50/1

mide (400mg/kg) were dissolved in dimethylformamide (1ml/kg) and were injected intraperitoneally or into the loose subcutaneous tissues of the neck respectively. Control rats received equal volumes of the appropriate solvent(s) by the same route(s).

Tryptophan pyrrolase activity was determined in liver homogenates (Badawy & Evans, 1975) in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin (2μM). The apoenzyme activity, obtained by difference, was used to calculate the haem-saturation ratio (holoenzyme activity/apoenzyme activity), which indicates the extent of saturation of the apoenzyme with haem (see Badawy, 1979). 5-Aminolaevulinate synthase activity was determined in liver homogenates by a modification (Badawy & Morgan, 1980) of the procedures of Yoda *et al.* (1974) and De Matteis (1971). Statistical analysis of results was performed by Student's *t* test.

Results and Discussion

The results of experiments with adrenalectomized and control (sham-operated) rats are shown in Table 1. Starvation of sham-operated rats for 48h increased the holoenzyme and total enzyme activities of liver tryptophan pyrrolase by 180 and 189% respectively ($P<0.001$). These similar increases did not therefore alter the haem-saturation ratio of the enzyme. This type of enhancement is characteristic of a hormonal induction mechanism (see also Badawy, 1979), and is probably corticosterone-mediated because it can be prevented by adrenalectomy (Table 1) and by actinomycin D (Badawy, 1977). Administration of cortisol to starved sham-operated rats increased both holoenzyme and total pyrrolase activities by 50% ($P=0.005$–0.001) and did not therefore alter the haem-saturation ratio. Liver 5-aminolaevulinate synthase activity in fed rats is half of that in starved rats (Bock *et al.*, 1971), and this is confirmed by our results with sham-operated animals (Table 1). In agreement with the finding by Marver *et al.* (1966), cortisol does not exert a significant effect on synthase activity in control (sham-operated) rats (Table 1), and this is consistent with the inability of the hormone to alter the haem-saturation ratio of tryptophan pyrrolase. Although this ratio is generally inversely related to synthase activity, an exception is provided by the above starvation-induced enhancement of the latter enzyme activity in the absence of an altered haem-saturation ratio of tryptophan pyrrolase. This exception may be explained by: (1) starvation enhancing synthase activity by increasing the utilization by tryptophan pyrrolase of the haem pool

Table 1. *Effects of administration of 2-allyl-2-isopropylacetamide, cortisol, or both, on liver tryptophan pyrrolase and 5-aminolaevulinate synthase activities in starved adrenalectomized and sham-operated rats*

Adrenalectomized and sham-operated rats were starved for 48h before death. 2-Allyl-2-isopropylacetamide (AIA; 400mg/kg) and cortisol acetate (20mg/kg) were injected either separately or together and the animals were killed 5h later. When given separately, each injection was accompanied by an injection of the solvent dimethylformamide (1ml/kg) by the other route. Control rats received two injections of the solvent. The results with fed sham-operated rats are included for comparison. Activities of liver 5-aminolaevulinate synthase and tryptophan pyrrolase and the haem-saturation ratio of the latter enzyme 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 line 2 should be compared with those in line 1, those in lines 3 and 4 should be compared with those in line 2 and those in line 5 should be compared with those in line 4. Similarly, the results in line 6 should be compared with those in line 2, those in lines 7 and 8 should be compared with those in line 6 and those in line 9 should be compared with those in line 8. The significance of differences is indicated as follows: *$P<0.05$; †$P<0.01$; ††$P<0.005$; †††$P<0.001$.

	Treatment of animals			Tryptophan pyrrolase activity (μmol of kynurenine formed/h per g wet wt. of liver)		Tryptophan pyrrolase haem-saturation ratio	Synthase activity (nmol of 5-aminolaevulinate formed/min per g wet wt. of liver)
	Surgery	Nutrition	Injections	Holoenzyme	Total enzyme		
1	Sham-operation	Fed	Nil	2.0 ± 0.03	4.5 ± 0.13	0.82 ± 0.02	0.38 ± 0.06
2	Sham-operation	Starved	Solvents	5.6 ± 0.20†††	13.0 ± 0.34†††	0.76 ± 0.07	0.72 ± 0.12*
3			AIA	3.2 ± 0.11†††	12.7 ± 1.34	0.34 ± 0.04††	2.89 ± 0.50†
4			Cortisol	8.4 ± 0.59††	19.4 ± 0.43†††	0.76 ± 0.06	0.56 ± 0.03
5			AIA + cortisol	4.9 ± 0.53††	19.2 ± 0.91	0.34 ± 0.03†††	2.79 ± 0.37†††
6	Adrenalectomy	Starved	Solvents	2.2 ± 0.09†††	4.5 ± 0.25†††	0.96 ± 0.22	0.44 ± 0.04*
7			AIA	1.9 ± 0.06*	4.4 ± 0.09	0.76 ± 0.07	0.99 ± 0.09††
8			Cortisol	9.6 ± 0.91†††	21.8 ± 2.19†††	0.79 ± 0.06	0.50 ± 0.09
9			AIA + cortisol	6.0 ± 0.78*	21.8 ± 2.27	0.38 ± 0.01††	3.31 ± 0.40†††

Table 2. *Effects of administration of allopurinol, 2-allyl-2-isopropylacetamide or both on the activities of liver tryptophan pyrrolase and 5-aminolaevulinate synthase in starved intact rats*

Locally bred intact rats were starved for 48h before death. Allopurinol (20mg/kg) or an equal volume (4ml/kg) of 0.9% NaCl was injected intraperitoneally 20min before a subcutaneous injection of either 2-allyl-2-isopropylacetamide (AIA; 400mg/kg) or an equal volume (1ml/kg) of dimethylformamide; the animals were killed 5h after the second injection. Activities of 5-aminolaevulinate synthase and tryptophan pyrrolase and the haem-saturation ratio of the latter enzyme were determined as described in the Materials and Methods section. Values are means ± S.E.M. for each group of four rats. The results with allopurinol alone or 2-allyl-2-isopropylacetamide alone should be compared with those obtained with the solvents, whereas those with the two drugs should be compared with those with allopurinol alone. The significance of differences is indicated as follows: $^{**}P<0.025$; $^{***}P<0.02$; †$P<0.01$; ††$P<0.005$; †††$P<0.001$.

Treatment	Tryptophan pyrrolase activity (μmol of kynurenine formed/h per g wet wt. of liver)		Tryptophan pyrrolase haem-saturation ratio	Synthase activity (nmol of 5-aminolaevulinate formed/min per g wet wt. of liver)
	Holoenzyme	Total enzyme		
Solvents	5.4 ± 0.39	13.5 ± 0.57	0.67 ± 0.03	1.65 ± 0.08
Allopurinol	1.9 ± 0.05†††	4.4 ± 0.23†††	0.76 ± 0.07	0.88 ± 0.08†††
AIA	3.6 ± 0.37***	13.8 ± 1.07	0.35 ± 0.02†††	5.03 ± 0.09†††
AIA + allopurinol	2.1 ± 0.17	6.1 ± 0.27††	0.52 ± 0.04**	3.24 ± 0.40†

involved in synthase regulation, because reversal of this latter effect by allopurinol prevents the effect of starvation on synthase activity (see Table 2); (2) under certain other experimental conditions (A. N. Welch & A. A.-B. Badawy, unpublished work), it can be shown that the haem saturation of tryptophan pyrrolase is not simply a passive expression of the regulatory haem pool, but is actively involved in determining the availability of this pool for synthase regulation.

Administration of 2-allyl-2-isopropylacetamide to starved sham-operated rats decreased the holo-(tryptophan pyrrolase) activity and the haem-saturation ratio by 43–55% ($P=0.005$–0.001) and increased 5-aminolaevulinate synthase activity 4-fold ($P<0.01$). This latter effect is less than that (10-fold) reported by Marver *et al.* (1966). Administration of cortisol to sham-operated rats did not modify the extent of synthase enhancement by 2-allyl-2-isopropylacetamide (Table 1), confirming the finding by Marver *et al.* (1966). Under these conditions, cortisol did not alter the extent of haem depletion by the porphyrogen.

The starvation-induced enhancement of pyrrolase and synthase activities was prevented by adrenalectomy (Table 1), and the values observed were similar to those in untreated fed control rats. Cortisol administration to starved adrenalectomized rats increased the pyrrolase activities to values similar to those observed in hormone-treated sham-operated animals. Cortisol, however, did not alter the haem-saturation ratio or the synthase activity. In agreement with the finding by Marver *et al.* (1966), synthase activity was enhanced only 2-fold by 2-allyl-2-isopropylacetamide administration to adrenalectomized rats (Table 1). Under these conditions, holo-(tryptophan pyrrolase) activity was decreased (only 14%; $P<0.05$) by the porphyrogen. These results suggest that the failure of this porphyrogen to enhance markedly synthase activity in adrenalectomized rats may be due to its moderate depletion of haem. This porphyrogen does not decrease the activity of holo-(tryptophan pyrrolase) of untreated fed rats, but it does decrease the haem-saturation ratio in these animals by enhancing the total enzyme activity by a hormonal-type mechanism and by simultaneously preventing the conjugation of the newly synthesized apoenzyme with haem, presumably by increasing the destruction of the latter (Badawy & Evans, 1973*b*). 2-Allyl-2-isopropylacetamide decreases the holo-(tryptophan pyrrolase) activity that had been elevated in fed rats by administration of cortisol and 5-aminolaevulinate (Badawy & Evans, 1973*b*). It is therefore possible that the moderate decrease in the holoenzyme activity caused by the porphyrogen in adrenalectomized rats (Table 1) may be explained by the haem pool of the basal pyrrolase (that found in fed rats) being resistant to depletion. By contrast, when the pool of pyrrolase haem is increased by administration of cortisol to adrenalectomized rats (Table 1), 2-allyl-2-isopropylacetamide becomes capable of depleting it to the same relative extent observed in sham-operated animals. Under these conditions, cortisol renders the porphyrogen capable of enhancing synthase activity to a value not significantly different ($P>0.10$) from that in control animals.

The decreased ability of 2-allyl-2-isopropylacetamide to deplete pyrrolase haem in adrenalectomized rats, and the reversal of this decrease by cortisol, could therefore explain the observed

changes in synthase activity. The possibility that metabolism of the porphyrogen by the mixed-function-oxidase system to the active (haem-depleting) metabolite(s) may be impaired by adrenalectomy cannot be ruled out. However, adrenalectomy does not always impair drug metabolism by this system (for a review, see Kato, 1977), and it is known that such surgery causes only moderate decreases in cytochrome *P*-450 concentration, which cannot be reversed by repeated daily injections of cortisol (Castro *et al.*, 1970). These authors found, however, that such doses of cortisol reverse the adrenalectomy-induced decreases in ethylmorphine metabolism and in NADPH-dependent aspects of the mixed-function-oxidase system. The possible effects of a single dose of cortisol on these aspects have not been examined, and further work is clearly required to clarify these points.

The effects of adrenalectomy on the synthase and pyrrolase activities described above could be reproduced in starved intact rats by administration of allopurinol, a specific inhibitor of the conjugation of apo-(tryptophan pyrrolase) with haem in the liver of fed rats (Badawy & Evans, 1973*a*). The results in Table 2 show that allopurinol reversed the starvation-induced increases in pyrrolase activity and also decreased synthase activity by 47% ($P < 0.001$). This suggests that the pyrrolase haem pool in starved rats may regulate synthase activity (see above). In the absence of allopurinol, 2-allyl-2-isopropylacetamide decreased holo-(tryptophan pyrrolase) activity and the haem-saturation ratio by 33–48% and enhanced synthase activity by about 3-fold ($P < 0.001$), thus confirming the results in Table 1 for sham-operated rats. However, in the presence of allopurinol, the effects of the porphyrogen on synthase activity and pyrrolase haem were similar to those observed in porphyrogen-treated adrenalectomized rats; synthase enhancement was only 2-fold and saturation of pyrrolase with haem (as determined by the saturation ratio) was decreased by only 22% ($P < 0.025$). The results of these experiments with allopurinol therefore provide further support for the hypothesis that the permissive effect of cortisol on the enhancement of synthase activity by 2-allyl-2-isopropylacetamide in adrenalectomized rats involves changes in the amount of haem associated with liver tryptophan pyrrolase caused by hormonal induction of this latter enzyme.

We are grateful to the Wellcome Trustees for their support, Dr. M. Evans for his interest, Mr. A. Dacey for animal maintenance, Dr. L. Rowlands of the South Glamorgan Institute of Higher Education (Cardiff) for providing technical assistance by his students, and Dr. G. E. Lovatt and Mrs. C. Harper for generous gifts of allopurinol and 2-allyl-2-isopropylacetamide respectively.

References

Badawy, A. A.-B. (1977) *Life Sci.* **21**, 755–767
Badawy, A. A.-B. (1978) *Biochem. J.* **172**, 487–494
Badawy, A. A.-B. (1979) *Biochem. Soc. Trans.* **7**, 575–583
Badawy, A. A.-B. & Evans, M. (1973*a*) *Biochem. J.* **133**, 585–591
Badawy, A. A.-B. & Evans, M. (1973*b*) *Biochem. J.* **136**, 885–892
Badawy, A. A.-B. & Evans, M. (1975) *Biochem. J.* **150**, 511–520
Badawy, A. A.-B. & Morgan, C. J. (1980) *Biochem. J.* **186**, 763–772
Bock, K. W., Krauss, E. & Fröhling, W. (1971) *Eur. J. Biochem.* **23**, 366–371
Castro, J. A., Greene, F. E., Gigon, P., Sesame, H. & Gillette, J. R. (1970) *Biochem. Pharmacol.* **19**, 2461–2467
De Matteis, F. (1971) *Biochem. J.* **124**, 767–777
De Matteis, F. (1975) in *Enzyme Induction* (Parke, D. V., ed.), pp. 185–205, Plenum Press, London
Granick, S., Sinclair, P., Sassa, S. & Grieninger, G. (1975) *J. Biol. Chem.* **250**, 9215–9225
Kato, R. (1977) *Xenobiotica* **7**, 25–92
Marver, H. S., Collins, A. & Tschudy, D. P. (1966) *Biochem. J.* **99**, 31c–33c
Wetterberg, L., Geller, E. & Yuwiler, A. (1970) *Biochem. Pharmacol.* **19**, 2833–2838
Yoda, B., Schacter, B. A. & Israels, L. G. (1974) *Biochim. Biophys. Acta* **372**, 478–481