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Tryptophan pyrrolase in haem regulation

The mechanism of the opposite effects of tryptophan on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase

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1. Administration of tryptophan to starved rats causes a rapid decrease in liver 5-aminolaevulinate synthase activity associated with an increase in the haem saturation of tryptophan pyrrolase. Both effects are maximally produced at 30 min by a 100 mg/kg body wt. dose of tryptophan. 2. Pb^{2+} prevents both effects. 3. Prevention by allopurinol or benserazide of the tryptophan-induced increase in the haem saturation of tryptophan pyrrolase renders this haem available for further repression of synthase synthesis. 4. The opposite effects on synthase activity and pyrrolase saturation with haem caused by administration of 5-aminolaevulinate, but not those by that of haematin, are potentiated by tryptophan. 5. It is suggested that tryptophan decreases 5-aminolaevulinate synthase activity and causes the initial increase in the haem saturation of tryptophan pyrrolase by enhancing the conversion of 5-aminolaevulinate into haem by a process requiring protein synthesis.

There is considerable evidence suggesting that, in addition to being the most sensitive hepatic haemoprotein for assessing small changes in haem concentration, rat liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) utilizes the small, readily exchangeable and rapidly-turning-over pool(s) of haem involved in the feedback regulation of haem synthesis, exerted at the 5-aminolaevulinate synthase (EC 2.3.1.37) step of the haem-biosynthetic pathway (Badawy, 1979, and references cited therein; Badawy & Morgan, 1980; Morgan & Badawy, 1980; Welch & Badawy, 1980). We have observed a complex inverse relationship between the haem saturation of tryptophan pyrrolase and the activity of 5-aminolaevulinate synthase in which the former apparently participates in determining the availability of the regulatory-haem pool (Welch & Badawy, 1980).

One aspect of this inverse relationship (the decrease in 5-aminolaevulinate synthase activity observed when pyrrolase saturation with haem is increased), which has resulted from studies with 5-aminolaevulinate, endotoxin and haematin (Druyan & Kelly, 1972; Bissell & Hammaker, 1977; Welch & Badawy, 1980), is at variance with the work of Marver *et al.* (1966), who reported that tryptophan, which had previously been shown to increase the haem saturation of tryptophan pyrrolase (Greengard & Feigelson, 1961; Knox, 1966), causes a marked enhancement of 5-aminolaevulinate synthase activity. Marver *et al.* (1966) suggested that tryptophan causes this latter effect by removing the negative feedback control of synthase synthesis as a result of increasing haem utilization by apo-(tryptophan pyrrolase). It is, however, difficult to see how a simple increase in the utilization of (presumably preformed) haem by a haemoprotein could be sensitive to blockade by translational inhibitors (see e.g. Badawy & Evans, 1975). We have therefore re-examined the above suggestion of Marver *et al.* (1966) by performing a detailed study of the effects of tryptophan administration 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.

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Materials and methods

Chemicals

Actinomycin D, allopurinol (4-hydroxypyrazolo-[3,4-*d*]pyrimidine) and the aromatic amino acid

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decarboxylase inhibitor benserazide [*N*-(seryl)-*N*-(2,3,4-trihydroxybenzyl)hydrazine; compound Ro4-4602] were gifts from Merck, Sharp and Dohme (Hoddesdon, Herts., U.K.), the Wellcome Foundation (London NW1 2BP, U.K.) and Roche Products (Welwyn Garden City, Herts., U.K.) respectively. 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

Rats (160g ± 6%) were locally bred and were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water. The animals were starved for 48h before being killed between 13:00 and 14:30h by stunning and cervical dislocation. Most experiments were performed with male Wistar rats, although a limited number of experiments were carried out with male and female rats of the Sprague–Dawley strain.

All compounds were administered intraperitoneally. Haematin hydrochloride (2mg/kg body wt.) was dissolved in dimethylformamide (1ml/kg body wt.) and lead acetate (10mg/kg body wt.) in water (1ml/kg body wt.). Control rats received an equal volume of the appropriate solvent. All other compounds were dissolved in 0.9% (w/v) NaCl and were given in volumes of 2ml/kg body wt. each (except allopurinol and tryptophan, which were given in volumes of 4 and 20ml/kg body wt. respectively) and in the following doses: actinomycin D (0.7mg/kg body wt.), allopurinol (20mg/kg body wt.), 5-aminolaevulinate hydrochloride (15mg/kg body wt.), benserazide (400mg/kg body wt.), cycloheximide (10mg/kg body wt.), puromycin (35mg/kg body wt.), tryptophan (50–750mg/kg body wt.). The allopurinol and tryptophan solutions for injection were prepared as described by Badawy & Evans (1973, 1975) respectively. Control rats received an equal volume of 0.9% NaCl.

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 2μM-haematin. The saturation of the enzyme with haem was expressed as the percentage saturation (100 × holoenzyme activity/total enzyme activity).

5-Aminolaevulinate synthase activity was determined in liver homogenates by a method (Badawy & Morgan, 1980) based on colorimetric determination of the product (De Matteis, 1971). Interfering indoles (derived from tryptophan) were removed by shaking the combined supernatants of duplicate samples from each liver assay (combined volume 8ml) with 0.6g of a mixture of deactivated charcoal and celite (7:5, w/w) and centrifugation (800*g* for 10min). This decreased apparent values by up to 15% and was quicker and more effective than the column procedure of Marver *et al.* (1966).

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

Results and discussion

Effects of tryptophan on liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase in Sprague–Dawley rats

When we found that tryptophan administration to male Wistar rats decreased liver 5-aminolaevulinate synthase activity, it was thought that the marked increase observed by Marver *et al.* (1966) may be specific to female Sprague–Dawley rats. We therefore repeated our experiments with male and female Sprague–Dawley rats. The effects of tryptophan on synthase and pyrrolase activities were therefore

Table 1. *Effects of tryptophan administration to Sprague–Dawley rats on liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities*

Male and female rats of the above strain were starved for 48h, and received an injection of either tryptophan (500mg/kg body wt.) or an equal volume (20ml/kg body wt.) of 0.9% (w/v) NaCl at 4h, before being killed. Synthase and pyrrolase activities and the haem saturation 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 observed in tryptophan-treated rats are compared with those in control (0.9% NaCl-treated) animals, and the significance of the differences is indicated as follows: *$P < 0.01$; **$P < 0.005$; ***$P < 0.001$.

Sex	Treatment	Tryptophan pyrrolase activity (μmol of kynurenine formed/h per g wet wt. of liver): Holoenzyme	Tryptophan pyrrolase activity (μmol of kynurenine formed/h per g wet wt. of liver): Total enzyme	Saturation of pyrrolase with haem (%)	Synthase activity (nmol of 5-aminolaevulinate formed/min per g wet wt. of liver)
Male	0.9% NaCl	3.4 ± 0.17	7.6 ± 0.33	45 ± 2	0.83 ± 0.11
	Tryptophan	7.9 ± 0.56***	11.5 ± 0.65**	69 ± 5**	0.34 ± 0.04*
Female	0.9% NaCl	2.3 ± 0.07	5.2 ± 0.08	44 ± 1	0.63 ± 0.03
	Tryptophan	10.3 ± 0.44***	15.2 ± 0.91***	68 ± 3***	0.31 ± 0.02***

examined at the time interval (4h) at which the above authors observed the maximum enhancement. The results in Table 1 show that tryptophan decreased synthase activity in male and female Sprague–Dawley rats by 59 and 51% respectively ($P<0.001$). Under these conditions, tryptophan produced its classical substrate-type enhancement of pyrrolase activity in both sexes of this strain of rats; it increased the haem saturation of the enzyme from a control value of 44–45% to values of 68–69%. It is noteworthy that pyrrolase activities of starved female rats of the above strain were 32% ($P<0.001$) lower than those of males, and that the tryptophan enhancement of the female-enzyme activities was more pronounced than that observed in males. The above findings therefore exclude the possibility that tryptophan influences synthase activity by a sex- or rat-strain-dependent mechanism. Accordingly, all subsequent experiments were performed with male Wistar rats.

Failure of tryptophan to influence in vitro the activity of rat liver 5-aminolaevulinate synthase

Addition *in vitro* of tryptophan (2mM) to liver homogenates from 48h-starved rats did not cause any significant changes ($P>0.10$) in 5-aminolaevulinate synthase activity. Synthase activities (in nmol of 5-aminolaevulinate formed/min per g wet wt. of liver ± S.E.M. for each group of homogenates from four different rats) were as follows: control homogenates (0.51 ± 0.01); tryptophan-treated homogenates (0.49 ± 0.02). These results therefore exclude the possibility that tryptophan exerts a direct effect on 5-aminolaevulinate synthase activity.

Time course of the effects of tryptophan administration on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase

The time course of these effects of a 500mg/kg body wt. dose of tryptophan is shown in Fig. 1. As early as 15min after tryptophan administration, synthase activity was decreased by 39% ($P<0.001$). The decrease was maximum (62%; $P<0.001$) at 30min. Synthase activity then started to recover slowly, reaching values no longer different significantly from those of control rats at 12h.

This decrease in synthase activity exhibited a reciprocal relationship with the tryptophan-induced increase in the haem saturation of tryptophan pyrrolase (Fig. 1). The latter was maximum (98%; $P<0.001$) at 30min, after which time it was decreased, reaching a 50% value at 8h. Normal saturation was then observed at 12h after tryptophan administration. The increase in this saturation at 30min was caused only by the enhancement of the holo-(tryptophan pyrrolase) activity, whereas all subsequent increases were associated with an enhancement of both the holoenzyme and total pyrrolase activities, which were maximally increased (by 6- and 3.1-fold respectively; $P<0.001$) at 4h after tryptophan administration (results not shown).

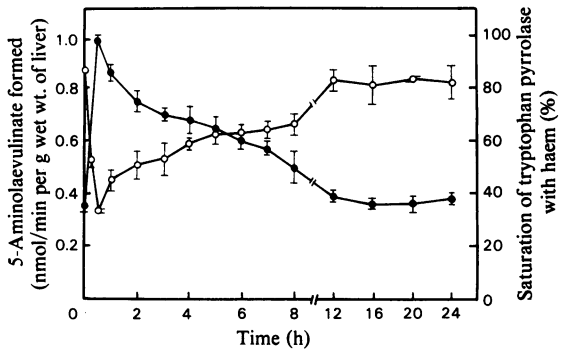

Fig. 1. *Time course of the effects of administration of tryptophan on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase*

Tryptophan (500mg/kg body wt.) was administered at zero-time to 48h-starved rats, which were then killed at various times. Synthase and pyrrolase activities and the haem saturation 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. Symbols: O, 5-aminolaevulinate synthase activity; ●, the haem saturation of tryptophan pyrrolase.

Effects of administration of various doses of tryptophan on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase

These effects were examined at 30min. The mean activity of 5-aminolaevulinate synthase (in nmol of 5-aminolaevulinate formed/min per g wet wt. of liver ± S.E.M. for each group of four rats) in control (0.9% NaCl-treated) rats of 0.75 ± 0.08 was altered to values of 0.67 ± 0.08, 0.48 ± 0.02, 0.42 ± 0.04, 0.44 ± 0.03, 0.45 ± 0.05 and 0.45 ± 0.04 by doses of tryptophan (in mg/kg body wt.) of 50, 75, 100, 200, 350 and 500 respectively. The decrease in synthase activity caused by the 50mg/kg body wt. dose of tryptophan was not significant, whereas that caused by larger doses was ($P<0.025$). It is also clear from these results that the maximum decrease in synthase activity was caused by the 100mg/kg body wt. dose of tryptophan. This latter dose also caused the largest increase in the haem saturation of tryptophan pyrrolase; the saturation (%) was increased from a control value (mean ± S.E.M. for each group of four rats) of 40 ± 6 to 97 ± 2 by this dose. Doses of tryptophan of 50, 75, 200, 350, 500 and 750mg/kg body wt. increased the saturation to

values of 52 ± 2 ($P > 0.05$), 67 ± 1, 98 ± 2, 96 ± 3, 98 ± 3 and 97 ± 1% ($P < 0.005$) respectively.

The results described so far therefore demonstrate the ability of administered tryptophan to decrease rat liver 5-aminolaevulinate synthase activity irrespective of sex or strain of the animals, and provide further support to the inverse relationship (see the introduction) between this decrease and the increase in the haem saturation of tryptophan pyrrolase. The difference between our results and those of Marver *et al.* (1966) may be explained by the possibility that the animals used by these latter authors respond differently. This is further suggested by the finding that, whereas the haem-destroying porphyrogen 2-allyl-2-isopropylacetamide causes a prolonged depletion of tryptophan pyrrolase haem in our rats (Badawy & Morgan, 1980), it caused a marked increase in the haem saturation of the enzyme in livers of rats used by Marver *et al.* (1966).

Mechanism of the tryptophan-induced early increase in haem availability

It is now well established that tryptophan enhances pyrrolase activity by causing an initial increase in the haem saturation of the endogenous (preformed) apoenzyme followed by an overall increase in enzyme activity that represents the combined results of synthesis at the normal rate plus stabilization by a mechanism requiring the presence of tryptophan itself rather than any changes in enzyme activity (Greengard & Feigelson, 1961; Schimke *et al.*, 1965; Knox, 1966; Schimke, 1969; Badawy & Evans, 1975). Although tryptophan does not increase pyrrolase synthesis (Schimke *et al.*, 1965), it is known that translational inhibitors such as cycloheximide and puromycin, but not the transcriptional inhibitor actinomycin D, are capable of blocking the tryptophan enhancement of pyrrolase activity in both fed (see e.g. Badawy & Evans, 1975) and 48h-starved (results not shown) rats. The latter authors explained this controversy by suggesting that tryptophan may act on pyrrolase activity by increasing haem synthesis, and quoted in support of this suggestion the findings that the amino acid enhances 5-aminolaevulinate synthase activity (Marver *et al.*, 1966) and increases liver microsomal-haem concentration (Greengard & Feigelson, 1961). However, since we have now shown that tryptophan decreases synthase activity, our results can only be compatible with an increase in microsomal-haem concentration if the amino acid stimulates haem synthesis at a step(s) beyond that catalysed by 5-aminolaevulinate synthase.

If tryptophan increases the conversion of 5-aminolaevulinate into haem, it should augment the effects of administered 5-aminolaevulinate on synthase activity and the haem saturation of tryptophan pyrrolase, provided that both agents are administered in doses causing submaximal changes in these two parameters. That this is so is suggested by the results in Table 2, which show that the combined treatment resulted in full (100%) saturation of pyrrolase with haem and an almost total (95%) abolition of synthase activity. To exclude the possibility that tryptophan could have acted by

Table 2. *Effects of administration of 5-aminolaevulinate, haematin and tryptophan on rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities*

Rats were starved for 48h before death and received an injection of 5-aminolaevulinate hydrochloride (15mg/kg body wt. in 2ml of 0.9% NaCl), haematin hydrochloride (2mg/kg body wt. in 1ml of dimethylformamide) or tryptophan (75mg/kg body wt. in 20ml of 0.9% NaCl) either separately or in the combinations given below. The animals were killed at 0.5, 1 or 2h after administration of tryptophan, 5-aminolaevulinate or haematin respectively. Synthase and pyrrolase activities and the haem saturation 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 the above three compounds are compared with those obtained in control rats (those treated with the appropriate solvents) and the significance of the differences is indicated as follows: †$P < 0.05$; ††$P < 0.02$; *$P < 0.01$; **$P < 0.005$; ***$P < 0.001$.

Treatment groups	Tryptophan pyrrolase activity (μmol of kynurenine formed/h per g wet wt. of liver)		Saturation of pyrrolase with haem (%)	Synthase activity (nmol of 5-aminolaevulinate formed/min per g wet wt. of liver)
	Holoenzyme	Total enzyme		
Control	4.1 ± 0.33	10.0 ± 0.24	41 ± 3	0.88 ± 0.08
Tryptophan	6.6 ± 0.39**	10.7 ± 0.83	62 ± 2**	0.66 ± 0.02†
5-Aminolaevulinate	5.8 ± 0.25*	10.2 ± 0.66	57 ± 3*	0.24 ± 0.04***
Tryptophan + 5-aminolaevulinate	10.3 ± 0.61***	10.3 ± 0.60	100 ± 1***	0.04 ± 0.02***
Control	3.9 ± 0.22	9.5 ± 0.35	41 ± 3	0.80 ± 0.07
Tryptophan	6.3 ± 0.24*	10.3 ± 0.47	61 ± 2**	0.27 ± 0.02***
Haematin	7.6 ± 0.33***	10.9 ± 0.44†	70 ± 3***	0.46 ± 0.02**
Tryptophan + haematin	6.7 ± 0.69*	9.7 ± 0.54	69 ± 5**	0.51 ± 0.04††

decreasing the degradation of haem newly synthesized from 5-aminolaevulinate, similar experiments were performed in which the latter compound was substituted with a dose of haematin hydrochloride (2mg/kg body wt.) that had previously been shown (Welch & Badawy, 1980) to cause, at 2h, submaximal changes in synthase activity and the haem saturation of tryptophan pyrrolase. The results in Table 2 show that the effects on these two parameters of the combined administration of tryptophan plus haematin were not greater than those of either compound alone, and thus exclude the above possibility.

Further support to the hypothesis that tryptophan enhances the conversion of 5-aminolaevulinate into haem is provided by the finding (Table 3) that the increase in the haem saturation of tryptophan pyrrolase and the accompanying decrease in synthase activity observed at 30min after administration of tryptophan (500mg/kg) were abolished by pretreatment of rats with lead acetate. Pb^{2+} influences the activities of several enzymes of the haem-biosynthetic (and degradative) pathway, notably those of 5-aminolaevulinate dehydratase (EC 4.2.1.24) and protohaem ferro-lyase (EC 4.99.1.1) (see Goldberg, 1972; Maxwell & Meyer, 1976; Sassa, 1978) and has previously been shown (Badawy, 1977) to decrease the haem saturation of tryptophan pyrrolase in normal fed rats and in those in which this saturation has been increased by administration of the dehydratase stimulator Al^{3+}. As in fed rats, Pb^{2+} administration to 48h-starved control rats (those not given tryptophan) also decreased the haem saturation of tryptophan pyrrolase, but did not alter 5-aminolaevulinate synthase activity (Table 3). This latter finding obtained 1h after Pb^{2+} administration is in contrast with the moderate increase that occurs at later time intervals (Maxwell & Meyer, 1976).

The results in Tables 2 and 3 therefore strongly suggest that tryptophan increases the availability of haem for tryptophan pyrrolase and 5-aminolaevulinate synthase by enhancing the conversion of 5-aminolaevulinate into haem, probably by stimulating 5-aminolaevulinate dehydratase activity, although further work is required to locate the precise point(s) at which the amino acid stimulates the above conversion. These findings are also compatible with the ability of translational inhibitors to block the tryptophan enhancement of pyrrolase activity (Badawy & Evans, 1975).

Modification of the tryptophan-induced increase in haem availability

Welch & Badawy (1980) reported that prevention of the conjugation of apo-(tryptophan pyrrolase) with exogenously administered haematin by allopurinol renders this haem available for further repression of 5-aminolaevulinate synthase synthesis. To find out if allopurinol (and the other pyrrolase inhibitor benserazide) exerts a similar effect on the tryptophan-induced increase in haem availability, experiments whose results are shown in Table 4 were performed. Both allopurinol and benserazide prevented the tryptophan-induced increase in the haem saturation of the pyrrolase and, instead of preventing the accompanying decrease in synthase activity, they potentiated it by 25–26% ($P<0.02$). This latter finding, as that in rats treated with allopurinol and haematin (Welch & Badawy, 1980), therefore suggests that the relationship between the haem saturation of tryptophan pyrrolase and the activity of 5-aminolaevulinate synthase is not always an inverse one, but that the manner with which

Table 3. *Prevention by lead acetate of the effects of tryptophan on rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities*

Rats were starved for 48h before death. The animals received an injection of either lead acetate (10mg/kg body wt.) or an equal volume (1ml/kg body wt.) of water at 30min before a similar injection of either tryptophan (500mg/kg body wt.) or an equal volume (20ml/kg body wt.) of 0.9% (w/v) NaCl, and were killed 30min after the second injection. Synthase and pyrrolase activities and the haem saturation 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 obtained in rats treated with tryptophan, lead acetate or both are compared with those observed in control (0.9% NaCl- and water-treated) animals and the significance of differences is indicated as follows: †$P<0.05$; ***$P<0.001$.

Treatment	Tryptophan pyrrolase activity (μmol of kynurenine formed/h per g wet wt. of liver)		Saturation of pyrrolase with haem (%)	Synthase activity (nmol of 5-aminolaevulinate formed/min per g wet wt. of liver)
	Holoenzyme	Total enzyme		
Control	5.3 ± 0.30	10.9 ± 0.68	49 ± 2	1.02 ± 0.14
Lead acetate	2.4 ± 0.11***	10.8 ± 0.85	22 ± 1***	0.96 ± 0.06
Tryptophan	10.2 ± 0.76***	10.5 ± 0.79	97 ± 2***	0.53 ± 0.10†
Lead acetate + tryptophan	5.5 ± 0.36	10.8 ± 0.32	51 ± 2	0.83 ± 0.01

Table 4. *Effects of allopurinol and benserazide on the tryptophan-induced changes in rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities*

Starved rats received at 30min before death an intraperitoneal injection of either tryptophan (500mg/kg body wt.) or an equal volume (20ml/kg body wt.) of 0.9% (w/v) NaCl. The animals also received, at 20min before these treatments, a similar injection of allopurinol (20mg/kg body wt.), benserazide (400mg/kg body wt.) or an equal volume (4ml/kg body wt.) of 0.9% NaCl. 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. Pyrrolase activities are expressed in μmol of kynurenine formed/h per g wet wt. of liver, whereas that of synthase is in nmol of 5-aminolaevulinate formed/min per g wet wt. of liver. The values observed in tryptophan-treated rats in the first line of results are compared with those in 0.9% NaCl-treated animals. The values in allopurinol- or benserazide-pretreated rats are compared with those in 0.9% NaCl-pretreated animals. The significance of differences is indicated as follows: †††$P < 0.02$; *$P < 0.01$; **$P < 0.005$; ***$P < 0.001$.

		Pyrrolase activity			Pyrrolase activity		
Pretreatment	Treatment ...	Holoenzyme 0.9% NaCl	Total enzyme 0.9% NaCl	Synthase activity 0.9% NaCl	Holoenzyme Tryptophan	Total enzyme Tryptophan	Synthase activity Tryptophan
0.9% NaCl		4.3 ± 0.30	8.9 ± 0.65	1.20 ± 0.10	8.3 ± 0.49***	9.8 ± 0.79	0.60 ± 0.04**
Allopurinol		2.5 ± 0.33*	3.7 ± 0.20***	0.67 ± 0.07**	3.5 ± 0.25***	3.6 ± 0.30***	0.44 ± 0.03†††
Benserazide		1.8 ± 0.10***	3.0 ± 0.05***	0.63 ± 0.03***	2.2 ± 0.24***	3.5 ± 0.13***	0.45 ± 0.04†††

the above saturation is altered determines the direction of change in synthase activity. The results in Table 4 also show that allopurinol and benserazide decreased pyrrolase and synthase activities in control (0.9% NaCl-treated) rats. The allopurinol effects have previously been reported (Morgan & Badawy, 1980), as has been the benserazide inhibition of pyrrolase activity (Young *et al.*, 1978). The allopurinol-induced decrease in synthase activity of starved rats has previously been suggested (Morgan & Badawy, 1980) to involve prevention of haem utilization by the newly synthesized apo-(tryptophan pyrrolase), thus rendering this haem available for repression of synthase synthesis. A similar explanation may apply to the effect of benserazide, but further work is required to examine these possibilities.

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