Stimulation of liver tryptophan pyrrolase during heat exposure

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1. Exposure of rats to heat ($39 \pm 1^\circ\text{C}$) stimulated liver tryptophan pyrrolase 2-fold between 3 and 48 h. 2. Plasma corticosterone increased 2-fold after 1 h of heat exposure and decreased to a low value of 50% by 16 h. 3. The effect of heat exposure on the enzyme was obtained in adrenalectomized animals. 4. Stimulation by cortisol and tryptophan of the enzyme was also obtained in heat exposure, and the effects seemed to be additive. 5. The concentration of tryptophan in the liver remained unchanged, and that in the plasma decreased to about 50% at 8 h exposure to heat and reverted to normal by 46 h. 6. Simultaneous administration of noradrenaline to heat-exposed rats had no effect, whereas that of thyroxine partly prevented the stimulation of the enzyme activity. 7. Hypothyroid conditions obtained by thyroidectomy or treatment with propylthiouracil significantly stimulated the enzyme activity. 8. Cycloheximide treatment of heat-exposed rats did not prevent the stimulation of the enzyme activity. 9. The results indicate that the effect of heat exposure on liver tryptophan pyrrolase is obtained, due to the accompanying hypothyroid condition, by increasing the activity of the existing protein by a mechanism possibly different from those known at present.

The activity of tryptophan pyrrolase, the key enzyme in the catabolism of tryptophan, is known to be stimulated by treatment of rats with a variety of agents, e.g. cortisol (Knox & Mehler, 1951), tryptophan (Knox, 1951), drugs (Ruddon & Cohen, 1970), toxins (Szot & Murphy, 1970), psychopharmacological compounds (Nomura, 1965), salicylate (Badawy & Smith, 1971) and ethanol (Badawy & Evans, 1973). A similar effect on tryptophan pyrrolase was obtained when the animals were subjected to conditions of stress, such as immobilization (Curzon & Green, 1969), cold exposure (Sitaramam & Ramasarma, 1975a) and hypobaria (Inamdar *et al.*, 1972), but not hypoxia (Susheela *et al.*, 1972). The nature of these stimulations falls essentially into three categories. The first is the induction of the enzyme mediated by cortisol through an increase in mRNA and protein synthesis *de novo* (Danesch *et al.*, 1983). The second is represented by tryptophan treatment, wherein stimulation is obtained by a combination of increased polyribosomal aggregation (Fleck *et al.*, 1965), increased tryptophyl-tRNA (Munro, 1970) and stabilization of pre-formed enzyme (for a review see Schimke, 1969). A

variation of this is obtained on treatment with haem (Badawy & Evans, 1973, 1975) and haem precursor (Wetterberg *et al.*, 1969), but without stabilization of the apoenzyme. The little-known third mode involves the autonomic nervous system in which stimulation of the enzyme activity was obtained on spinal section at C₇₋₈ (Vaptzarova *et al.*, 1969). This may be accounted by a reversal of inhibition of cortisol induction by noradrenaline (Sitaramam & Ramasarma, 1974), identified to function through α -adrenergic receptors (Sitaramam & Ramasarma, 1975b). Increases in concentrations of corticosterone in both adrenals and plasma had been found in rats exposed to environmental stress conditions (Boulourd, 1966). Evidence was obtained that under conditions of hypobaric stress (Inamdar *et al.*, 1972) and cold stress (Sitaramam & Ramasarma, 1975a) the elevated concentrations of corticosteroids were responsible for the stimulation of tryptophan pyrrolase. In the present paper, we show that exposure of rats to heat stress also stimulated hepatic tryptophan pyrrolase. The nature of this stimulation is different from any of the mechanisms described so far, and appears to involve

activation of the existing protein beyond that obtained by saturation with haem cofactor and by preincubation with tryptophan in the assay.

Materials and methods

Chemicals

Thyroxine was obtained from BDH Chemicals, Poole, Dorset, U.K., and noradrenaline from Koch-Light Laboratories, Colnbrook, Bucks., U.K. Cycloheximide, cortisol acetate, haematin hydrochloride, tryptophan, propylthiouracil, xanthine oxidase and xanthine were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Animals and treatment

Male albino rats of Wistar strain (100–120g) were used. They were fed on standard Hindustan-Lever pellet diet *ad libitum*. All the animals in the experimental and control groups were treated identically. The animals were kept in ventilated rooms maintaining the natural light-darkness cycles at ambient temperature (mean values: 20–25°C, night 10–15°C). The experimental group of animals were exposed to a temperature of $39 \pm 1^\circ\text{C}$ in a ventilated heat chamber. All killings were done by cervical dislocation between 10:00 and 11:00h, except in the experiments on determination of plasma concentrations of tryptophan and corticosterone, in which the time was 15:00h. Results are means $\pm$ S.D. of independent analyses of four to six rats in each group. The level of significance was calculated by Student's *t* test, and a *P* value less than 0.05 was considered significant.

Groups of rats were adrenalectomized under diethyl ether anaesthesia, allowed to revive and then maintained for 7 days, during which period they were given 0.9% NaCl to drink instead of water. One group was exposed to heat stress for a period of 48 h. Two groups of control animals were simultaneously processed.

Preparation of liver homogenates

The liver was removed immediately after the animals were killed, and was homogenized in 2 vol. of 1.15% KCl at 0°C in a Potter-Elvehjem-type glass homogenizer. The homogenates were centrifuged at 3600g for 40 min in a refrigerated Sorvall RC 2B centrifuge, and the supernatants were used for the assay of tryptophan pyrrolase after dilution with 6 vol. of 1.15% KCl.

Determination of tryptophan pyrrolase activity

The activity of tryptophan pyrrolase was determined in liver homogenates by measuring the formation of kynurenine from L-tryptophan by the method of Knox & Auerbach (1955), with modifi-

cations as given by Sitaramam & Ramasarma (1975a). The activity of holoenzyme was obtained by performing the assay in the absence of the cofactor, haematin. The reaction mixture consisted of potassium phosphate buffer, pH 7.0 (0.1M), L-tryptophan (1mM), haematin (0.04mM) and the liver homogenate in a total volume of 2ml. This mixture was preincubated at 37°C for 30 min without shaking to activate the enzyme. Into one set of flasks, 10% (w/v) trichloroacetic acid (1ml each) was added, and these served as 'blanks'. To all the flasks an additional amount of L-tryptophan (5mM; 2ml) was added. The incubation of the flasks was continued at 37°C for 20 min with shaking, and the reaction was stopped by adding 10% trichloroacetic acid (1ml each). The mixture was centrifuged, the pH of the supernatant was adjusted to 7.0 and kynurenine formed was determined by measuring A_{365} . The activity was calculated from the difference between the test and blank samples and expressed as nmol of kynurenine formed/h per mg of protein. Protein in the liver homogenates was measured by the biuret method (Gornall *et al.*, 1949).

Determination of corticosterone and tryptophan

The concentration of corticosterone was determined by the method of Glick *et al.* (1964) in plasma obtained by centrifugation of anticoagulant-treated blood (0.1ml of mixture of 0.8% potassium oxalate and 1.2% ammonium oxalate/ml of blood). The concentration of tryptophan was determined by the method of Bloxam & Warren (1974) in trichloroacetic acid extracts of plasma and liver homogenates. Samples of corticosterone or tryptophan, simultaneously processed identically in each experiment, were used as standards for calculations.

Results

Effects of heat exposure on liver tryptophan pyrrolase

Groups of rats were exposed to heat stress as described in the Materials and methods section for various periods of time. The livers of these animals were processed for determining tryptophan pyrrolase activity. The results in Fig. 1 show that, as early as 3 h after heat exposure, the enzyme activity increased about 2-fold. This appears to be almost the maximum for heat exposure, and remained at this value until about 48 h of continuous exposure. On further exposure, the activity reverted to the basal value by 96 h exposure. On prolonged heat exposure (8 days), the activity was 17.6 ± 3.6 nmol/h per mg of protein. A similar pattern was found for the holoenzyme. The small dip obtained at 12 h was statistically not significant compared with other values between 3 and 48 h.

Fig. 1. Effect of heat exposure on liver tryptophan pyrrolase. Groups of rats were exposed to heat for various periods of time, after which they were killed. The livers were removed and tryptophan pyrrolase was assayed with (●) or without (○) addition of haem. The values are means $\pm$ S.D. ($n = 4$), and the increases at 3–48 h periods of exposure are significant ($P < 0.05$).

Effect of heat exposure on concentrations of corticosterone and tryptophan

In view of the well-known stimulation of tryptophan pyrrolase on administering cortisol or tryptophan, it was decided to test whether their concentrations increased in heat-exposed animals. A 2-fold increase was observed in plasma corticosterone concentration after 1 h exposure to heat (Fig. 2), but rapidly decreased and reached a low value (50% of control) on continued exposure. Thus the activity of tryptophan pyrrolase remained high at time periods where corticosterone concentration was actually low. Therefore it was suspected that the increase in amount of tryptophan pyrrolase may not be due to elevated corticosterone concentrations.

The total tryptophan concentrations were measured in both liver and plasma of rats exposed to heat. The data in Fig. 2 show that tryptophan concentration remained unchanged in liver, but that in plasma decreased to about 50% of control at 8 h and reverted to the normal value by 46 h of heat exposure. All these results indicated that the increase in tryptophan concentration could not explain the increase in the enzyme activity on heat exposure.

Effect of adrenalectomy

If corticosteroids were responsible, adrenalectomy under these conditions should block the increase in enzyme activity, as shown for hypobarbia (Inamdar *et al.*, 1972) and cold exposure

Fig. 2. Effect of heat exposure on concentrations of corticosterone and tryptophan.

Groups of rats were exposed to heat for various periods of time, after which they were killed and the livers and blood samples were collected. Corticosterone in the plasma (□) and tryptophan in the plasma (●) and the liver (○) were estimated. The values are means $\pm$ S.D. ($n = 4$), and the changes for corticosterone at 1 h and for plasma tryptophan between 5 and 17 h are significant ($P < 0.05$).

(Sitaramam & Ramasarma, 1975a). Exposure to heat produced nearly the same effect in adrenalectomized and intact animals (Table 1a). Adrenalectomy by itself led to a slight decrease in enzyme activity. These experiments therefore, ruled out the involvement of corticosteroids as agents responsible for increased tryptophan pyrrolase under heat stress.

Effect of administration of cortisol

In the next set of experiments, the presence of a cortisol-induced increase in tryptophan pyrrolase was tested. Groups of rats were given cortisol (10 mg/rat) intraperitoneally 6 h before being killed. Another set of animals exposed to heat for a total period of 18 h were also given cortisol (10 mg/rat) 6 h before being killed. The results in Table 1(b) show that cortisol treatment caused a 5-fold increase in the enzyme activity. In heat-exposed animals the increase was only 2-fold, but from a much higher control value. Although the change in enzyme activity was 281% in controls, compared with 125% on heat exposure, the actual differences in specific-activity units are 30 and 22, for control and cortisol-treated rats respectively, showing that the effect of heat was additive.

Table 1. *Effects of adrenalectomy and administration of cortisol, noradrenaline, thyroxine, tryptophan and cycloheximide*

In each set of experiments, control groups of rats were monitored for the effects of heat exposure for a period of 18 h, unless otherwise stated. Under identical conditions another group of rats was treated as specified and tested for effects of heat exposure. (a) Rats were adrenalectomized under diethyl ether anaesthesia and were exposed to heat for 48 h after a 7-day revival period. (b) Cortisol acetate (10 mg/rat) was given intraperitoneally, 6 h before rats were killed. (c) Noradrenaline (0.1 mg/rat) or thyroxine (0.2 mg/rat) was given intraperitoneally just before heat exposure. (d) L-Tryptophan (50 mg/rat) was given intraperitoneally as a suspension in water 6 h before rats were killed. (e) Cycloheximide (0.15 mg/rat) was given intraperitoneally 30 min before heat exposure and again at 9 h before rats were killed. The values given are means $\pm$ S.D. of independent analyses of four to six animals in each group: * $P < 0.01$; ‡ $P < 0.05$.

Treatment	Tryptophan pyrrolase activity (nmol/h per mg of protein)	
	Control	Heat-exposed
(a) Intact animals	20.5 $\pm$ 1.7	44.3 $\pm$ 8.1*
Adrenalectomized	14.8 $\pm$ 1.4	33.3 $\pm$ 11.9*
(b) None	16.5 $\pm$ 1.3	46.3 $\pm$ 2.1*
Cortisol	88.1 $\pm$ 24.3	109.9 $\pm$ 14.7
(c) None	21.0 $\pm$ 1.7	56.1 $\pm$ 12.0*
Noradrenaline	19.2 $\pm$ 3.8	46.8 $\pm$ 12.6*
Thyroxine	16.0 $\pm$ 2.0	28.4 $\pm$ 6.8‡
(d) None	16.5 $\pm$ 1.3	46.3 $\pm$ 2.1*
Tryptophan	41.8 $\pm$ 7.7	65.6 $\pm$ 0.6‡
(e) None	22.6 $\pm$ 4.5	44.0 $\pm$ 1.6*
Cycloheximide	24.5 $\pm$ 4.1	40.6 $\pm$ 5.9‡

Effect of administration of noradrenaline

It was shown in this laboratory that noradrenaline treatment decreased the cortisol-induced stimulation of tryptophan pyrrolase activity (Sitaramam & Ramasarma, 1974). Noradrenaline treatment did not affect the stimulation by heat exposure (Table 1c). This is another difference between stimulations by cortisol and heat exposure.

Effect of administration of tryptophan

Tryptophan (50 mg/rat) was injected intraperitoneally 6 h before rats were killed. One set of animals were also exposed to heat stress for 18 h and were given tryptophan as for the control groups. The results in Table 1(d) show that about a 3-fold increase in tryptophan pyrrolase was obtained after tryptophan administration. Further stimulation of the enzyme activity was obtained on heat exposure. The effect of heat exposure seems

Table 2. *Effect of thyroidectomy*

Groups of rats were thyroidectomized and killed after the specified period. A control group of sham-operated rats was also killed on each day. Values are means $\pm$ S.D. ($n = 4$): ‡ $P < 0.05$.

Time after operation (days)	Tryptophan pyrrolase activity (nmol/h per mg of protein)	
	Sham-operated	Thyroidectomized
1	18.5 $\pm$ 1.9	36.2 $\pm$ 3.8‡
2	26.7 $\pm$ 5.6	40.3 $\pm$ 3.8‡
3	21.2 $\pm$ 1.6	43.0 $\pm$ 12.8‡

Table 3. *Effect of administration of propylthiouracil*

Groups of rats were given propylthiouracil (15 mg/rat per day in 0.9% NaCl) intraperitoneally and the control group were given 0.9% NaCl for 3 days. The animals were killed 24 h after the last injection. Values given are means $\pm$ S.D. ($n = 4$): ‡ $P < 0.05$.

Period of treatment with propylthiouracil (days)	Tryptophan pyrrolase activity (nmol/h per mg of protein)
Control	18.6 $\pm$ 2.7
1	18.0 $\pm$ 4.0
2	31.4 $\pm$ 2.3‡
3	23.0 $\pm$ 2.4‡

additive, although the increase caused by heat exposure was somewhat smaller in tryptophan-treated animals.

Effect of administration of thyroxine

Exposure of rats to heat decreased thyroid activity and the circulating thyroid-hormone concentration (Collins & Weiner, 1968). Under the experimental conditions of heat exposure used in the present study, the serum concentration of thyroxine was kept decreased at 50% of the control value ($6.4 \pm 0.2 \mu\text{g}/100 \text{ ml}$) (Swaroop & Ramasarma, 1985). Hence the effect of administration of thyroxine to rats before exposure to heat was tested. After 6 h of thyroxine treatment there was a small decrease in control animals, and the increase obtained during heat stress was partially prevented (Table 1c). These results gave the first indication of involvement of thyroid hormone in regulation of tryptophan pyrrolase activity.

Effect of thyroidectomy and administration of propylthiouracil

The stimulation of tryptophan pyrrolase in heat exposure appeared to be due to release of inhibition by thyroxine. It was therefore decided to test the effects of hypothyroid conditions. Groups of

Table 4. *Comparison of effects on tryptophan pyrrolase under environmental conditions*

Information is collected from Sitaramam & Ramasarma (1974, 1975a) for cold exposure, from Inamdar *et al.* (1972) for hypobaric exposure.

	Cold exposure (0–5°C)	Hypobaric exposure [51 kPa (0.5 atm)]	Heat exposure (38–40°C)
Extent of stimulation	3-fold	3-fold	2-fold
Period of exposure for maximum effect	8 h	8 h	3 h
Adrenalectomy	Blocks stimulation	Blocks stimulation	Stimulation unaffected
Cycloheximide treatment	Blocks stimulation	Blocks stimulation	Stimulation unaffected
Noradrenaline treatment	Reverses stress effect	Reverses stress effect	No change in stress effect

thyroidectomized rats were killed after specified periods of time, and tryptophan pyrrolase activity was measured and compared with that in sham-operated controls. Increases of about 2-fold in enzyme activity, comparable with that in heat exposure, were obtained (Table 2). In the other hypothyroid condition, obtained by daily injections of propylthiouracil, tryptophan pyrrolase activity also increased, albeit to a smaller extent (Table 3).

Effect of administration of cycloheximide

The stimulation of tryptophan pyrrolase obtained on administration of cortisol or tryptophan or on exposure to hypobaria or cold was found to be sensitive to cycloheximide, an inhibitor of protein synthesis. Therefore the effect of cycloheximide on the increase in tryptophan pyrrolase activity after heat exposure was investigated. Administration of cycloheximide 30 min before the start of heat exposure and again 9 h before the rats were killed, in a total experimental period of 18 h of heat exposure, was used because in earlier experiments it was found that protein synthesis was inhibited for a period of 12 h after a single injection of the drug. The results in Table 1(e) show that cycloheximide treatment did not prevent the increase in enzyme activity in heat-exposed animals, and therefore indicate an alteration in activity of the existing enzyme.

Discussion

The results of the present investigation show that none of the known mechanisms, dependent on cortisol, tryptophan or noradrenaline, is operative in the effect of heat exposure. Further, the involvement of a new factor, thyroid hormone, is introduced in this investigation. Thus, the activity of tryptophan pyrrolase is now shown to be inversely related to the thyroid status of the animal. This effect, however, is obtained by modulating the activity of the existing protein and not by inducing new enzyme synthesis.

The assay of the enzyme in the present experiments included two types of activation: addition of the cofactor, haematin, to saturate all the free apoenzyme, and also activation of the enzyme by preincubation with tryptophan which reduces chemically and activates the enzyme protein. With these treatments, it is assumed that maximum activity of the existing protein is measured. Since in heat exposure more active enzyme was obtained without apparent induction, there must be other means of activating the enzyme further. Reducing agents such as borohydride, ascorbate and xanthine/xanthine oxidase were known to activate the enzyme. This seems to be obtained by reduction of the catalytic site, by superoxide or hydroperoxide, because of its known sensitivity to superoxide dismutase and catalase (Fiegelson & Brady, 1974). Treatment with xanthine/xanthine oxidase stimulated the activity by another 50% over and above that already obtained by preincubation with haem and tryptophan. Under heat exposure such an activation seems possible. Investigations in this laboratory on the effects of exposure to environmental stress conditions have helped in understanding regulation of tryptophan pyrrolase. Comparison of the three conditions of cold, hypobaria and heat given in Table 4 indicates that heat exposure produced an effect different from that with the other two conditions.

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