Effect of Environmental Stress of Low Pressure on Tyrosine Aminotransferase and Phenylalanine 4-Hydroxylase Activities in the Rat

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1. Tyrosine aminotransferase activity in the liver increased about fourfold after 9h, on exposure of rats to stress of low pressure. 2. The phenylalanine hydroxylase activity increased about 60% on exposure for 24h or more. 3. An environmental pressure decrease of about 0.033 MN/m² is needed to increase the activity of tyrosine aminotransferase. 4. Adrenalectomy completely abolished the increase in activity of tyrosine aminotransferase obtained on exposure to low pressure. 5. Treatment with cycloheximide or actinomycin D prevented the increase in activity of tyrosine aminotransferase. 6. Treatment with cycloheximide at the early part of exposure to stress prevented the increase in activity of phenylalanine hydroxylase obtained after 24h.

It is well established that exposure of animals to low pressure causes stimulation of the sympatho-adrenal system resulting in altered noradrenaline (norepinephrine) metabolism (Cantu *et al.*, 1966; Downing *et al.*, 1963; Downing & Siegel, 1963; Goldman & Harrison, 1970). The catecholamines released in higher quantities into the blood are apparently important in the process of adaptation to the hypoxic stress. For instance, the alveolar–arterial oxygen gradient is known to be decreased on exposure of rats to high altitudes. It is caused by the increased amount of catecholamines released into the blood (Kreuzer, 1962). Catecholamine metabolism in the central nervous system is also known to be affected on exposure to low pressure. Hurvitz *et al.* (1971) observed a decrease in noradrenaline and dopamine (3,4-dihydroxyphenethylamine) concentrations in brain on exposure of rats to a simulated altitude of 7000m. Such changes in the metabolism of catecholamines may be involved in the inability of animals to thermoregulate at high altitudes (Gellhorn, 1937; Baumel *et al.*, 1969), in the changes in the conditioned avoidance performance (Hurvitz *et al.*, 1971) and in the altered sensitivity of drugs acting on the central nervous system (Robinson *et al.*, 1969; Merrit & Medina, 1968).

Since catecholamines are derived from tyrosine, the metabolism of this amino acid under conditions of hypoxic stress assumes great importance. Excluding protein synthesis, the major pathway for the metabolism of tyrosine involves tyrosine aminotransferase (EC 2.6.1.5.). In view of the altered metabolism of noradrenaline and corticosteroids, both of which are known to control the activity of tyrosine aminotransferase (Black & Axelrod, 1970; Mavrides & Lane, 1960), a study of the activity of this enzyme under conditions of low pressure seems

worth while. Apart from the dietary sources, tyrosine can be obtained from phenylalanine by hydroxylation catalysed by phenylalanine 4-hydroxylase (EC 1.14.16.1) (Kaufman, 1957). Since this is an oxygen-dependent enzyme, the exposure of animals to low pressure and the consequent hypoxia may be expected to change its activity. Also, study of the activity of tyrosine aminotransferase in the brain under conditions of low pressure is important in view of the known dependence of synthesis of catecholamines on the concentration of tyrosine (Wurtman *et al.*, 1974), which in turn is controlled by the activity of this enzyme (Gibb & Webb, 1969). The results given in the present paper show that the activity of tyrosine aminotransferase in the liver begins to increase within hours but that of phenylalanine hydroxylase responds only slowly after a day of exposure to the stress.

Materials and Methods

Chemicals

Actinomycin D, cycloheximide and 6,7-dimethyl-5,6,7,8-tetrahydropterin were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A., Upjohn Co., Kalamazoo, Mich., U.S.A., and Calbiochem Co., Los Angeles, Calif., U.S.A., respectively.

Animals and exposure to low pressure

Male albino Wistar rats (weighing 150–200g) were obtained from the Institute stock colony. They were fed on Hindustan Lever pellet diet consisting of 24% protein, 4% fat, 50% carbohydrate and other nutrients. Water was given *ad libitum*. They were exposed to a pressure of 0.047 MN/m², corresponding

to an altitude of about 6100m, in a decompression chamber. The flow of air inside the chamber was maintained by evacuation from one side and a leak on the other. Low pressure was obtained in the chamber in less than 3 min after the start of evacuation and was maintained at a constant value (Inamdar *et al.*, 1972). Five animals or more were used in each group and the mean $\pm$ S.D. values were employed for the calculation of *P* values by Student's *t* test.

Preparation of tissue extracts

At the end of the specified periods, atmospheric pressure was restored in the chamber and the animals were removed and killed by stunning and decapitation. They were usually killed between 09:30h and 10:30h and the livers and brains were removed. The livers were homogenized in 2vol. (v/w) of cold (0°C) 1.15% (w/v) KCl in a Potter-Elvehjem-type glass homogenizer. The homogenates were centrifuged at 36000g for 40min in a refrigerated Sorval RC 2B centrifuge. The supernatant was diluted with 6vol. of 1.15% KCl and used for the assay of the enzymes.

The brains were usually stored for 24h at -20°C before the assay for tyrosine aminotransferase activity. The brains were homogenized in 2vol. of cold 0.2M-potassium phosphate buffer, pH7.2, containing 1% (v/v) of Triton X-100, and the homogenates were used directly for the assay of the enzyme.

Assay of enzyme activities

Tyrosine aminotransferase activity in the liver was assayed as described in a previous paper (Namboodiri & Ramasarma, 1973). Tyrosine aminotransferase activity in the brain was assayed as described by Diamondstone (1966), with some modifications. The assay system contained 400 μ mol of potassium phosphate buffer, pH7.2, 11 μ mol of L-tyrosine, 20 μ mol of 2-oxoglutarate, 40nmol of pyridoxal phosphate, 3 μ mol of diethyldithiocarbamate and the enzyme in a total volume of 2.5ml. The assay system without 2-oxoglutarate was incubated for 5min at 37°C. The reaction was started with the addition of 2-oxoglutarate and incubated for 20min at 37°C. The reaction was then stopped with 1ml of 10% (w/v) trichloroacetic acid. To the deproteinized solution 0.5ml of 2.5M-NaOH was added and the mixture was then kept at 37°C for another 30min. The extinction of the solution was then measured at 331nm. The activity was expressed as nmol of product formed/min per g wet wt. of brain.

Phenylalanine hydroxylase activity was assayed by the procedure described by Bublitz (1969), with slight modifications. The assay system contained 100 μ mol of Tris-HCl buffer, pH7.2, 10 μ mol of freshly prepared dithiothreitol, 0.45 μ mol of cofactor 6,7-dimethyl-5,6,7,8-tetrahydropterin, 2 μ mol of L-

phenylalanine and the enzyme extract in a total volume of 1ml. The complete system except L-phenylalanine was preincubated at 30°C for 5min, L-phenylalanine was then added and incubation was continued for 20min at 30°C in a metabolic shaker. The reaction was stopped by the addition of 2ml of 10% (w/v) trichloroacetic acid. Tyrosine was estimated colorimetrically in 2ml samples of the deproteinized solution (Udenfriend & Cooper, 1952). Protein was measured by the biuret reaction (Gornall *et al.*, 1949). The activity is expressed as nmol of product formed/min per mg of protein.

Adrenalectomy

Adrenalectomy was achieved by dorsal approach and the animals were kept for 5-7 days before being used in the experiments, during which time they were given 0.9% NaCl to drink. The completeness of adrenalectomy was checked by the inability of rats selected randomly to survive in cold beyond 4-6h and by examination of retroperitoneal tissues *post mortem*.

Treatment with cycloheximide and actinomycin D

Cycloheximide and actinomycin D were given in 1ml of 0.9% NaCl intraperitoneally half an hour before the beginning of exposure. In the case of repeated injections of cycloheximide, the break of exposure was for about 15min for the second injection.

Results

Time-course of changes in the activities of tyrosine aminotransferase and phenylalanine hydroxylase in rats under low pressure

Groups of rats were exposed to low pressure for stated periods up to 96h. The activity of tyrosine aminotransferase in the liver increased rapidly in 2h and reached a maximum of about fourfold in about 9h (Table 1). The activity decreased on subsequent exposure but was still about 40% higher than the controls at 96h of exposure. Phenylalanine hydroxylase activity, on the other hand, increased only after the exposure of animals for 24h. The increase obtained was about 70% and it was stabilized, at about the same value, on continued exposure up to 96h. The contrast between the fast and slow responses of the two enzymes can be more clearly seen in Fig. 1, where activities are represented as percentages of normobaric controls. It should be mentioned that during the first day of exposure to low pressure the animals consumed much less food than the control animals. Starvation for 24h, however, does not increase the activity of this enzyme expressed on the

Table 1. *Changes in the activities of tyrosine aminotransferase and phenylalanine hydroxylase on exposure of rats to low pressure*

Groups of rats were exposed to a pressure of 0.047 MN/m² in a decompression chamber for the periods indicated. The results are means $\pm$ S.D. from independent analyses from five to ten rats per group. The increase in activities are significant where marked * ($P < 0.01$).

Period of exposure (h)	Liver tyrosine aminotransferase (nmol/min per mg of protein)	Liver phenylalanine hydroxylase (nmol/min per mg of protein)	Brain tyrosine aminotransferase (nmol/min per g wet wt.)
0	9.5 $\pm$ 1.1	9.0 $\pm$ 0.8	135 $\pm$ 6
1	10.5 $\pm$ 1.6	—	—
2	22.3 $\pm$ 1.7*	—	—
4	28.6 $\pm$ 4.4*	—	—
9	42.4 $\pm$ 2.1*	9.9 $\pm$ 1.1	136 $\pm$ 7
13	35.6 $\pm$ 2.2*	10.5 $\pm$ 1.3	134 $\pm$ 4
24	21.0 $\pm$ 1.8*	15.4 $\pm$ 1.4*	128 $\pm$ 4
37	20.1 $\pm$ 1.3*	13.6 $\pm$ 0.7*	135 $\pm$ 5
96	13.1 $\pm$ 3.0*	12.9 $\pm$ 0.4*	139 $\pm$ 4

Fig. 1. *Increase in the activities of tyrosine aminotransferase and phenylalanine hydroxylase in the liver of rats exposed to low pressure*

Groups of rats were exposed to low pressure for the periods indicated in logarithmic scale on the abscissa. The activities of tyrosine aminotransferase (●) and of phenylalanine hydroxylase (○) in the liver were then assayed. Enzyme activities are expressed as percentages of control values from animals killed at the same time.

basis of unit protein (Castells & Shirali, 1971) and therefore the above increase appears to be due to the stress *per se*. The activity of tyrosine aminotransferase in the brain did not show any change on exposure for up to 96 h (Table 1).

Effect of different degrees of low pressure

It was decided to test the differential atmospheric pressure required to show the above stimulation of the enzyme activity. This was done only at one time-interval and with respect to the stimulation of tyrosine aminotransferase activity in the liver. The rats

Table 2. *Effect of degree of stress due to low pressure on the increase in tyrosine aminotransferase activity in rat liver*

Groups of rats were exposed for 4 h in a decompression chamber set at different pressures as indicated. The values represent means $\pm$ S.D. of independent analyses of six rats in each group. The increase in activities are significant where marked * ($P < 0.01$).

Pressure in the chamber (MN/m ²)	Difference from the atmospheric pressure (MN/m ²)	Liver tyrosine aminotransferase (nmol/min per mg of protein)
0.093	Nil	9.5 $\pm$ 1.1
0.073	0.020	8.3 $\pm$ 0.6
0.066	0.027	10.7 $\pm$ 1.4
0.060	0.033	23.3 $\pm$ 4.8*
0.047	0.046	28.6 $\pm$ 4.4*

were exposed to different pressures for a period of 4 h. The results in Table 2 show that a difference from ambient pressure of about 0.033 MN/m² was sufficient to elicit the response. The maximum response was obtained with 0.047 MN/m² difference. The animals could not tolerate the stress of pressure lower than this for 4 h.

Effect of adrenalectomy

In view of the known induction of tyrosine aminotransferase by cortisol it was decided to test whether the increased concentration of corticosteroids known to occur in stress conditions was responsible for the observed effect. Therefore the effect of low pressure on adrenalectomized animals was studied. The results in Table 3 show that the hypobaric-induced increase in tyrosine aminotransferase activity was not observed in adrenalectomized animals. Adrenalectomized animals are in many respects different from

intact ones and, among other functions affected, a decrease in the activity of the sympathetic nervous system is of significance (Ramey & Goldstein, 1957). If there was another factor involved in the induction process, which may have changed on low-pressure exposure but required permissive concentration of corticosteroids, then the stimulation of the enzyme activity would not be observed in the above experiment. Therefore in the same set of experiments two other groups of animals were supplemented with small concentrations of cortisol, which in intact animals showed no induction of the enzyme, with the expectation that this supplement would normalize other corticoid-dependent functions. Exposure to low pressure would not elicit increased cortico-

steroids in the absence of adrenals. In such animals no difference in the induction was obtained between the control and low-pressure-exposed groups (Table 3).

Effect of inhibitors of protein synthesis

To test whether or not the observed stimulation of activity was obtained by increasing the synthesis of the enzyme protein, the inhibitors of protein synthesis cycloheximide and actinomycin D were used. Rats were given intraperitoneally cycloheximide (250 µg/rat) or actinomycin D (150 µg/rat), amounts known to prevent protein synthesis for at least 4 h (Young *et al.*, 1963). After 30 min at ambient pressure, the rats were transferred to the low-pressure chamber for a further period of 3½ h. The control rats were given the same amounts of inhibitors and left at atmospheric pressure for the total period. The activity of tyrosine aminotransferase was measured at the end of 4 h after the injection of the inhibitors in all cases. With both inhibitors the stimulation of the activity due to the low pressure was abolished, indicating that the increased protein synthesis was responsible for the increased activity (Table 4). It may be noted that the small stimulation of the enzyme obtained by cycloheximide treatment at ambient pressure was abolished on exposure to low pressure.

In another set of experiments rats were given two injections of cycloheximide (separated by an interval of 6 h; each injection 150 µg/rat), and the exposure to low pressure was started 30 min after the first injection and continued for 24 h. The control rats were also given the same amount of cycloheximide at the above intervals and left at ambient pressure. The increase in activity of phenylalanine hydroxylase obtained on exposure for 24 h to low pressure was abolished in animals treated with cycloheximide (Table 4). That

Table 3. *Effect of adrenalectomy on the increase in tyrosine aminotransferase activity in the livers of rats exposed to low pressure*

Groups of adrenalectomized rats were exposed to low pressure (0.047 MN/m²) for 4 h. Where mentioned cortisol dispersed in 0.9% NaCl was injected intraperitoneally 30 min before exposure to low pressure. The values given are the means ± s.d. of independent analyses of six rats in each group.

Treatment	Liver tyrosine aminotransferase (nmol/min per mg of protein)	
	Control	Low pressure
Nil	9.3 ± 1.0	28.6 ± 4.4
Adrenalectomized	8.2 ± 1.5	7.6 ± 1.0
Adrenalectomized + cortisol (0.3 mg/rat)	16.4 ± 1.9	18.0 ± 2.6
Adrenalectomized + cortisol (0.6 mg/rat)	29.3 ± 7.6	31.3 ± 6.3

Table 4. *Effect of treatment with inhibitors of protein synthesis on the increase in the activities of tyrosine aminotransferase and phenylalanine hydroxylase in the livers of rats exposed to low pressure*

Groups of rats were injected intraperitoneally with cycloheximide (250 µg/rat) or actinomycin D (150 µg/rat). After being kept for 30 min at ambient pressure, one set of rats was transferred to a decompression chamber set at 0.047 MN/m² and exposed to the low pressure for 3½ h. Tyrosine aminotransferase activity was then assayed. In another set of experiments two groups of rats were injected with cycloheximide (150 µg/rat, each dose) at two time-intervals of 0 and 6 h. One group was exposed to low pressure 30 min after the first injection and the exposure was continued for 24 h. Phenylalanine hydroxylase activity was then assayed. The values given are means ± s.d. of independent analyses of six rats in each group. The changes are significant ($P < 0.01$) where marked * for exposure to low pressure and † for drug treatment.

Treatment	Liver tyrosine aminotransferase (nmol/min per mg of protein)		Liver phenylalanine hydroxylase (nmol/min per mg of protein)	
	Control	Low pressure	Control	Low pressure
Nil	9.5 ± 1.1	27.6 ± 3.2*	9.1 ± 1.0	15.4 ± 1.4*
Cycloheximide	13.5 ± 1.1†	9.7 ± 0.9*†	7.1 ± 0.3†	6.9 ± 0.5†
Actinomycin D	7.5 ± 0.6	11.9 ± 0.6*†	—	—

protein synthesis is involved is indicated, but it is noteworthy that the inhibitor was not present throughout the period of exposure, i.e. the inhibitory effect on protein synthesis would not persist beyond 10–12h of the experimental period (Verbin & Farber, 1967).

Discussion

The experiments described above show that tyrosine aminotransferase in the liver is induced on exposure of rats to low pressure and appears to be stabilized at a higher concentration on continued exposure. But this cannot be taken as a general stress response because other stress conditions such as hypoxia alone (Susheela *et al.*, 1972) or shaking (Geller *et al.*, 1964) did not bring about any change in the activity of this enzyme. Also, the nature of the change in the activity of this enzyme appears to differ from stress to stress, as discussed below.

Adrenalectomy was able to abolish the induction of this enzyme under low pressure, prompting further studies on the involvement of adrenal steroids in the induction process. It is known that adrenalectomized animals are more sensitive to corticosteroids (Rosen *et al.*, 1963). As expected, the small concentrations of cortisol were able to induce the enzyme in such animals (Table 3). This phenomenon can be explained either as a change in the nature of receptors (Feldman, 1974) or removal of another adrenal factor that may interfere with the process. Under the conditions of adrenalectomy and cortisol supplement at permissive concentration and simultaneous low-pressure exposure, the increase in endogenous corticosteroids is expected to be prevented. Therefore it may be that no other factor besides corticosteroids is involved in the induction process in low-pressure exposure. In cold exposure, on the other hand, similar experiments suggested the involvement of an additional factor of possible adrenergic origin (Namboodiri & Ramasarma, 1973). This is a notable difference between the two environmental stress conditions of low pressure and cold exposure in the nature of induction of this enzyme.

There are instances where tyrosine aminotransferase was not induced even when there was an increased secretion of steroids (Geller *et al.*, 1964). The activity of this enzyme decreased on prolonged exposure to the cold even when the steroid concentrations were high (Roulouard, 1966). One explanation for these observations would be that the increase in some factors, such as growth hormone or noradrenaline, with opposite effect to that of cortisol on the induction of tyrosine aminotransferase may simultaneously have increased, thus nullifying the effect of corticosteroids.

The results of experiments on treatment with cycloheximide provide another instance of a drug

action being modified or abolished by environmental stress. In normal conditions cycloheximide treatment stimulates tyrosine aminotransferase activity to a small but significant extent by a still unknown mechanism (Fiala & Fiala, 1966). This action of cycloheximide, which was confirmed in our experiment, is lost under low pressure. It may be noted that exposure to low pressure has a similar effect on the induction of hepatic δ -aminolaevulinic synthetase by 3,5-dicarbethoxy-1,4-dihydrocollidine (Amruthavalli & Ramasarma, 1973). Adenine nucleotides have been implicated as the causative agents for this inhibition. Whether a similar mechanism is operative in the case of the tyrosine aminotransferase system and cycloheximide is worthy of further investigation.

Tyrosine aminotransferase in the brain is important in regulating the concentration of tyrosine, which in turn can control the synthesis of catecholamines in the brain (Gibb & Webb, 1969; Wurtman *et al.*, 1974). Conflicting reports are available on the nature of the regulation of this enzyme *in vivo*. Gibb & Webb (1969) have observed a decrease in the activity of brain tyrosine aminotransferase in several conditions associated with a decreased concentration of noradrenaline in the brain. They have also shown that the normal enzyme activity was restored on re-establishing the concentration of noradrenaline by administering 3,4-dihydroxyphenylalanine. Zigmond & Wilson (1973), on the other hand, failed to find any change in the enzyme activity in conditions associated with changes in the concentration of noradrenaline in the brain. Cymerman *et al.* (1972) reported that the concentration of catecholamines in the brain decreased by about 40% on exposure of rats to low pressure. The results in the present paper show that there is no change in the activity of tyrosine aminotransferase in the brain under conditions of low pressure, in contrast with the increase in that of the hepatic enzyme. This type of differential response of the same enzyme in different tissues is observed under other stress conditions also.

The major route for the catabolism of phenylalanine involves its conversion into tyrosine (Kaufman, 1971). Very little is known about the regulation of phenylalanine hydroxylase *in vivo*. McGee *et al.* (1972) have shown that there is a definite correlation between the amount of this enzyme measured *in vitro* and the capacity of the animal to convert phenylalanine into tyrosine. So the increased activity of the enzyme in low pressure may mean an increased capacity on the part of the animal to convert phenylalanine into tyrosine. It can also be interpreted as a possible adaptive change to meet the decreased availability of oxygen, a substrate for the reaction. A similar adaptive increase in the activities of other hydroxylases, namely tyrosine hydroxylase (Klain, 1972) and tryptophan hydroxylase (Inamdar *et al.*, 1972), was observed under these conditions.

Cortisol treatment induced phenylalanine hydroxylase in the livers of 8–14-day-old rats but not in adult animals (McGee *et al.*, 1972). If cortisol is a factor involved in the increased activity obtained on exposure to low pressure in adult animals in the present experiments, it cannot be the only one. Our studies with cycloheximide suggest that some initial event involving protein synthesis is required for the increase in activity obtained at a later period; and this may be the explanation for the delay before phenylalanine hydroxylase activity increases. Another possibility is that the delay is due to a long half-life for the enzyme, as proposed by Schimke & Doyle (1970). Whatever the explanation, the fast response of tyrosine aminotransferase activity and the slow response of phenylalanine hydroxylase activity to the same original stimulus provide an interesting contrast in adaptation to the stress. Further studies on the mechanism of this notable phenomenon may lead to a better understanding of the absence of this enzyme in phenylketonuria and the regulation of this enzyme during adaptation.

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