INHIBITION OF CORTISOL-MEDIATED INDUCTION OF HEPATIC TRYPTOPHAN-2,3-DIOXYGENASE BY NOREPINEPHRINE

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SUMMARY: Norepinephrine inhibits cortisol-mediated induction of hepatic tryptophan pyrrolase in rats. During cold exposure the stabilization of this enzyme appears to occur by an interaction of corticoids and norepinephrine on the induction process.

On exposure of rats to low environmental temperature, a rapid response of induction of hepatic tryptophan-2,3-dioxygenase (EC.1.13.1.12) (tryptophan pyrrolase) was observed (V. Sitaramam, T. Ramasarma, unpublished results). This, in the early phases upto 8 hr. exposure, was shown to be directly related to the concomitant increase in circulating corticosteroids known to occur (1). In these studies one interesting observation was the decrease in the levels of hepatic tryptophan pyrrolase on prolonged exposure notwithstanding the continued higher levels of corticoids. This effect could be more clearly shown in adrenalectomized animals supplemented with low doses of exogenous cortisol which on simultaneous cold exposure led to significant decrease in the cortisol-mediated induction of the enzyme. Similar inhibitory effect observed by Berry *et al.* both in cold (2) and hypobaria (3) was ascribed to bacterial endotoxins. In this communication results are presented which indicate that cortisol and norepinephrine, with mutually opposing effects on the induction process, are responsible to determine the levels of the enzyme in cold exposure.

EXPERIMENTAL

Male albino rats in the weight range 170±20 gms of Wistar strain of the

Institute Colony were used. The rats were fed with Hindustan lever pellet diet containing 24% protein, 4% fat and 50% carbohydrates and the required vitamins and minerals. All animals were maintained in controlled environmental light darkness cycles (lights 6 A.M. - 8 P.M., dark 8 P.M. - 6 A.M.). All injections were given intraperitoneally as solutions in distilled water. Half an hour after the injections the animals were exposed to cold at 0-2°C in a ventilated chamber for 3 hr. All animals were sacrificed by cervical dislocation between 10.00 - 11.00 A.M. to avoid interference due to circadian variations in the enzyme levels. Each group had atleast 6 animals and the results are analyzed for statistical significance. In each set of experiments, comparisons were made with proper controls run simultaneously, as the values showed variations between experiments.

Livers were homogenized in 1.15% (w/v) KCl at 0°C using 2 ml per gram tissue. The homogenates were centrifuged at 36,000 g in Sorvall RC 2B centrifuge for 40 min. and the clear supernatant was aspirated with a needle and was assayed for enzyme activity. The assay was performed according to Knox & Auerbach (4), with the following modifications: (a) preincubation was carried out with the substrate for 30 min. to overcome the preparative artifact due to oxidative inactivation (5), (b) microsomal fraction (6) and exogenous heme were added in the assay system to obtain maximal activity. Kynurenine formed was measured in a Unicam Spectrophotometer by its absorption at 365 nm. Protein was estimated by the biuret method (7) using bovine serum albumin as standard. Enzyme activity was expressed as nanomoles of kynurenine formed $-hr^{-1}-mg^{-1}$ protein.

RESULTS

Exposure of rats to cold (0-2°) for 8 hr. increased the hepatic tryptophan pyrrolase about 4-fold and this on continued exposure decreased to about 3-fold the initial value at 12 hr. (Table 1). The increased levels compared to the initial level as well as the decrease between 8 hr. and 12 hr. are statistically significant. Treatment with cycloheximide between 8-12 hr. period of cold exposure abolished the elevation in enzyme activity. This suggested that continued higher rates of synthesis were obtained in this phase. Therefore

Table 1: Hepatic tryptophan pyrrolase levels in continued cold exposure

	Treatment	No. of animals	Enzyme activity nmoles/hr/mg. protein (mean±S.E.M.)	P value
1.	Control (23-25°)	6	22.7±5.7	<0.01
	Cold exposed (0-2°, 8 hr)	7	82.0±9.0	
	Cold exposed (0-2°, 12 hr)	8	59.4±5.5	<0.05
2.	Cold exposed (0-2°, 12 hr)	7	96.4±7.9	<0.01
	Cold exposed (0-2°, 12 hr) + Cycloheximide (8-12 hr)	8	19.4±1.7	

Groups of Wistar albino rats were exposed to cold (0-2°) for the intervals of time indicated in a temperature-controlled ventilated chamber. Cycloheximide (250 µg/rat) was injected (i.p.) to rats after an exposure of 8 hr. to cold and then exposure to cold was continued for another 4 hr. The control animals in this experiment (also cold exposed) were given equal volume of saline at the same time.

the fall in enzyme activity after 8 hr. of exposure to cold may be due to altered synthesis or degradation or both.

In the second set of experiments, the animals were given saturating doses of cortisol for maximum induction of the enzyme. By this approach the influence of alterations in endogenous corticoids due to the labile pituitary adrenal axis was rendered negligible. On simultaneous exposure to cold for a brief period of 3 hr. along with cortisol treatment, the cortisol-mediated induction of the enzyme was found to be inhibited (Table 2). Increased activity of sympathetic nervous system during cold exposure is well established (8). Therefore, whether norepinephrine was responsible for the above inhibitory effect was next investigated. Norepinephrine given alone by intraperitoneal injection showed slight decrease in enzyme activity and higher concentrations were lethal.

Table 2: Effect of norepinephrine on cortisol-induced hepatic tryptophan pyrrolase

	Treatment	No. of animals	Enzyme activity nmoles/hr/mg. protein (mean±S.E.M.)	P value
1.	Control	6	163.0±5.8	<0.01
	Cold exposed	6	101.2±2.5	
2.	Control	6	176.2±8.2	N.S.
	Norepinephrine	7	152.0±8.9	
3.	Control	7	123.9±7.7	<0.01
	Norepinephrine + Pargyline	8	65.5±6.6	
4.	Control	5	131.3±7.9	N.S.
	Theophylline	6	122.1±10.6	
5.	Control	3	149.7±14.0	<0.05
	Norepinephrine + Pargyline	5	93.0±15.0	<0.01
	Norepinephrine + Pargyline + Propranalol	5	57.6±3.5	<0.05

The experiments were carried out at room temperature (23-25°) except where specified as cold exposure (0-2°). All the animals received 10 mg of cortisol (Sigma Chem. Co., U.S.A.) by injection (i.p.) 3 hr. before killing. The experimental period was 3 hr. in all cases. The dosages used per animal were: Norepinephrine, (Koch Light, U.K.) 0.2 mg; Pargyline, (Abbot Co. Ltd., U.S.A.) 5 mg; Theophylline (Sigma Chem. Co., U.S.A.) 15 mg; DL-Propranalol, (A gift from Dr. R.M.Marchbanks, London) 2.5 mg. N.S. - not significant.

But at the same dose given along with pargyline, an inhibitor of monoamine oxidase, the inhibition of the induction was clearly seen (Table 2). Pargyline by itself was found to have no effect under the conditions employed. These

experiments indicate that increased norepinephrine may have a counteracting effect on the induction process mediated by cortisol.

The experiment with theophylline showed no apparent change and therefore the inhibitory process may not involve cyclic AMP, as in the case of induction of tyrosine aminotransferase (9). This is further confirmed by the non-reversal of the inhibition on simultaneous treatment with a β-blocker, propranalol, along with norepinephrine and pargyline, and instead there was an unexplained enhancement of the inhibition (Table 2).

The experimental approach adopted here for the study of liver enzymes is considered acceptable in view of the known responses of liver plasma membranes to catechol amines and the autonomic drugs (10). Increasing evidence is becoming available on the participation of norepinephrine and the adrenergic receptors on the control of hepatic enzyme levels e.g., serine dehydratase (11), ornithine decarboxylase (12) and also tyrosine aminotransferase (13) depending on the time after administration. The present work gives, for the first time, evidence for an inhibitory role in the case of tryptophan pyrrolase also. Further, the increase in hepatic tryptophan pyrrolase on spinal section at C_{7-8} in both intact and adrenalectomized rats (14) can readily be explained as due to elimination of the inhibitory control by autonomic nervous system via norepinephrine.

These results suggest that norepinephrine regulates the cortisol-mediated induction of the enzyme, at the level of synthesis, by a mechanism possibly not involving the β-receptor and cyclic AMP. The interplay of the two hormones presumably determines the stabilized level of hepatic tryptophan pyrrolase in acclimation.

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