

ANALYSIS OF THE INFLUENCE OF SELECTED
NEUROTROPIC AGENTS ON HEPATIC METABOLISM IN
RELATION TO ENDOTOXICOSISM. K. AGARWAL*

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(Received 26 September 1973; accepted 13 February 1974)

Abstract—A number of neurotropic agents were tested for influence on hepatic metabolism in relation to their ability to alter sensitivity to bacterial endotoxin which is known to derange both neurotransmission and also the homeostasis of selected functions in the liver. Like endotoxin, dihydroxyphenylalanine (DOPA) lowered hepatic tryptophan pyrrolase (TP) activity within 6 hr and this effect was specific since neither of the two other analogues parachlorophenylalanine (PCPA) and phenylalanine, nor serotonin and LSD-25, depressed the hepatic TP. The LSD-25 or PCPA, but not phenylalanine, DOPA or serotonin, increased hepatic tyrosine transaminase (TT). Contrary to endotoxin, neither agent lowered liver glycogen levels in 4 hr but these decreased 18 hr after phenylalanine or LSD-25. HgCl_2 , which alters host response to endotoxin lethality like PCPA, increased the endogenous TT and TP activities, decreased liver glycogen, and permitted residual induction of these functions by exogenous hormone. The organic mercurial PCMB (which only minimally alters endotoxin toxicity) was less toxic than HgCl_2 for these hepatic functions. The uptake and distribution of cortisone were not altered by either mercurial. These results, in conjunction with those on the lethal effects of endotoxin, indicate the need to reconsider the molecular mechanisms of endotoxic reactions.

LIVER is a major tissue that detoxifies, metabolizes and eliminates substances that may or may not have liver as the focal point of attack. Indeed, the origin of some of these bioactive materials may actually be traced back to the liver. Thus appropriate hepatic adjustments are likely to dictate survival or fatality in the event of stress. Our past studies on the biochemical characterization of the stress response have attempted to accommodate the fact that certain agents, such as bacterial endotoxins, (also zymosan or glucan), are present only in the Kupffer cells of the liver and yet they modulate selected functions believed to be localized in the parenchymal cells (for a brief review see ref. 1).

Neurotoxic manifestations accompany endotoxic shock² which is reported to alter the tryptophan→serotonin pathway in the liver (see review in ref. 3). The vasoactive amine imbalance associated with endotoxin poisoning, moreover, could further contribute to derangement in hepatic homeostasis.⁴ In the present studies it was assessed whether substances such as parachlorophenylalanine (PCPA), lysergic acid diethylamide (LSD-25), dihydroxyphenylalanine (DOPA), all of which exhibit neurotropic properties, and inhibit designated steps of the aforementioned pathway, could alter the course of endotoxiosis, and if this could be accompanied with the type of changes in metabolism seen in livers from endotoxin poisoned animals.

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EXPERIMENTAL

Endotoxin (lot 569787) was purchased from Difco Labs. The following compounds were obtained from Calbiochem: *p*-chlorophenylalanine hydrochloride (PCPA; lot 0441), L-phenylalanine (lot 901856), L-dihydroxyphenylalanine (DOPA; lot 079024), and *p*-chloromercuribenzoate (PCMB; lot 01847). Mercury (II) chloride (HgCl_2 ; lot 1475874) and serotonin creatinine sulphate (SER; lot 70156273) were products of Merck. Cortisone acetate was purchased from Roussel Labs. (Paris) and lysergic acid tartaric salt (LSD-25) was a gift from Sandoz (courtesy J. Chosson, A. Pons and R. A. Chatain). 1,2- ^3H -cortisone (6 Ci/mM) was purchased from CEA, Saclay, France.

All materials were injected intraperitoneally in a volume of 0.5 ml. Cortisone and endotoxin were suspended in isotonic, nonpyrogenic saline; all others were dissolved in pyrogen free water (Meram, Paris). The amounts and injection schedules are given at the appropriate places in the paper.

Hepatic tyrosine transaminase (TT) activity was determined by the colorimetric procedure described by Rosen *et al*⁶ as adapted to mice.⁷ The activities are calculated as μg of *p*-hydroxyphenylpyruvic acid formed/mg tissue/10 min.

Liver tryptophan pyrrolase (TP) activity was measured by the procedure of Knox⁸ as previously described.⁹ Data are presented as μmoles of kynurenine formed/g of fresh liver/hr.

The glycogen concentration was assessed by the technique of Kemp and Kits van Heijningen,¹⁰ and is expressed as mg/100 mg tissues.

The distribution of ^3H -cortisone was studied by a procedure previously described in detail.¹¹ Additional details are given in the text. All samples were placed in Insta-gel (Packard) and counted in an Inter technique SL-30 liquid scintillation spectrometer. Corrections for quenching and background were made as before.¹¹

A standard error was calculated for data in all experiments. A probability level of 2 per cent or less, as estimated by Student's *t*-test, was considered statistically significant.

In some experiments, survival of male, Swiss, albino mice (26 ± 2 g) was recorded for 48 hr after challenge with 250 μg endotoxin. The dose at which 50 per cent of the animals survived (LD_{50}) was calculated according to Reed and Muench.¹² Animals were housed on vermiculite bedding and food and water were available *ad lib.* at all times unless otherwise indicated.

RESULTS

It is clear from the data in Fig. 1 that liver TP activity exhibited transient increase (maximum at 4 hr) after PCPA or saline although this could reflect endogenous corticoid release. Such a rise was not observed in DOPA injected animals which exhibited a significantly low level of hepatic TP (minimum at 6 hr; $P < 0.001$), and this remained less than control 12 hr later. Thus, just as DOPA lowers tryptophan hydroxylase,⁵ so does it inhibit liver tryptophan pyrrolase. PCPA did not lower TP activity at any time period.

The TT activity appeared to exhibit diurnal rhythm in saline injected control mice. The stimulatory effect observed shortly after PCPA was not further characterized although similar studies in adrenalectomised animals should reveal any contribution of the pituitary-adrenal axis under these conditions.

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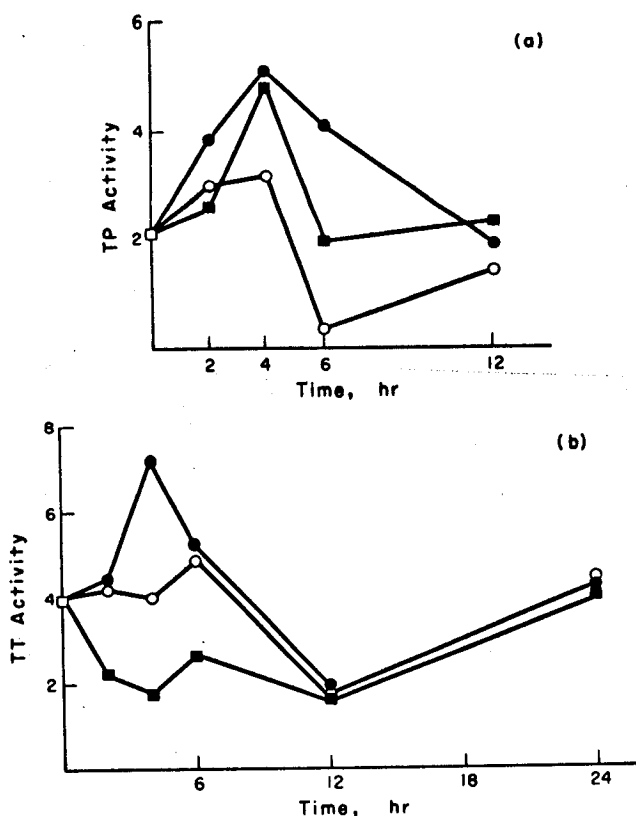


FIG. 1. Change with time in enzyme activities from livers of mice pretreated with the neurotropic material. Animals were given, intraperitoneally, 0.5 ml saline (■), 10 mg *p*-chlorophenylalanine (●), or 10 mg dihydroxyphenylalanine (○). Thereafter, livers were assayed for tryptophan pyrrolase (a) or tyrosine transaminase (b) activity at the indicated time points. All values are average of 5-6 separate determinations. The standard errors were left out to keep the figures readily comprehensible. Relevant statistics is presented in the text.

Data in Table 1 show that TP activity was unchanged 4 or 18 hr after administration of either LSD-25 or phenylalanine. The inhibitory effect of dihydroxyphenylalanine on liver TP (Fig. 1) would therefore appear to be specific since neither phenylalanine (Table 1) nor its parachloro-analogue (PCPA, Fig. 1) lowered liver TP. Since endotoxin lethality is not altered by DOPA (Table 1; ref. 13) contrary to previous beliefs,³ hepatic TP levels do not show parallelism with the state of host resistance to endotoxin. Also, PCPA sensitized to endotoxin (Table 1; ref. 13) without lowering TP activity (Fig. 1). Additionally, serotonin appeared to stimulate liver TP ($P < 0.05$) but it did not increase resistance against endotoxin (Table 1).

Data in Table 2 show that TT activity was elevated 4 hr after LSD-25 ($P < 0.05$) but it was not determined whether this represents induction by corticoids possibly released in response to the injected materials. The TT levels were normal 18 hr after either substance.

TABLE 1. INFLUENCE OF SELECTED NEUROTROPIC AGENTS ON LIVER TRYPTOPHAN PYRROLASE ACTIVITY IN RELATION TO ENDOTOXIN LETHALITY IN MICE

Treatment*	TP Activity		Living/total†
	4 hr	18 hr	
Saline	1.58 ± 0.05	1.50 ± 0.03	68/126
LSD	1.98 ± 0.02	1.63 ± 0.03	6/10
PHE	1.56 ± 0.01	1.42 ± 0.02	21/44
SER	1.08 ± 0.02	2.26 ± 0.02	6/10
DOPA			20/28
PCPA			15/59

* Animals were given either 0.5 ml saline (i.p.), 1 mg LSD (i.p.), 5 mg serotonin (i.p.) or 10 mg (i.p.) of any one of the other indicated materials. Liver TP activity was determined as previously described.⁹ For DOPA and PCPA see Fig. 1. All values are average of five separate determinations ± the standard error.

† One hr after the indicated treatment, animals were challenged with endotoxin (250 µg i.p.). Survival was recorded until 48 hr.

Data in Table 3 show that liver glycogen did not differ from the control level 4 hr after either of the indicated materials. A decrease in liver glycogen levels, however, was observed 18 hr after phenylalanine ($P < 0.01$) or LSD ($P < 0.02$). Thus, sensitization to endotoxin following PCPA pretreatment (Table 1) was not due to low hepatic energy reserves. Decrease in hepatic glycogen by LSD or phenylalanine (Table 3), moreover, was not associated with impaired resistance against the lethal effects of endotoxin (Table 1). This is important since endotoxin poisoned animals consistently exhibit low hepatic glycogen reserves and carbohydrate metabolism has been implicated to play a causal role in the outcome of endotoxemia.³

In an earlier study¹³ it was shown that PCPA sensitization to endotoxin lethality was similar to that seen after HgCl_2 (but not after PCMB) pretreatment under comparable conditions. Data in Table 4 show that HgCl_2 , but not its aromatic analogue PCMB, decreased the hepatic glycogen levels within 6 hr and the glyconeogenic action of cortisone (given 1 hr after the mercurial) was impaired by both substances. Hepatic glycogen levels were normal 24 hr after either mercurial, or the mercurial

plus the horrogenous TT and was more effective in mice given HgCl_2 .

Thus, HgCl_2 as the inducer may explain the protection against sepsis comparable to endotoxin. HgCl_2 actually

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TABLE 2. INFLUENCE OF SELECTED NEUROTROPIC DRUGS ON LIVER TYROSINE TRANSAMINASE ACTIVITY

Treatment*	TT Activity	
	4 hr	18 hr
Saline	4.74 ± 1.08	4.88 ± 0.57
LSD	8.96 ± 0.84	4.03 ± 0.54
PHE	3.11 ± 1.71	3.41 ± 0.13
SER	7.63 ± 1.39	4.47 ± 0.26

* The tyrosine transaminase activity was determined as previously described,⁷ after the animals had been injected as for experiments in Table 1 and Fig. 1. All values are average of five separate determinations ± the standard error.

TABLE 4. INFLUENCE

Treatment*

Saline
 HgCl_2
 HgCl_2 +
Cortisone
PCMB
PCMB +
Cortisone
Cortisone

* Animals were assayed for endotoxin or the mercurial. All values represent

TABLE 3. INFLUENCE OF SELECTED NEUROTROPIC SUBSTANCES ON HEPATIC GLYCOGEN CONCENTRATIONS

Treatment	Glycogen (mg %)	
	4 hr	18 hr
Saline	7.94 $\pm$ 0.39	13.29 $\pm$ 0.57
PCPA	8.84 $\pm$ 0.50	12.98 $\pm$ 0.49
DOPA	7.51 $\pm$ 0.51	11.07 $\pm$ 0.84
PHE	7.31 $\pm$ 0.76	9.58 $\pm$ 1.07
LSD	7.81 $\pm$ 0.71	10.88 $\pm$ 0.58
SER	6.73 $\pm$ 0.50	11.20 $\pm$ 1.01

* Details of injections are given in Table 1 and Fig. 1. The glycogen concentrations were determined by the method described earlier.¹⁰ All values are average of 5-7 separate determinations $\pm$ the standard error.

plus the hormone. Data in Table 4 further show that HgCl_4 increased the endogenous TT and TP levels to a greater extent than PCMB. Furthermore, the hormone was more effective in inducing these enzymes in PCMB pretreated animals than in mice given HgCl_2 .

Thus, HgCl_2 was more toxic than PCMB both on the endogenous levels as well as the inductive effect of exogenous cortisone, on all the three systems tested and this may explain why HgCl_2 sensitizes to endotoxin lethality and prevents hormonal protection against endotoxic death. It has been established that PCMB and HgCl_2 possess comparable toxicities for normal mice¹³. In any event, effects on host resistance to endotoxin lethality cannot be related to depletion of hepatic TP levels since HgCl_2 actually increased this enzyme in the liver.

Attention was directed next to hepatic uptake and distribution of cortisone under these conditions since binding of the hormone to "specific" intracellular receptors is said to constitute the first step in the array of events leading to the maintenance of homeostasis by glucocorticoids (brief review in ref. 11). Data in Table 5 show that the nucleo/cytoplasmic ratio of cortisone bound to specific receptors was identical

TABLE 4. INFLUENCE OF SELECTED MERCURIALS ON THREE HEPATIC FUNCTIONS SENSITIVE TO CORTISONE OR ENDOTOXIN IN NORMAL MICE

Treatment*	Glycogen (mg %)		TT Activity 6 hr	TP Activity 6 hr
	24 hr	6 hr		
Saline	12.50 $\pm$ 0.84	7.94 $\pm$ 0.39	4.74 $\pm$ 1.08	1.58 $\pm$ 0.05
HgCl_2	10.35 $\pm$ 1.01	3.52 $\pm$ 0.79	14.91 $\pm$ 1.81	4.54 $\pm$ 0.10
HgCl_2 + Cortisone	12.38 $\pm$ 0.68	4.13 $\pm$ 1.08	17.41 $\pm$ 0.18	4.93 $\pm$ 0.05
PCMB	10.32 $\pm$ 0.50	7.87 $\pm$ 1.26	7.77 $\pm$ 1.59	2.27 $\pm$ 0.25
PCMB + Cortisone	13.88 $\pm$ 0.57	8.96 $\pm$ 0.80	14.86 $\pm$ 0.66	3.89 $\pm$ 0.22
Cortisone	11.35 $\pm$ 0.43	10.35 $\pm$ 1.35	13.37 $\pm$ 0.41	4.43 $\pm$ 0.13

* Animals were given 200 μg of either HgCl_2 or PCMB alone (i.p.) or 1 mg cortisone (i.p.) 1 hr later. They were assayed 6 or 24 hr after the mercurial. In concurrent experiments, animals were given only endotoxin or the hormone plus the toxin to reproduce previous results³ but these are not presented here. All values represent average of 6-7 individual determinations $\pm$ the standard error.

TABLE 5. DISTRIBUTION OF CORTISONE IN LIVERS OF MICE PRETREATED WITH MERCURIALS

	Counts/min/mg protein*			
	No carrier		With carrier	
	Cytoplasm	Nuclei	Cytoplasm	Nuclei
Saline	5110 $\pm$ 676	1402 $\pm$ 150	3794 $\pm$ 331	915 $\pm$ 73
HgCl ₂	4577 $\pm$ 518	1580 $\pm$ 150	3920 $\pm$ 290	963 $\pm$ 72
PCMB	5026 $\pm$ 271	1624 $\pm$ 78	3385 $\pm$ 668	933 $\pm$ 196

* One hr after saline, 200 μ g of HgCl₂ or PCMB (all i.p.) animals were given a 10 min pulse of either 5 μ Ci of 1,2-³H-cortisone alone or with 1 mg, cold cortisone acetate. Protein concentrations were determined by the method of Biuret. All values represent average of 6-7 individual determinations $\pm$ the standard error.

in saline, HgCl₂ or PCMB pretreated groups, although somewhat more steroid got bound to the nuclear fraction with cortisone of very high specific activity. Thus, contrary to a previous report *in vitro*,¹⁴ HgCl₂ did not inhibit association of steroid with the receptor *in vivo*. The sensitizing effect of HgCl₂ on endotoxin lethality,¹³ therefore, cannot be explained as a possible inhibition of cortisone distribution in the target organ. This is important since endotoxin and cortisone appear to compete for the same receptor/effector entities in tissues.¹¹

DISCUSSION

The results described in this paper show that among the neurotropic substances, only dihydroxyphenylalanine decreased liver TP activity and that this effect was specific since the other two analogues, phenylalanine and parachlorophenylalanine, were ineffective in this regard. Thus, just as DOPA inhibits tryptophan hydroxylase,⁵ and tyrosine transaminase *in vivo*,¹⁵ so does it lower liver TP activity.

The increase in TT activity after LSD-25 or PCPA, but not after PHE, serotonin or DOPA, may be attributed to endogenous corticoid release but no attempts were made to reproduce these results in adrenalectomized mice. However, it must be borne in mind that liver glycogen levels were not altered by either agent within the first 4 hr and were below normal 18 hr after PHE or LSD-25. Thus, the contribution of endogenous corticoids, under these conditions, remains conjectural. All factors controlling enzyme induction or liver glyconeogenesis have not yet been elucidated but it is evident that hepatic homeostasis is susceptible to alteration by selected neurotropic drugs.

Although endotoxin is reported to alter neurotransmission,² the putative neurotransmitters hitherto tested do not modify endotoxin lethality.¹³ PCPA was the only neurotropic agent that sensitized to endotoxin but this was also observed with HgCl₂. So there is no direct evidence to believe the idea² that altered neurotransmission may play a determining role in endotoxemia. It is, however, conceivable that continual internal adjustments in such parameters may initiate a series of positive, circular events with progressive worsening of critical processes in various tissues. Whatever the nature of the supposed targets, it is clear that derangement of the tryptophan-serotonin pathway (as obtained after endotoxin) does not appear to be causally

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related to the eventual outcome of endotoxic shock, as had previously been suggested.³ Thus, DOPA inhibited this pathway at two steps viz. hepatic TP (Fig. 1) and tryptophan hydroxylase⁵ without influencing endotoxin lethality (Table 1). Contrarily, PCPA sensitized to endotoxin (Table 1), and altered hormonal protection against lethality,¹³ but it did not influence liver TP to any considerable extent (Fig. 1). Serotonin, moreover, appeared to increase hepatic TP without augmenting host resistance against endotoxin death (Table 1). The role of TP as a factor that may determine the course of endotoxemia has also been obviated by other approaches.^{3,16,17}

The outcome of endotoxin poisoning after HgCl₂ is similar to that after PCPA. Both materials sensitize to endotoxin lethality and prevent hormonal protection against endotoxic death.¹³ Contrary to its effects *in vitro*,¹⁸ the inorganic mercurial actually increased liver TT and TP levels *in vivo*. This further argues against the contribution of liver TP as a possible factor determining endotoxic death.

Nearly half a century ago it was hypothesized that hepatic glycogen levels are important determinants of endotoxic reactions,³ and glucocorticoid protection against endotoxic death was explained as an amelioration by the glycogenic action of the hormone, of hepatic glycogen depletion by endotoxin. This sort of thesis is supportable with results after HgCl₂, and further fortified by the limited effectiveness of PCMB both on endotoxin toxicity¹³ and liver glycogen (Table 4). However, experiments with PCPA do not support the idea that liver glycogen levels be faithful indicator of endotoxic reactions.

Since cortisone remains one of the few pharmacological agents that can impart protection against endotoxic death, and since cortisone and endotoxin appear to compete for common intracellular sites, it was hoped that alterations in this parameter may explain the results with HgCl₂, especially since the mercurial is reported to disrupt cortisone-receptor association *in vitro*.¹⁴ Unfortunately, this was not true of the situation *in vivo* (Table 5). This further alienates the extrapolation *in vivo* of conclusions drawn from studies *in vitro*. Furthermore, the nucleocytoplasmic index of bound cortisone may not be as faithful an indicator of sensitivity to corticoid action as has hitherto been accepted (review in ref. 11).

In conclusion, the studies described here further support the possibility that features sensitive to the inductive effect of cortisone may be critically important in host response to endotoxin. However, the existing hypotheses apparently do not suffice to explain all experimental evidence. But it is to be hoped that consideration of such exceptions will lead to a search for a unified mechanism of endotoxic reactions.

Acknowledgements—Thanks are due to Edith Bourgeois for technical assistance and to various colleagues for helpful discussions.

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