Bull. Environ. Contam. Toxicol. (1987) 38:561–564

Environmental Contamination and Toxicology

Brain Ammonia Metabolism in Hexachlorophene-Induced Encephalopathy

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In spite of long and extensive use of hexachlorophene (HCP) as an antibacterial and antifungal agent in a wide variety of cosmetics, medical and home care products since 1949 (Gump & Walter 1968), it was only in 1971 that the toxic potential of this compound was recognized; mainly because of the HCP-induced neuropathologic changes observed by kimbrough *et al.* Since then, several studies concerned with the neurotoxicity of HCP have been conducted, however the biochemical mechanism of HCP action remained obscure. HCP is reported to exert toxic effects on non nervous tissues as well. Freeze fracture studies showed that administration of an acute dose of 30 mg HCP/kg causes disorganization in tight junctions, increase in number of gap junctions and disruption of mitochondria in the liver of rats (Robenek *et al.* 1980). When HCP was given intragastrically at doses of 60 mg/kg/day for 1 week, mice exhibited degenerative changes in liver (Prasad 1986). Since hepatic degeneration is most commonly associated with abnormal ammonia metabolism and brain function, an attempt has been made in the present study to investigate the cerebral ammonia, glutamate and related metabolite patterns in mice during HCP-induced neurotoxicity.

MATERIALS AND METHODS

Male mice, *Mus booduga* (Gray) maintained under laboratory conditions (temperature 30 ± 2°C; relative humidity 75% and a light period of 12 h) with free access to standard food pellets (Hindustan Lever Limited) and water were employed in the present investigation.

Hexachlorophene (HCP), [2,2'-methylenebis (3,4,6 trichloro phenol)] purchased from Sigma Chemicals, USA; was administered to experimental mice in minimum quantities of corn oil (Sigma) intragastrically at sublethal doses of 60 mg/kg/day for 1 week. Mice were weighed daily and checked for behavioural changes. The control animals were given corn oil alone. On the eighth day, blood was collected in pre-chilled heparinized vials and the brains were rapidly chilled after isolation. The blood and brain samples were immediately processed for biochemical analyses.

The ammonia concentration in blood and brain was determined by the

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method of Sadasivudu *et al.* (1977). The glutamic acid, glutamine and γ -amino butyric acid (GABA) levels were estimated after pre-column derivatization with *o*-phthaldialdehyde, by a high-pressure liquid chromatographic (HPLC) method proposed by Rajendra (1986). All the reagents used were of HPLC grade. The HPLC system (Waters Associates, MA, USA) consisted of an automated gradient controller (Model 680), two solvent delivery systems (Model M45), a fluorescence detector (Model 420) and a Rheodyne injection valve 7125 with a filling loop. The fluorometric measurements were made at an extinction wave length of 338 nm and an emmission wave length of 425 nm with a 12 μl flow cell and fluorescent lamp. The brain/blood ammonia concentration ratios and GABA/glutamic acid ratios of the brain were obtained by simultaneous measurements from individual subjects as described earlier (Prasad 1986).

RESULTS AND DISCUSSION

Table 1. Deviations in blood and brain levels of ammonia, glutamate and related metabolites and certain biochemical ratios in mice following repeated HCP treatment

Parameter	Concentration (mM) Control	HCP treated	%Alteration
Ammonia			
Blood[a]	0.132 ± 0.01	0.271 ± 0.03[c]	+ 105.3
Brain[b]	0.207 ± 0.01	0.498 ± 0.03[c]	+ 140.6
Brain/Blood	1.61 ± 0.07	1.87 ± 0.17[d]	+ 16.5
Glutamic acid			
Brain[b]	2.99 ± 0.15	2.57 ± 0.21[c]	- 14.5
Glutamine			
Blood[a]	0.708 ± 0.06	1.53 ± 0.13[c]	+ 116.1
Brain[b]	5.41 ± 0.66	14.15 ± 2.02[c]	+ 161.6
Brain glutamine/ Brain glutamic acid	2.10 ± 0.31	5.11 ± 0.44[c]	+ 143.3
GABA			
Brain[b]	2.11 ± 0.16	2.64 ± 0.30[c]	+ 25.1
Brain GABA/ Brain glutamic acid	0.707 ± 0.14	1.03 ± 0.23[d]	+ 45.7

Values are mean ± SD of six observations [a]expressed in mM, [b]expressed in μ moles/gr fresh weight; [c]different from control with $P < 0.001$; [d]different from control with $P < 0.01$.

Symptoms of HCP intoxication were mainly neurological. Daily administration of 60 mg HCP/kg produced hind limb weakness in mice within 3-5 days which was progressed to paralysis after 5 days treatment. After 6-7 days treatment animals became drousy, exhibited listlessness and weighed less than controls.

There was a steep rise in blood and brain ammonia levels following 7 days HCP treatment. The brain/blood ammonia concentration ratios of control and HCP intoxicated mice are presented in table 1. In control mice the ratios were in a range of 1.6 ± 0.07 which are in agreement with the findings of other investigators (Ehrlich *et al.* 1980; Benjamin 1982) who have reported values greater than unity in rats. These ratios were significantly ($P < 0.001$) increased (16.5%) in HCP intoxicated subjects, denoting relatively higher accumulation of ammonia in brain than in blood.

Histopathological studies in our laboratory demonstrated the swelling, membrane disruption and nuclear degeneration in hepatocytes of mice given 60 mg HCP/kg/day for 1 week (Prasad 1986). These findings are compatable with the idea that the impairment of hepatic ammonia detoxication function could be involved in the rise of blood and brain ammonia levels. The data further showed a conspicuous rise in glutamine and GABA levels and depletion of glutamate levels in brain which were statistically significant ($P < 0.001$). The glutamine/ glutamic acid ratios of brain were high in intoxicated mice probably due to the reversible fixation of excess ammonia as glutamine.

The reasons for relatively more accumulation of ammonia in brain than in blood were not understood. Studies of Prasad (1986) negated the possibility of endogenous ammonia generation, since the major ammonogenic enzymes of the brain, AMP deaminase, glutaminase and glutamate dehydrogenase have showed either reduced or unaltered activities following different time durations of HCP administration. Hence, an increased uptake of ammonia from the hyperammonemic blood might be involved. The main factors that influence the uptake of ammonia by the brain include the blood ammonia concentrations (Lockwood *et al.* 1979) and the integrity of the blood-brain barrier (Ehrlich *et al.* 1980). The effect of HCP on the blood-brain barrier is not understood. The nitroxide spin label quenching experiments of Rakhit and Hanig (1984) have showed that the HCP alters permeability properties of the brain membranes. Further, hepatic dysfunction is known to cause morphological changes in astrocytes (Norenberg *et al.* 1974) and therefore may reflect in altered blood-brain barrier function (Bradbury 1975). Similar sort of perturbations in blood-brain barrier along with hyperammonemia might deserve consideration in the increased accumulation of ammonia in HCP intoxicated mouse brain.

Whatever the explanation for the accumulation of ammonia in the brains of HCP intoxicated mice, such a change may have implications in the neurotoxicity of HCP, since ammonia is known to affect the brain function in a multifaceted fashion (Benjamin 1982). Moreover, the accumulation of GABA and increased GABA/glutamic acid ratios are indicative of depressed state of the brain, which may have contributory significance in the expression of HCP neurotoxicity.

Acknowledgments. The authors are grateful to Prof. E.W. Banister, Dept. of Kinesiology, Simon Fraser University, Burnaby, Canada for his help in HPLC of free amino acids. Supported by the Council of Scientific and Industrial Research, New Delhi, India by a Senior Research Fellowship to Dr. G.V.Prasad.

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Received August 28, 1986; Accepted November 24, 1986