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Aminogram of Mouse Skeletal Muscle: Deviations in Response to Repeated Hexachlorophene Treatment

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Although hexachlorophene (HCP) was used extensively as a bactericide and fungicide in soaps, cosmetics and other personal care products as well as in veterinary medical and industrial applications, the use of this bisphenol has declined recently because of its inherent neurotoxic effects (Kimbrough 1976). Neurotoxicity of HCP has been extensively investigated, however, reports on the effects of HCP on non nervous tissues are scanty. We have recently drawn attention towards the implications of HCP-induced peripheral metabolic changes in the operational failure of biochemical homeostatic mechanisms (Prasad 1986; Prasad et al. 1986). In view of the dynamic role of skeletal muscle in the amino acid metabolism, we examine in this study the effects of repeated HCP administration on the free amino acid patterns of the skeletal muscle.

MATERIALS AND METHODS

Healthy male mice, Mus booduga (Gray), maintained under laboratory conditions (temperature 30 + 2°C; relative humidity 75% and a light period of 12 h) with full access to food and water were employed in the present study.

Hexachlorophene (2,2'-methylenebis (3,4,6-trichlorophenol) (99.8% pure) obtained from Sigma Chemical Co., St.Louis, Mo., USA, was dissolved in minimum quantities of corn oil and was administered orally with a stomach tube connected to a glass syringe, at a sublethal dosage of 60 mg/kg/day for 7 consecutive days. The control mice were given an equal quantity of the vehicle alone (50 µl). On the eighth day the control and HCP administered mice were sacrificed and the gastrocnemius muscles were rapidly isolated and homogenized (approximately 50 mg/ml) in methanol to extract free amino acids. The extraction was repeated thrice to ensure the complete recovery of tissue free amino acids.

The concentrations of individual free amino acids were detected by a high-performance liquid chromatography (HPLC), after pre-column derivatization with o-phthaldialdehyde (Rajendra 1986).

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The HPLC system (Waters Associates, MA, USA) consisted of a Model-680 automated gradient controller, Model-M45 solvent delivery systems, a Model-420 fluorescence detector and a Rheodyne injection valve 7125 with a 20-µl filling loop.

After derivatization, 20 µl of amino acid standard/extract was injected into an ultrasphere 3 µ ODS (7.5 cm X 4.5 mm ID) reversed phase column (Beckman instrument, Inc., CA, USA) which was used in conjunction with a stainless steel guard column (20 X 3.9 mm ID) packed with Waters corasil C-18 packing material and a pre-column filter (2 µm). The column was maintained at room temperature (21°C). The chromatographic conditions and elution procedure were as described earlier (Rajendra 1986). The fluorimetric measurements were made at an excitation wave length of 338 nm and emission wave length of 425 nm with a 12- µl flow cell and a fluorescent lamp. The chromatographic peaks were rocorded and integrated by a SP 4100 computing integrator system (Spectra Physics, San Jose, CA, USA).

The skeletal muscle samples from control and HCP administered mice were homogenized to 5% (w/v) in 0.25 M sucrose and centrifuged (2000 X g)for 15 min. The aliquots were used for the assay of alanine aminotransferase, aspartate aminotransferase (Sigma 1979) and glutamate dehydrogenase (Lee and Lardy 1965). Glutaminase and glutamine synthetase were assayed as described by Meister (1955) and Rowe et al. (1970) respectively. The protein concentrations were determined using crystalline bovine serum albumin as standard (Lowry et al. 1951).

Six replicates were maintained for each observation on the enzyme assays and HPLC of free amino acids. Statistics were performed by student's t-test.

RESULTS AND DISCUSSION

Oral administration of HCP at a dosage of 60 mg/kg/day for 7 consecutive days resulted in significant changes in the concentration of different free amino acids of mouse skeletal muscle. Typical high-performance liquid chromatograms derived from o-phthaldialdehyde derivatives of the standard and tissue free amino acids are shown in figure 1. The pathological aminogram (Fig 2) presents the deviation from the respective controls of individual free amino acid pool sizes of the skeletal muscle of mouse subjected to HCP treatment. Among neutral amino acids phenylalanine (PHE), tyrosine (TYR), leucine (LEU), isoleucine (ILE), valine (VAL), threonine (THR), asparagine (ASN) and glutamine (GLN) have showed significant ($P < 0.01$) elevation, while the serine (SER) levels were not significantly affected. The rise in branched chain amino acids (LEU, ILE,& VAL) and aromatic amino acids PHE and TYR were highly conspicuous as compared to all the other amino acids. Changes in the acidic amino acids were denoted by elevation of glutamic acid (GLU) and fall in aspartic acid (ASP) concentrations which were statistically significant ($P < 0.01$). Alanine (ALA) and

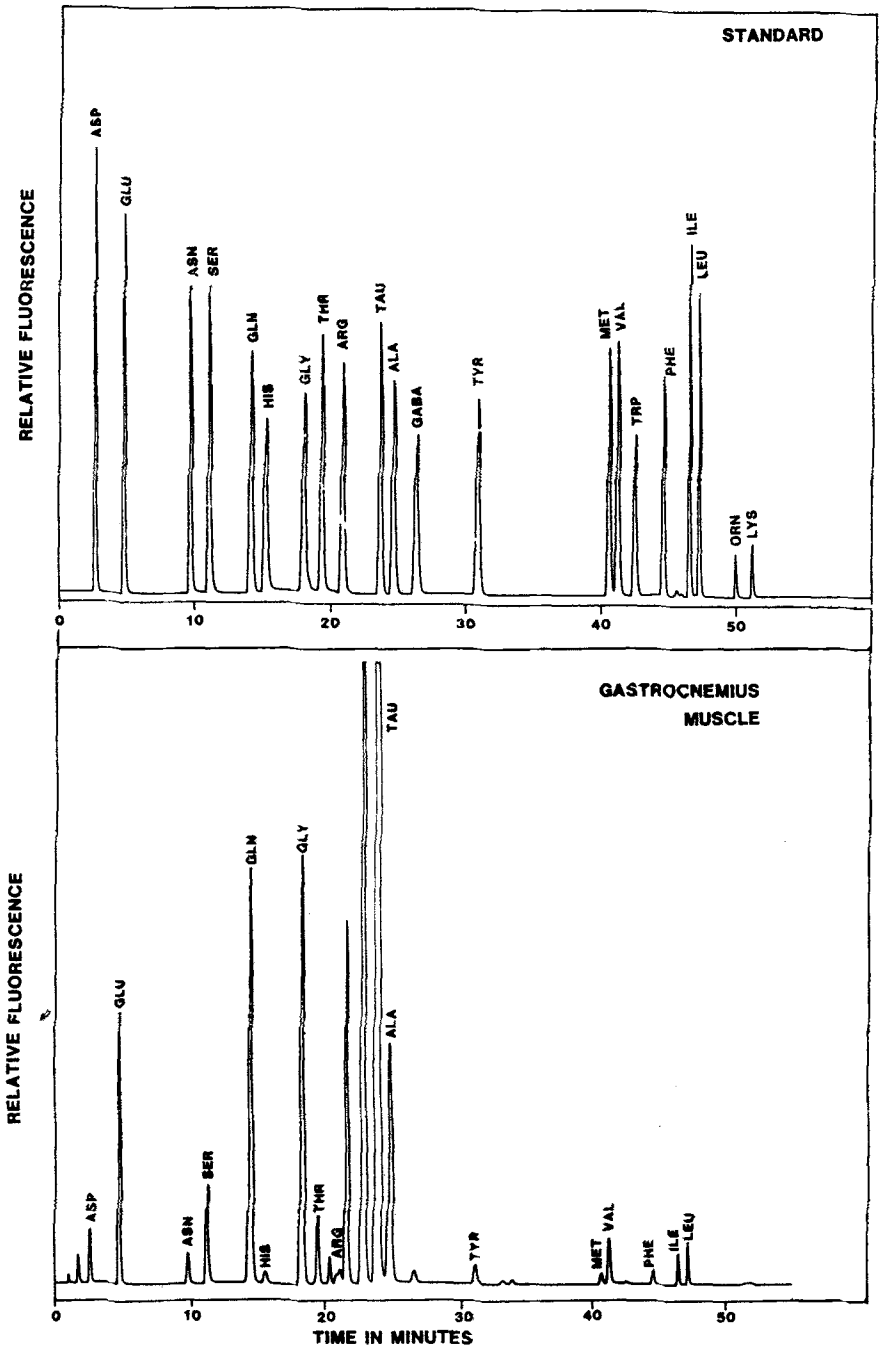

Figure 1. Typical high-performance liquid chromatograms of free amino acids, derived from standard amino acid mixture and mouse skeletal muscle extract.

glycine (GLY) levels were not affected significantly (Fig 2).

The results revealed a general trend of elevation of the majority of the amino acids leading up in a pronounced rise in total free amino acids in skeletal muscle after repeated HCP treatment. The abnormal free amino acid patterns observed in this study can be attributed to the loss of hind limb function with a subsequent reduction in the muscle mass (Hanig et al. 1976). Others have demonstrated sciatic nerve lesions and reduced nerve conduction velocities in HCP intoxicated rats (Maxwell and Pamela 1979). These may be of interest because the loss of neural influence

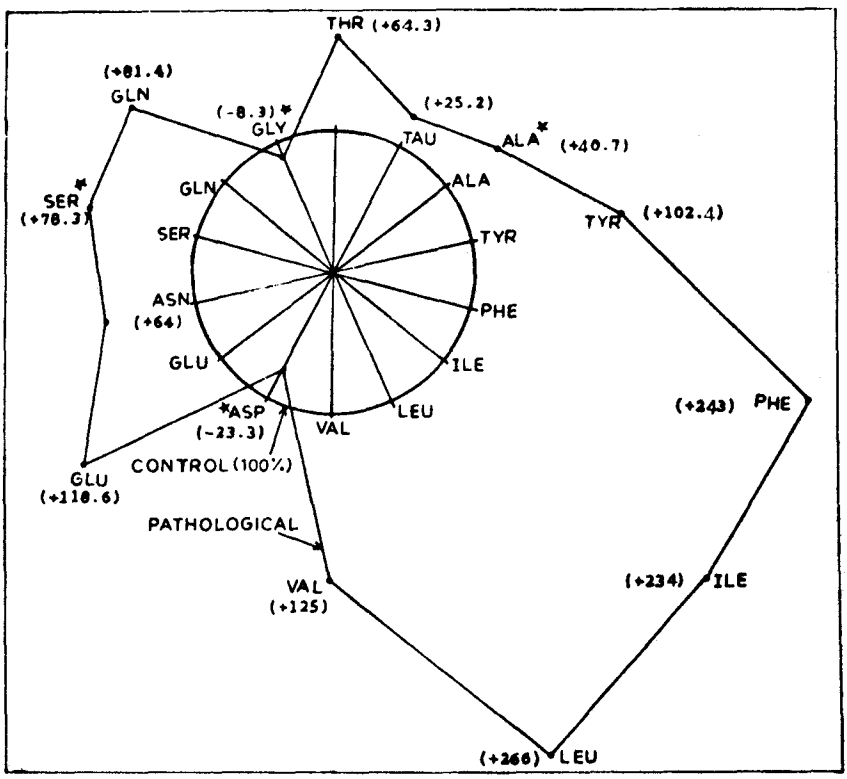

Figure 2. Deviations in mouse skeletal muscle aminogram following repeated HCP treatment. Values in parentheses are per cent changes over controls. The statistical significance was set at $P < 0.01$. The asterisk (*) denotes insignificant deviation from control.

triggers proteolysis in muscle (Gutman 1973). In consonance with these findings, Prasad (1986) reported the loss of myofibrillar and sarcoplasmic proteins after 7 days HCP treatment suggesting that the lysis of these protein fractions could have a contributory role in the rise of free amino acid levels in skeletal muscle.

The reasons for the abnormal rise in branched chain (BCAA) and aromatic (AAA) amino acids were not clearly understood. Under

normal conditions the AAA are primarily metabolized in liver, in contrast the BCAA are selectively excluded from hepatic uptake and are metabolized predominantly in skeletal muscle (Adibi 1980; Munro 1983). Microscopic examination of the HCP intoxicated mouse liver revealed swelling and membrane disruption in the majority of hepatocytes, some of them exhibiting degenerated nuclei, further, these perturbations were associated with a sharp rise in serum transaminases and manifestation of hyperammonemia which are indicative of hepatic dysfunction (Prasad 1986). These pathological changes raised the possibility of the impairment of hepatic AAA metabolism, to be involved in the rise of AAA in the blood with a subsequent rise in skeletal muscle. The degenerative changes in the liver may deserve consideration also in the abnormal rise in BCAA, predictively by manifesting a state of hormonal imbalance. Krass et al. (1974) demonstrated that half of the insulin secreted by the pancreas is removed by the liver, consequently animals with degenerated liver contain excessive insulin which is known to stimulate the influx of BCAA into the skeletal muscle (Munro 1983). However, our unpublished results showed a significant rise in the BCAA levels in the brain and liver. It appears from these findings that there may be an impairment in BCAA catabolism. Such a possibility has been supported by the potent inhibitory efficacy of HCP on various dehydrogenase enzymes (Wang and Buhler 1978). A similar inhibitory effect on branched chain keto acid dehydrogenases which irreversably commit the BCAA to the oxidative cycle in muscle (Adibi 1980) can be predicted, since HCP is known to accumulate in skeletal muscle in significant quantities following oral treatment (Miller et al. 1978).

Data presented in table 1 show the effect of repeated HCP treatment on the enzymes associated with amino acid metabolism. The aspartate aminotransferase (AAT) and glutamate dehydrogenase showed significant ($P < 0.001$) elevation, while alanine aminotransferase (AlAT) showed reduction in activity levels after HCP treatment. Glutaminase activity was not significantly affected. A significant drop in aspartic acid despite a generalized elevation of the majority of free amino acids and observed elevation in AAT activity and glutamate concentration may be indicative of the diversion of aspartic acid nitrogen towards glutamate formation. However, reductive amination and amidation of 2-oxoglutarate and glutamate respectively appear to be primarily responsible for the elevation of glutamic acid and glutamine as evidenced by a significant rise in GDH and glutamine synthetase activities. Insignificant deviation in alanine and reduced AlAT activities may suggest decreased rates of alanine formation. The pattern of changes observed in the amino acid profiles and amino acid-enzymes of atleast the glutamate family appears to be secondary to hyperammonemia manifested as a result of multifaceted action HCP on liver (Prasad 1986). The changes were well in line with typical metabolic alterations observed under various experimental situations of hyperammonemia (Benjamin 1982). Thus multiple factors may underlie the sharp deviations of amino acid levels in skeletal muscle. Further, the abnormal transamination and deamination

Table 1. Effect of repeated HCP treatment on the specific activities of certain amino acid enzymes of the mouse skeletal muscle.

Enzyme	Specific activity Control	HCP treated
Alanine aminotransferase (μ moles of pyruvate/mg protein/h)	0.399 $\pm$0.044	0.319 $\pm$0.035[b] (-20.05)
Aspartate aminotransferase (μ moles of pyruvate/mg protein/h)	6.54 $\pm$0.54	7.72 $\pm$0.73[b] (+18.04)
Glutamate dehydrogenase (n moles of formazan/mg protein/h)	55.0 $\pm$3.0	70.0 $\pm$6.0[b] (+27.3)
Glutaminase (μ moles of ammonia/mg protein/h)	0.149 $\pm$0.014	0.157 $\pm$0.01[c] (+5.37)
Glutamine synthetase (μ moles of γ-glutamyl hydroxamate/mg protein/h)	0.497 $\pm$0.06	1.03 $\pm$0.24[a] (+107.2)

Values are mean $\pm$ S.D. of six experiments, values in the parentheses are per cent deviations over controls. Student's t-test: a) $P < 0.001$ b) $P < 0.01$ c) Insignificant difference from controls.

patterns are reflective of the disturbance in the overall amino acid metabolism in skeletal muscle.

To sum up the results, it can be envisaged that abnormal rise in free amino acids of the skeletal muscle may be due to the increased mobilization of tissue protein and failure of the liver and skeletal muscle to utilize the free amino acids for gluconeogenesis and/or for oxidation purposes. However it is not understood whether these changes are secondary and/or due to the direct effect of HCP that accumulates significantly in skeletal muscle following oral administration (Miller et al. 1978). Whatever the reasons for the abnormal amino acid pattern in skeletal muscle during HCP intoxication, such a change and other perturbations such as depletion of hepatic enzymes of amino acid metabolism and loss of muscle protein (Prasad 1986) are reflective of disturbed muscle-liver metabolic interrelationship which is indispensable for the maintenance of homeostasis.

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