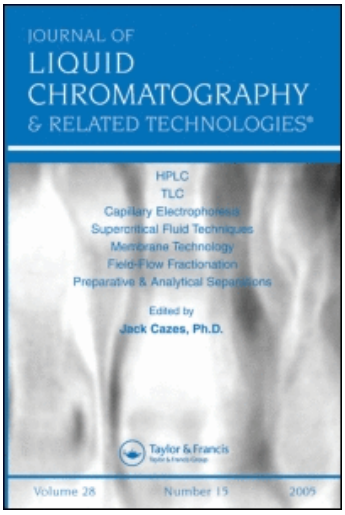

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High Performance Liquid Chromatographic Determination of Free Amino Acids from Carbaryl-and Thiram- Intoxicated Mice by Pre-Column Derivatization with O-Phthaldialdehyde

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF BRAIN FREE AMINO ACIDS FROM CARBARYL- AND THIRAM- INTOXICATED MICE BY PRE-COLUMN DERIVATIZATION WITH O-PHTHALDIALDEHYDE

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ABSTRACT

Precolumn derivatization with OPA was used for the analysis of brain free amino acids from mice after the administration of different doses of carbaryl and thiram for different durations. In general, there was a dose dependent decrease in amino acids, except that threonine, taurine, alanine, arginine and ornithine were found to increase with 1/4 LD_{50} dose of carbaryl, and glycine and lysine increased with 1/4 LD_{50} dose of thiram. Threonine, taurine and GABA levels were elevated with all the doses of thiram. These studies suggested that these two pesticides produced an imbalance of the amino acids in brain which may be due to carbamylation of proteins.

INTRODUCTION

Carbaryl (1-naphthyl - N - methyl carbamate) and thiram (Tetra methyl thiuram disulfide) are commonly

used for controlling insect pests. They may also affect the non target systems like animals and humans. Both the compounds[1,2] have been shown to cross the blood-brain barrier and thus cause carbamylation of protein. Carbaryl derives its toxic effects from being a potent cholinesterase inibitor[3]. Changes in amino acid content of brain may be responsible for certain diseases like Huntington's chorea[4] - caused by decreased GABA content, and Tay-Sachs disease[5].

Thus, it was thought pertinent to study the effect of these pesticides on brain free amino acids using pre-column derivatization of amino acids by O-phthaldialdehyde (OPA), because of some of the advantages such as high sensitivity, rapid quantitave analysis and good selectivity[6]. Since, relatively little information is available on the application of HPLC for amino acid analysis in animal tissues, the present study was undertaken.

MATERIALS AND METHODS

Adult Swiss Portan mice of either sex weighing 30 $\pm$ 5g obtained from the University Animal House, were maintained at 25^{o}C and fed on the normal rat diet.

Carbaryl, of 99% purity was obtained from Union Carbide, USA and was used as such. Thiram, obtained from Fluka AG, Switzerland, was of 99% purity.

Both the pesticides in 2% propanol-2 were given intraperitoneally to 16h fasted mice. The doses of carbaryl and thiram, and the time of sacrifice of animals by decapitation, after the administration of the toxicants is given below:-

Dose ($\mu M\ Kg^{-1}$ body wt.) and schedule of administration of the toxicant	Time of sacrifice after the administration of the toxicant. (h)
Carbaryl	
1/4 LD_{50} (29.9), single i.p.	0.75, 4 and 168
1/4 LD_{50} (29.9), single i.p. once daily for three consecutive days.	72
1/2 LD_{50} (59.7), single i.p.	0.75 and 4
LD_{50} (119.0), single i.p.	0.75
Thiram	
1/4 LD_{50} (670), single i.p.	0.75, 4 and 168
1/4 LD_{50} (670), single i.p. once daily for three consecutive days.	72
1/2 LD_{50} (1330), single i.p.	0.75
LD_{50} (2660), single i.p.	0.75

Determination of free amino acids by HPLC.

Brain free amino acids were extracted, derivatized and estimated according to the method of Rajendra[6] with slight modification. Brains excised from mice at surgery, were immediately chilled and

homogenized (approximately 50 mg ml^{-1} in methanol). The homogenate was centrifuged at 2000 x g at 4°C for 15 min. The extraction of amino acids was repeated twice by the same procedure and the supernatants obtained were pooled. The pooled supernatant was evaporated to dryness on a boiling water bath. The residue was cooled and two ml of double distilled water was added to it and kept in waterbath at 37°C for 5 min. Six ml chloroform was then added and mixed on a vortex mixer. The upper layer was carefully drawn out by a Pasteur pipette and 20 µl of HCl (1:1 diluted) was added. The mixture was then evaporated to dryness. The residue was resuspended in 100 µl of distilled water, and used immediately.

The amino acid analysis was done according to the method given by waters Technical Bulletin, whose instrument was used.

Column used : C_{18} µ bondapak (reversed phase)

Mobile phase

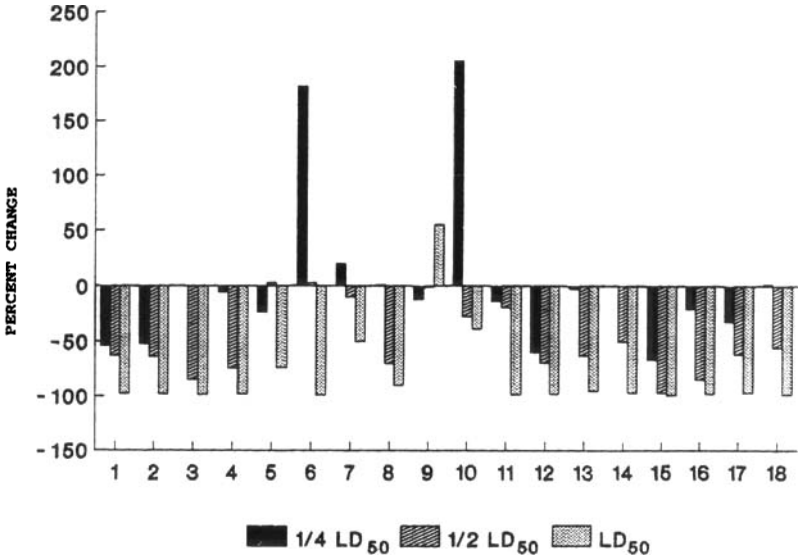
A) Methanol : Tetrahydrofuran : 0.005M Sodium acetate, pH 7.2 (5:5:90).

B) Methanol : 0.05M sodium acetate, pH 7.2 (80:20)

Detector model : 420 AC.

Extinction : 338nm; Emission : 425 nm

Flow rate was maintained at 1ml min^{-1}.



1- Aspartate; 2- Glutamate; 3- Serine; 4- Histidine; 5- Glycine; 6- Threonine; 7- Taurine; 8- Alanine; 9- GABA; 10- Arginine; 11- Tyrosine; 12- Methionine; 13- Valine; 14- Phenyl alanine; 15- Isoleucine; 16- Leucine; 17- Lysine; 18- Ornithine

FIGURE 1. Percent Change in Brain Free Amino Acids of Mice Intoxicated with Different Doses of Carbaryl.

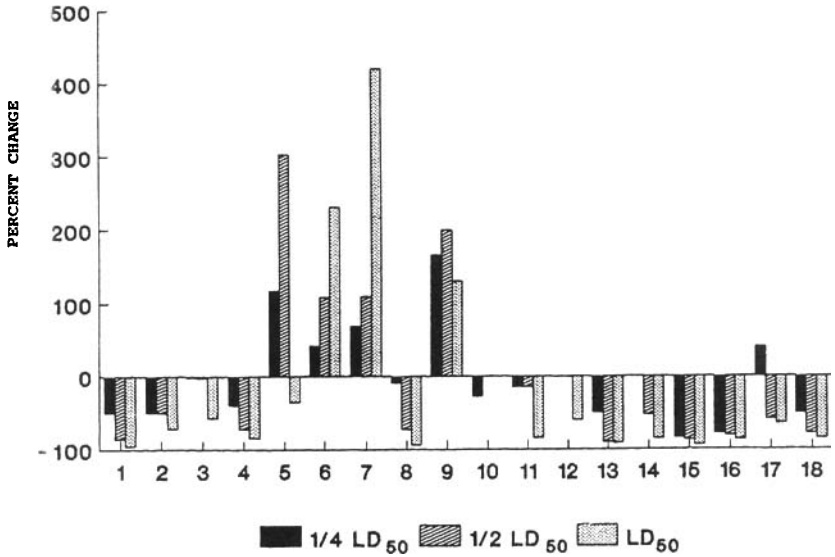
Quantitation

Flourescence response was measured at a sensitivity dial setting of 4 on the detector. Amino acid concentration was quantified from the ratio of peak height of the chromatogram curve against an external standard.

RESULTS

Dose Related Changes

From the results, it could be seen that carbaryl (Fig. 1) decreased the content of amino acids



1- Aspartate; 2- Glutamate; 3- Serine; 4- Histidine; 5- Glycine; 6- Threonine; 7- Taurine; 8- Alanine; 9- GABA; 10- Arginine; 11- Tyrosine; 12- Methionine; 13- Valine; 14- Phenyl alanine; 15- Isoleucine; 16- leucine; 17- Lysine; 18- Ornithine

FIGURE 2. Percent Change in Brain Free Amino Acids of Mice Intoxicated with Different Doses of Thiram.

in a dose dependent manner - aspartate, glutamate, serine, histidine, glycine, γ - aminobutyric acid (GABA), tyrosine, methionine, valine, isoleucine, leucine and lysine. Threonine, taurine, alanine, arginine and ornithine were found to increase with 1/4 LD_{50} dose of carbaryl, while the rest of the two doses led to a decrease of these amino acids below the control. Serine and phenyl alanine were found to be unaffected at 1/4 LD_{50} dose of carbaryl and their concentration fell below the control with increasing doses (1/2 LD_{50} and LD_{50}) (Fig.1.).

TABLE 1.

Effect Of 1/4 LD_{50} Dose Of Carbaryl On Mice Brain Free Amino Acids After Different Durations Of Its Administration.

Name of the amino acid	Control	amino acid concentration at different durations(h) after the administration of carbaryl			
		0.75	4.0	168.0	72.0
Aspartate	2.00	4.00	0.90	1.00	0.62
Glutamate	2.00	0.92	0.70	0.91	0.51
Serine	1.30	0.96	1.28	0.91	0.73
Histidine	1.62	1.30	0.45	1.42	0.10
Glycine	0.34	1.53	0.24	0.28	0.11
Threonine	0.66	0.26	0.63	0.60	0.48
Taurine	0.10	1.86	0.15	0.10	0.06
Alanine	0.80	0.12	0.72	0.50	0.48
GABA	4.00	3.50	2.70	6.48	8.61
Arginine	0.18	0.55	0.13	0.30	0.13
Tyrosine	3.40	2.92	0.90	2.98	2.92
Methionine	2.26	0.90	2.26	1.13	1.67
Valine	2.26	2.20	1.45	2.00	2.26
Phenyl alanine	2.59	2.59	0.78	2.50	0.80
Isoleucine	3.00	1.01	0.86	1.12	0.05
Leucine	1.40	1.11	0.11	1.22	0.07
Lysine	4.76	3.22	1.82	3.12	2.52
Ornithine	6.00	6.11	0.89	6.00	2.96

Values are mean of three independent experiments. Amino acid content is expressed as $\mu M\ g^{-1}$ weight wet tissue.

Nearly similar results were obtained with thiram (Fig. 2), except that threonine and taurine were found to increase in a dose dependent manner and GABA content was more than control at all the dose levels. Glycine and lysine concetrations increased at 1/4 LD_{50} dose of thiram (Fig.2.).

TABLE 2.

Effect Of 1/4 LD_{50} Dose Of Thiram On Mice Brain Free Amino Acids After Different Durations Of Its Administration.

Name of the amino acid	Control	amino acid concentration at different durations(h) after the administration of thiram			
		0.75	4.0	168.0	72.0
Aspartate	2.00	1.00	1.00	1.00	1.00
Glutamate	2.00	1.00	1.00	1.00	1.00
Serine	1.30	1.29	1.29	1.29	0.46
Histidine	1.62	0.98	0.83	1.23	0.19
Glycine	0.34	0.74	0.36	0.40	0.17
Threonine	0.66	0.94	1.03	0.60	0.59
Taurine	0.10	0.17	0.18	0.13	0.04
Alanine	0.80	0.73	0.26	0.74	0.08
GABA	4.00	10.67	12.90	2.10	8.75
Arginine	0.18	0.13	0.25	0.16	0.35
Tyrosine	3.40	2.92	0.29	2.91	1.90
Methionine	2.26	2.25	2.25	2.20	2.92
Valine	2.26	1.13	2.20	1.14	1.58
Phenyl alanine	2.59	2.59	2.59	2.58	1.02
Isoleucine	3.00	0.48	0.63	0.60	2.21
Leucine	1.40	0.30	0.24	0.72	1.00
Lysine	4.76	6.72	5.88	6.00	3.72
Ornithine	6.00	2.96	3.74	3.00	2.51

Values are mean of three independent experiments. Amino acid content is expressed as $\mu M\ g^{-1}$ weight wet tissue.

Time related changes:

In general, with increase in duration of observation (Tables 1 and 2) to 4h, 168h and 72h, there was a fall in amino acid content with few exceptions, which were as follows. Taurine was still increased after 4h as compared to 0.75h after thiram (Table 2)

administration and GABA concentration increased after 168h and 72h after carbaryl (Table 1) intoxication. Taurine and ornithine contents were found to revert back to the control level after 168h and that of methionine after 4h of carbaryl administration. Arginine was found to increase after 168h of carbaryl intoxication. Lysine was increased after 168h of thiram administration.

DISCUSSION

As both carbaryl[1] and thiram[2] have been shown to cross the blood tissue barrier and even after 24h, radioactivity could be detected in tissues like liver, blood, kidney and spleen including brain, the observed changes in the amino acid content (Tables 1 and 2; Figs. 1 and 2) might be due to carbamylation of proteins. Excitatory amino acids like glutamate and aspartate cause membrane depolarization and increase the permeability of cellular membranes to sodium ions[7]. Thus an inhibition of these neurotransmitter amino acids, in brain as in present study may lead to hinderances in the nerve transmission and cellular metabolism.

Similarly, reduction of concentration of GABA - a presynaptic inhibitory transmitter, below the normal in brain induces convulsions[8] and hyperexcitability[9] whereas an increased concentration can be associated

with reduced electrical discharge[10,11]. GABA has been shown to have excitatory and depolarizing actions as well. Furthermore, alterations in the content of one amino acid may affect the metabolism of other amino acids. There is evidence that transport systems for amino acids may in fact be homeostatic mechanisms responding to alterations[12].

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