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Distribution of Hexokinase and ATPase in the Brain of DDT-Exposed Fish

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DDT toxicity was studied in relation to neural acetyl cholinesterase, ATPase reacting system, Na^+, Mg^+ and K^+ dependent ATPase (Chetty et al. 1978), brain ATPase (Srivastava et al. 1979), G-6-p dehydrogenase (Buhler and Benville 1969), gluconeogenic enzymes and non-specific phosphomonoesterases, tissue lipids and cholesterol content in various animal species including fish (Shaffi 1982). However, the studies regarding the effect of DDT on the metabolic compartmentation of the brain was not yet investigated on any fresh water Indian fish. In the present study, an attempt has been made to study the effect of sub-lethal dose of DDT on differential distribution of hexokinase, Na^+, K^+-ATPase and Mg^{2+} -ATPase in various regions of the brain in Labeo rohita (Ham), Clarias batrachus (Linn) and Channa Punctatus (Bloch) from a tropical environment on a comparative basis.

MATERIALS AND METHODS

Mature, healthy specimens of 18-20 cm were obtained from local fish market and acclimatized in the laboratory for 5 days. Six fish of each species were killed for basal cerebrum, cerebellum, pituitary region and medulla oblongata, hexokinase and adenosine triphosphatase level in L.rohita, C. batrachus and C. punctatus. Fish species were divided into two groups of equal numbers, namely, control and treated. The treated group was kept in a sub-lethal (0.035 mg/L) concentration of DDT for 5 hours. The preparation of tissue homogenates and enzyme assays of hexokinase, Na^+, K^+-ATPase was described elsewhere (Crane and Sole 1953; Somyogi 1964).

RESULTS AND DISCUSSION

It is evident from Table 1, the hexokinase, Na^+, K^+-ATPase and Mg^{2+}-ATPase responded differently to sub-lethal dose of DDT toxicity in various compartments of the brain in the investigated three fish species. Sub-lethal doses of DDT enhanced the hexokinase level more in the medulla oblongata of the C. punctatus

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(Table 1), followed by C. batrachus (Table 2), and L. rohita (Table 1) than in the pituitary region of C. punctatus (Table 3), L. rohita (Table 1), and C. batrachus (Table 2). The least rise in hexokinase was noticed in the cerebrum of L. rohita (Table 1), C. batrachus (Table 2), and C. punctatus (Table 3). The hexokinase fall in cerebellum was maximum in L. rohita, followed by C. punctatus and C. batrachus (Tables 1, 2 and 3).

The cerebrum in C. batrachus (Table 2), the cerebellum in L. rohita (Table 1), and the cerebrum in C. punctatus (Table 3) registered optimum rise in Na^+, K^+ ATPase. The cerebrum in L. rohita (Table 1), the medulla oblongata in C. batrachus (Table 2), and C. punctatus (Table 3) registered optimum fall in Mg^{2+}-ATPase. The pituitary region in L. rohita (Table 1), C. batrachus (Table 2), and C. punctatus (Table 3) registered greater rise in Mg^{2+}-ATPase than in cerebellum. Among the three fish species, maximum fall and rise in hexokinase, Na^+, K^+ ATPase and Mg^{2+}-ATPase activity was noticed more in L. rohita (Table 1) than in C. batrachus (Table 2) and C. Punctatus (Table 3).

Hexokinase activity was predominantly higher in the regions rich in neurons like the cerebral hemispheres and the cerebellum as compared to the medulla oblongata. The regions with high activity of hexokinase are well adapted for efficient aquisition and introduction of glucose into metabolism (Shaffi 1982). The result profiles suggest that hyperglycemia induced by DDT interfered with the glucose metabolism by inhibiting the first phosphorylating enzyme of the glycolytic pathway (Stohlman and Lille 1948). It seems that the cerebellum was the main target of DDT action as the change in hexokinase activity in the former was higher than the later. However, the hexokinase rise in medulla oblongata can be considered as an attempt to restore normalcy and to overcome the stress created by DDT.

The result profiles on ATPase show that DDT did affect membrane and mitochondrial functions of the animal species. Earlier reports have shown that in-vivo exposure of animals DDT alters the specific activity of subsequently isolated ATPase of various tissues and species (Cutkomp et al. 1971; Desaiah et al. 1975; Koch 1969).

Bratkowski and Matsumura (1972), as well as Matsumura and Patil (1969) reported that DDT is a potent inhibitor of Na^+, K^+-ATPase, while Koch (1969) reported that DDT is also a potent inhibitor of Mg^{2+}-ATPase. In the present investigation Na^+, K^+-ATPase inhibition was recorded in the medulla oblongata and the pituitary region and Mg^+-ATPase fall was noticed in the cerebrum and the medulla oblongata, which is in full agreement with the earlier results. The fall of ATPase in the above brain regions may be related to the interfering nature of DDT on the transport processes of cations across the membrane which may further influence the membrane potential (Shaffi 1979).

Table 1. Effect of Sub-lethal dose of DDT on brain enzymes in Labeo rohita (Ham.)

Region of the Brain	Control	Treated	% of Rise/Fall
(A) Hexokinase (μ moles of glucose/mg protein)			
Cerebrum	0.400 ±0.013	0.444 ±0.012	10^{R}*
Cerebellum	0.250 ±0.023	0.100 ±0.013	60^{F}*
Pituitary Thalamus & Hypothalamus	0.200 ±0.012	0.220 ±0.013	10.0^{R}*
Medulla Oblongata	0.170 ±0.014	0.280 ±0.019	39.2^{R}*
(B) Na^{+}, K^{+}-ATPase (μ moles of Pi/mg protein)			
Cerebrum	0.125 ±0.010	0.132 ±0.014	5.30^{R}*
Cerebellum	0.085 ±0.009	0.097 ±0.007	12.37^{R}*
Pituitary Thalamus & Hypothalamus	0.050 ±0.012	0.042 ±0.009	27.58^{F}*
Medulla Oblongata	0.0180 ±0.020	0.115 ±0.018	35.11^{F}*
(C) Mg^{2+}-ATPase (μ moles of Pi/mg protein)			
Cerebrum	0.090 ±0.017	0.056 ±0.011	37.77^{F}*
Cerebellum	0.054 ±0.008	0.060 ±0.012	10.00^{R}
Pituitary Thalamus & Hypothalamus	0.045 ±0.011	0.060 ±0.008	33.33^{R}*
Medulla Oblongata	0.072 ±0.014	0.045 ±0.011	37.50^{F}*

Values are ± SDM of 6 replicates. Student "t" test was performed. "t" was significant at $P<0.05$ level. * indicates for those differences that are significant only. Super scripts "R" indicates rise and "F" indicates fall.

Table 2. Effect of Sub-lethal dose of DDT on brain enzymes in Clarius batrachus (LINN)

Region of the Brain	Control	Treated	% of Rise/Fall
(A) Hexokinase (μ moles of glucose/mg protein)			
Cerebrum	0.230 ±0.011	0.250 ±0.001	8.0^{R}*
Cerebellum	0.180 ±0.019	0.100 ±0.021	44.4^{F}*
Pituitary Thalamus & Hypothalamus	0.130 ±0.025	0.140 ±0.024	7.14^{R}
Medulla Oblongata	0.110 ±0.019	0.190 ±0.022	42.10^{R}*
(B) Na^{+}, K^{+}-ATPase (μ moles of Pi/mg protein)			
Cerebrum	0.166 ±0.011	0.190 ±0.011	14.46^{R}*
Cerebellum	0.145 ±0.014	0.154 ±0.011	5.84^{R}
Pituitary Thalamus & Hypothalamus	0.106 ±0.010	0.082 ±0.008	22.64^{F}*
Medulla Oblongata	0.256 ±0.031	0.179 ±0.016	30.07^{F}
(C) Mg^{2+}-ATPase (μ moles of Pi/mg protein)			
Cerebrum	0.145 ±0.018	0.102 ±0.023	26.65^{F}*
Cerebellum	0.095 ±0.015	0.101 ±0.023	5.94^{R}
Pituitary Thalamus & Hypothalamus	0.065 ±0.009	0.080 ±0.010	23.077^{R}*
Medulla Oblongata	0.107 ±0.018	0.069 ±0.010	35.51^{F}*

Values are ± SDM of 6 replicates. Student "t" test was performed. "t" was significant at $P<0.05$ level. * indicates for those differences that are significant only. Super scripts "R" indicates rise and "F" indicates fall.

Table 3. Effect of Sub-lethal dose of DDT on brain enzymes in Channa punctatus (Bloch)

Region of the Brain	Control	Treated	% of Rise/Fall
(A) Hexokinase (μ moles of glucose/mg protein)			
Cerebrum	0.140 ±0.027	0.150 ±0.019	6.6^{R}
Cerebellum	0.100 ±0.020	0.050 ±0.011	50.0^{F}*
Pituitary Thalamus & Hypothalamus	0.073 ±0.009	0.090 ±0.010	23.29^{R}*
Medulla Oblongata	0.070 ±0.006	0.110 ±0.009	57.14^{R}*
(B) Na^{+}, K^{+}-ATPase (μ moles of Pi/mg protein)			
Cerebrum	0.275 ±0.014	0.298 ±0.005	9.39^{R}*
Cerebellum	0.250 ±0.012	0.272 ±0.016	7.80^{R}*
Pituitary Thalamus & Hypothalamus	0.201 ±0.019	0.168 ±0.013	16.41^{F}*
Medulla Oblongata	0.350 ±0.034	0.258 ±0.028	26.28^{F}*
(C) Mg^{2+}-ATPase (μ moles of Pi/mg protein)			
Cerebrum	0.215 ±0.012	0.144 ±0.022	20.93^{F}*
Cerebellum	0.137 ±0.024	0.144 ±0.023	4.86^{R}
Pituitary Thalamus & Hypothalamus	0.092 ±0.013	0.100 ±0.019	8.00^{R}
Medulla Oblongata	0.155 ±0.029	0.107 ±0.017	30.96^{F}*

Values are ± SDM of 6 replicates. Student "t" test was performed. "t" was significant at $P<0.05$ level. * indicates for those differences that are significant only. Super scripts "R" indicates rise and "F" indicates fall.

From the results, it is clear that the site of DDT action is the cerebellum and the medulla oblongata for hexokinase, the pituitary region and the medulla oblongata for Na^+, K^+-ATPase, and the cerebrum and the medulla oblongata for Mg^{2+}-ATPase in the three fish species. The fall and rise in hexokinase Na^+, K^+- ATPase and Mg^2+-ATPase in the four brain regions of the three fish species can be ascribed to toxicant precipitation and biochemical compartmentation.

REFERENCES

Bratkowski TA, Matsumura F (1972) Properties of brain ATPase sensitivity of DDT. J Environ Entomol 65: 1238-45

Buhler DR, Benville P (1969) Effect of feeding and of DDT on the activity of G-6-P dehydrogenase in two salmonids. J Fish Res Bd Can 26: 3209

Crane RK, Sole AA (1953) The association of hexokinase with particular fractions of brain and other tissue homogenates. J Biol Chem 202: 273-79

Cutkomp LK, Yap HH, Cheng EY, Koch RB (1971) ATPase activity in fish tissue homogenates and inhibitory effects of DDT on related compounds. Chem Biol Interact 6: 439-447

Desaiah D, Cutkomp LD, Koch RB, Jarvinen A (1975) DDT effects of continuous exposure on ATPase activity in fish, *Pimephases promelas*. Arch Environ Contam Toxicol 3: 132

Koch RB (1969) Chlorinated hydrocarbon insecticides : Inhibition of rabbit brain ATPase activities. Inhibition of rabbit brain ATPase activities. J Neurochemistry 16: 269-271

Matsumura F, Patil KC (1969) Adenine triphosphatase sensitivity of DDT in synapses of rat brain. Science 166: 171

Shaffi SA (1979) Biochemical compartmentation of fish tissues II. Non-specific phosphomonoesterases in brain. Experientia 35: 1280- 1282

Shaffi SA (1982) Biochemical compartmentation of fish tissues VI. hexokinase in brain. Acta Physiol Acad Sci Hung 60: 175-181

Somyogi J (1964) Preparation of Na^+, K^+-ATPase activated ATPase system of rat brain free from the Mg+-activated ATP hydrolyzing enzyme. Biochem Biopys Acta 92: 615

Srivastava LK, Baquer NZ, Habiulla M (1979) Effect of PP'DDT on hexokinase and ATPase in nervous system of cockroach, frog and rat. Ind J Exp Biol 17: 1204.

Stohlman EF, Lille ND (1948) The effect of DDT on the blood sugar and of glucose administration on the acute and chronic poisoning of DDT in rabbits. J Pharmacol Exptl Ther 93: 351