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Article

A study on membrane enzyme Na^+-K^+-ATPase in lindane exposed fish, *Channa punctatus*

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Abstract: Na^+-K^+-ATPase is a membrane-bound enzyme responsible for the transport of ions through the membrane and the immediate release of energy. This enzyme is known to be an early target for oxygen radical-induced damage to intact cells. Exposure of *C. punctatus* to subacute concentrations of lindane for 96 h caused a significant reduction in the activities of Na^+-K^+-ATPase in all the tissues of the fish tested, with the brain being maximally affected and the heart being the least affected organ at the highest concentration of lindane (0.1 mg/L). The effect of pesticides was concentration-dependent. The percent decrease in the activity of Na^+-K^+-ATPase in brain, gills, heart, kidney, liver, and muscle was found to be 36.7, 23.4, 19.2, 29, 22.9, and 29.7, respectively. The order of level of enzyme activity recorded was as follows: liver > gills > kidney > brain > muscle > heart in the control.

Keywords: lindane; Na-K-ATPase; organs

1. Introduction

The aquatic ecosystem is an open system exposed to all different kinds of pollutants, toxicants, and surfactants, thus polluting the aquatic environment. Their direct discharge without any pre-treatment either leads to large-scale destruction of aquatic life or accumulation in water, soil, or bioaccumulation in biotic aquata. Though the pollutants could be biodegradable or non-biodegradable, these pollutants sometimes decrease the rate of decay of biodegradables. Thus, the increase in the contaminants may allow their environmental persistence for a longer period. Excessive use of nitrate and phosphate fertilizers may lead to eutrophication, thereby reducing the amount of oxygen in the aquatic biota and increasing the biological oxygen demand of that water for a prolonged period. Industrial smokes, burning of wood, petroleum, and vehicle fumes all gave rise to gases like sulphur dioxide, nitrogen oxides, and carbon dioxide; lead and the particulate matter have all caused serious harm to the environment and humans [1–3]. Reports on the increased levels of heavy metals and pesticides and their effects on oxidative stress have been exhaustively documented [4–8]. Many bacteria like *Vibrio anguillarum*, *Aeromonas*, *Flavobacterium*, *Pseudomonas*, *Serratia*, and *Yersina*, etc. have been shown to grow in water, which has less oxygen, increased organic matter, and an unsuitable pH for aquatic life.

The presence of organic matter may be due to leakage from septic tanks or contamination by domestic sewage [9]. Aquatic life may suffer from diseases like fin rot, papilloma, hyper-neoplasia, gill diseases, etc. Gills are the main respiratory organs of fish; they regulate ion concentration and osmotic balance for survival in unfavorable concentrations [10]. A decrease in function of gills has been reported when exposed

to a pH of water less than or more than 7 [11]. Lindane is an organochlorine, hydrophobic, and highly persistent pesticide. Due to its lipophilic nature, it gets easily bioaccumulated in aquatic organisms. Lindane is strongly adsorbed on soils that contain a large amount of organic matter. It can move downward by capillary action through the soil with water from rainfall or artificial irrigation. In the UV light, it undergoes rapid dichlorination or degradation to form pentachlorocyclohexenes and tetrachlorocyclohexenes. The fish are able to bioaccumulate due to direct exposure to chemicals in water and ingestion of contaminated food or prey [12]. Their accumulation in low concentration in aquatic animals generates warning signals about the environment. Ultimately, in the long run, when these fish are eaten by humans or animals, the pesticide residues accumulate in different organs of the exposed living systems and hence may pose serious health problems [13]. The pesticide and its residues are known to get strongly adsorbed on clays and sediments of surface water where several fish populations reside for feeding.

Sodium Potassium ATPases (Na^+-K^+-ATPase, EC 3.6. 3.9) are membrane-bound sulfhydryl-containing oligomeric enzymes whose function is critical for the maintenance of cell viability. It works as an electrogenic P-type pump involved in monovalent ion transport across membranes, utilizing the energy of ATP hydrolysis. It pumps 3 sodium outside (extracellular) and 2 potassium inside the cell membrane (intracellular) for 1 ATP. The concentration gradient created due to ion transport across the membranes helps in other secondary transports [14,15]. This pump has many diverse functions like contractions, signalling, homeostasis (osmoregulation), and cell-cell adhesions [16,17]. It also governs many physiological processes like reabsorption, filtration, pH, electrolyte osmotic regulation by kidneys [18], sperm motility [19], and action potential in neurons [20–23]. This pump consists of 3 subunits *α, β,* and FXYD. The α subunit is for ion transport (catalytic) and is under the influence of the *β* (auxiliary) and FXYD (tissue-specific) subunits [24]. The *β*2 subunit of Na^+-K^+-ATPase is responsible for regulation of egg development in *Aedes aegypti***,** the causative organism of many vector-borne diseases like yellow fever, zika, dengue, and chicken guinea. The reduced expression of mRNA of *β*2 Na^+-K^+-ATPase in the knockdown mice showed less egg formation, which directly reduced their population and so less spread of disease [25]. In humans, decreased heart functions or heart failures have been reported due to a decrease in its activity [26]. In plants, it helps in nutrient uptake, root development, stomatal regulation, and response to environmental stresses. It helps plants to maintain proper ion balance, participate in nutrient absorption, and regulate turgor pressure in cells. In the environment, it acts as a marker for organisms facing challenges such as varying salinity, ion concentrations, or osmotic stress. In the present study, the effect of subacute concentrations of lindane (0.025, 0.05, 0.1 mg/L) on the activity of Na^+-K^+-ATPase was assessed in order to evaluate the perturbations in the transport of ions in different fish tissues exposed for 96 h.

2. Materials and methods

2.1. Experimental design and exposure to lindane

The healthy and active fish of 30–40 gm with no signs of any diseases or external

injury were equally distributed in four aquaria of 1 × 1 ft. The subacute concentrations of lindane (0.025, 0.05, 0.1 mg/L) prepared in acetone were used for the exposure of *C. punctatus* for 96 h. In the control group, an equal volume of acetone was added. All aquaria were constantly aerated during the period of exposure by the aerator, and the fish were fed properly. The water of all 4 aquaria was changed in 24 h and replenished with fresh lindane.

2.2. Preparation of cell-free extracts and biochemical assays

1) Protein determination: The quantitative estimation of total protein in various tissue extracts and solutions was done according to the known procedure [27]. The samples were homogenized 10% (w/v) in 0.05 m sodium phosphate buffer, pH 7.4, and centrifuged at 10,000 rpm for 10 min in a cold (4 ℃) condition. The supernatants were collected in labelled vials, and volume was noted. Determination of protein was done using a Folin-Ciocalteau reagent. The bovine serum albumin (BSA) was used as a standard. A blank was prepared, which contained all reagents but no protein. The intensity of the blue color was measured colorimetrically at 620
2) Assay of Na^+-K^+-ATPase activity: The Na^+-K^+-ATPase activity was determined as inorganic phosphorus (Pi) production using the method of Svobaca and Mossinger [28] and Fiske and Subbarow [29]. The reaction mixture is given in **Table 1**.

Table 1. Process for assay of Na^+-K^+-ATPase activity.

10% (w/v) tissue homogenized in 0.25 m sucrose (pH 7.4)	
15 min centrifugation 12,000 rpm at 4 ℃	
Supernatant collected	
1	**2**
0.2 mL of 200 mm KCl	
0.2 mL of 1 m NaCl	0.1 mL of 1000 mm $MgCl_2$
0.1 mL of 1000 mm $MgCl_2$	1.0 mL of 200 mm Tris buffer, pH 7.4
1.0 mL of 200 mm Tris buffer, pH 7.4	100–200 µg supernatant
0.2 mL distilled water	0.16 mL distilled water
100–200 µg supernatant	0.2 mL of 10 mm ouabain
Leave 5 min/35–37 ℃	
0.2 mL of 25 mm ATP (di sodium salt)	0.2 mL of NaCl + 0.2 mL of 25 mm ATP
Leave 15 min/35–37 ℃	
1 mL of 10%TCA	
3000 rpm/5 min	
Supernatant collected	
0.5 mL supernatant	
3.0 mL DW	
0.5 mL of 2.5% ammonium molybdate in 5N H_2SO_4	
0.2 mL of 1, 2, 3, 4 ANSA	
Vortexed/10 min	
Reading at 600 nm	
Difference between 1 and 2 gives activity of Na^+-K^+-ATPase in µmol Pi liberated/mg protein/h.	

ANSA = aminonaphthol sulphonic.

All reagents were obtained from Sigma Chemical Company, USA. The pesticide

used in the experiments was from Rallis India Limited. The double-distilled water was used for all biochemical experiments.

2.3. Statistical analysis

The values were presented as means ± standard error of mean (SEM) of observed data of three to five replicates. GraphPad Prism version 3.0 (GraphPad Prism Software Inc., San Diego, CA, USA) was used to analyze the data. Results obtained from treated and control fish were compared using the Turkey H test.

3. Results and discussion

Effect of lindane on the activity of Na^+-K^+-ATPase in different organs of lindane exposed fish

The data demonstrated the highest activity of Na^+-K^+-ATPase to be present in liver (41.67 ± 0.35 units/mg protein) and lowest in heart (16.73 ± 0.11 units/mg protein) of the control fish. The activities of Na^+-K^+-ATPase in other fish tissues such as gills, kidney, brain, and muscle were recorded as 33.78 ± 0.23, 32.87 ± 0.19, 30.29 ± 0.07, and 21.29 ± 0.09 units/mg protein, respectively. The order of level of enzyme activity recorded in the control fish tissues was as follows: liver > gills > kidney > brain > muscle > heart (**Table 2, Figure 1**).

Table 2. Effect of lindane (mg/L) on the specific activity of Na^+-K^+-ATPase (units/mg protein) in different tissues of *C. punctatus* exposed for 96 h.

Lindane	Gills	Heart	Kidney	Liver	Muscle	Brain
0	33.78 ± 0.23	16.73 ± 0.11	32.87 ± 0.19	41.67 ± 0.35	21.29 ± 0.09	30.29 ± 0.07
0.025	30.59 ± 0.21 (–9.42)	15.69 ± 0.09 (–6.21)	29.79 ± 0.18 (–9.37)	39.27 ± 0.33 (–5.76)	19.17 ± 0.08 (–9.95)	25.31 ± 0.05 (–16.44)
0.05	28.67 ± 0.22 (–14.98)	14.87 ± 0.07 (–11.10)	25.66 ± 0.15 (–21.97)	37.09 ± 0.32 (–11.01)	17.15 ± 0.07 (–19.47)	22.11 ± 0.06 (–27.02)
0.1	25.87 ± 0.20 (–23.41)	13.52 ± 0.08 (–19.19)	23.35 ± 0.16 (–28.94)	32.13 ± 0.29 (–22.92)	14.97 ± 0.07 (–29.70)	19.17 ± 0.04 (–36.68)

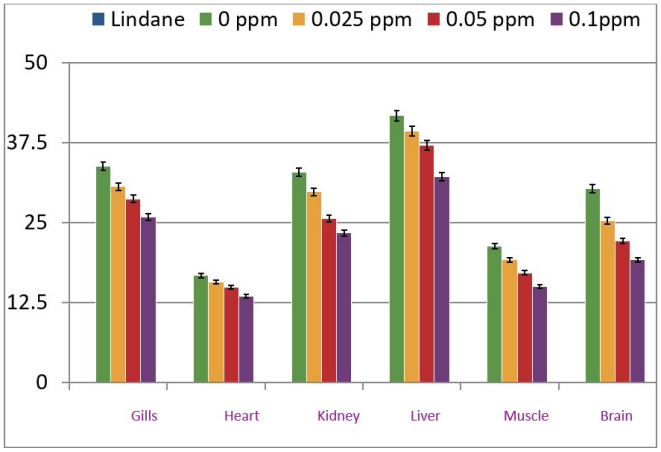

Figure 1. Lindane exposure at different concentrations (0, 0.025, 0.05, 0.1 ppm) on the specific activity of Na^+-K^+-ATPase (units/mg protein) in different tissues of *C. punctatus* exposed for 96 h.

Values are represented as µm of *Pi* released/h/mg wet weight of tissue. Each value represents the mean ± SEM of ten different observations. Values in parenthesis are percent change over control. The (–) sign represents a decrease over control. *h* represents time in hours. SEM = standard error of mean.

From the Turkeys HSD test all pairwise comparisons between different concentrations (0.025, 0.05, 0.1 mg/L) and control (0 mg/L) were statistically significant with $p < 0.05$. This indicates that there are significant differences in the mean values between the control and each of the concentrations, as well as between the different concentrations themselves for organs brain, muscle and liver. The non-significant differences were observed in the gills and kidney with 0.1 vs. 0.05mg/L and heart 0.05 vs. 0.025 mg/L (**Table 3**).

Table 3. Statistical inference of data based on pairwise comparisons between different concentrations of lindane (mg/L).

Lindane	Gills		Heart		Kidney		Liver		Muscle		Brain	
0.025 vs. 0	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.05 vs. 0	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.1 vs. 0	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.05 vs. 0.025	$P < 0.001$	S	$P = 0.34$	NS	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.1 vs. 0.025	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S
0.1 vs. 0.05	$P = 0.52$	NS	$P < 0.001$	S	$P = 0.16$	NS	$P < 0.001$	S	$P < 0.001$	S	$P < 0.001$	S

S = Significant, NS = Not significant.

The treatment of the fish with three subacute concentrations of lindane displayed a marked decrease in the activity of Na^+-K^+-ATPase in all the tissues tested, i.e., the brain was maximally affected at all of these concentrations. At the lowest concentration of lindane (0.025 mg/L), the fish tissues such as gills, kidneys, and muscles showed the same level of percent inhibition (about 9%–10%) in the enzyme activity (**Table 2**).

At the highest concentration of the pesticide (0.1 mg/L), the brain of the fish exhibited a maximum decrease of 37% in the enzyme activity, and the heart indicated a minimum decrease of 19% after 96 h of treatment duration. The decrease in the enzyme activity was in a concentration-dependent manner. The percent decrease in the activity of Na^+-K^+-ATPase in brain, gills, heart, kidney, liver, and muscle was found to be 36.7, 23.4, 19.2, 29, 22.9, and 29.7, respectively. The data indicated that the gills and liver were affected by lindane to a similar level, showing about a 23% decrease in enzyme activity. Further, the kidney and muscle displayed almost the same level of enzyme inhibition to about 29% when the fish was treated with lindane at a 0.1 mg/L concentration for 96 h. The extent of inhibition in the enzyme activity from these fish tissues treated with the highest pesticide concentration (0.1 mg/L) was found in the following order: brain > muscle = kidney > gills = liver > heart (**Table 2**).

This enzyme is known to be an early target for oxygen radical-induced damage to intact cells [30,31]. Sharma observed a significant reduction of liver ATPase activity in *C. gaucha* upon ensdosulphan exposure [32]. Oruc et al. reported a reduction of liver activity in *Tillapia zilli* and *O. niloticus* and suggested that increased

lipid peroxidation disturbed the anatomical integrity of the biomembrane and diminished its fluidity, leading to inhibition of activities of several membrane-bound enzymes, including Na^+-K^+-ATPase [33]. Very recently, cypermethrin at lethal (5.03 µg/L) and sublethal (1.02 µg/L) concentrations has been shown to cause significant alterations in the gills, liver, and muscle of the fish *Cirrhinus mrigala* [34].

Similar observations have been reported in *C. punctatus* exposed to pyrethroids [35] and *Clarias gariepinus* juvenile exposed to oxadiazon [35]. Increased activity in gills of silver catfish (*Rhamdia quelen)* was reported in water pH 9.0 and no significant change in kidney [36]. In a hypotonic environment, the kidneys of fish species excrete more dilute urine to maintain homeostasis [37], and in hypertonic they suffer from dehydration, and so they drink more sea water, excreting out extra salts [38,39]. At lethal concentration, the insecticide caused an increase in enzyme activity, whereas at sublethal concentration the enzyme activity decreased [40]. Maiti exposed the fish *Clarius batrachus* to 5.69 mg/L and 11.38 mg/L of chromium (lll) for 96 h and reported a decrease in activity in the brain [41–45]. This inactivity can lead to diverse alterations in the neurons, such as partial membrane depolarization, Ca^{+2} influx, altered neurotransmitter release, and even apoptosis, and all can be co-related to functional deficits in the brain [46].

4. Conclusions

The significance of sodium-potassium ATPase (Na^+-K^+-ATPase) in the environment is its role in maintaining cellular homeostasis both in plants and animals. It contributes to the adaptability and survival of organisms in diverse environmental conditions and serves as a sensitive indicator of environmental stress and toxicity. The exposure of the fish to varying subacute concentrations of lindane has caused significant perturbations in the activity of this enzyme in different organs of the fish tested. The results may serve as an indicator of pesticide contamination in water and better management of pesticide application in agricultural practices to secure environmental health.

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