GLYCOLYTIC OXIDATION IN FRESHWATER FISH, *SAROTHERODON MOSSAMBICUS* DURING BENTHIOCARB EXPOSURE

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ABSTRACT

Changes in glucose metabolism were studied in freshwater teleost fish, *Sarotherodon mossambicus*. A decrease in total carbohydrate and pyruvate levels and an increase in lactate levels were observed. The decrease in total carbohydrate levels is more pronounced in liver than in other tissues. Oxygen consumption decreased significantly with increase in the exposure period. However, the blood glucose level was not altered appreciably in fish exposed to sublethal concentration for 1 day but a significant decrease in blood glucose was seen in fish exposed for 2 and 10 days.

INTRODUCTION

PESTICIDE pollution of freshwater system poses a great threat to aquatic environment[1]. Organocarbamates and other pesticides enter the estuarine water through industrial effluent and other applications[2]. Organocarbamates cause many harmful effects such as ovarian atrophy[3], decreased

Table 1 *Changes in the levels of total carbohydrates (mol/g wet wt), pyruvate and lactic acid (mg/g wet wt) in selected tissues of fish after exposing to sublethal and lethal concentrations of benthiocarb*

			Sublethal concentration			Lethal concentration	
Tissues	Parameters	Control	1 day	2 day	10 day	1 day	2 day
Brain	Total carbohydrates	19.96 ± 1.16	16.97 ± 1.10 (− 14.98)	15.52 ± 1.31 (− 22.24)	10.69 ± 1.15 (− 46.44)	13.05 ± 1.01 (− 34.62)	8.96 ± 1.01 (− 55.11)
	Pyruvate	1.47 ± 0.08	1.24 ± 0.11 (− 15.65)	1.07 ± 0.07 (− 27.21)	0.751 ± 0.060 (− 48.91)	0.965 ± 0.113 (− 34 35)	0.392 ± 0.102 (− 73.33)
	Lactic acid	0.622 ± 0.020	0.699 ± 0.030 (+ 12.38)	0.736 ± 0.020 (+ 18.33)	0.774 ± 0.050 (+ 24.44)	0.747 ± 0.011 (+ 20.09)	0.819 ± 0.041 (+ 31.67)
Liver	Total carbohydrates	38.68 ± 1.05	33.74 ± 1.35 (− 12.77)	27.63 ± 1.51 (− 28.59)	15.79 ± 1.24 (− 59.18)	23.89 ± 1.20 (− 38.24)	15.37 ± 1.44 (− 60.26)
	Pyruvate	5.03 ± 0.63	4.39 ± 0.22 (− 12.72)	4.04 ± 0.18 (− 19.68)	2.29 ± 0.15 (− 54.47)	2.25 ± 0.17 (− 55.27)	0.672 ± 0.023 (− 86.64)
	Lactic acid	1.07 ± 0.02	1.73 ± 0.04 (+ 9.35)	1.27 ± 0.03 (+ 18.69)	1.71 ± 0.06 (+ 59.81)	1.43 ± 0.12 (+ 33.64)	1.96 ± 0.29 (+ 83.18)
Muscle	Total carbohydrates	17.35 ± 1.83	14.93 ± 0.72 (− 13.95)	12.83 ± 0.69 (− 26.05)	10.80 ± 0.67 (− 37.75)	12.72 ± 0.92 (− 26.69)	10.13 ± 0.25 (− 41.61)
	Pyruvate	0.717 ± 0.110	0.562 ± 0.040 (− 21.62)	0.492 ± 0.041 (− 31.38)	0.289 ± 0.031 (− 59.69)	0.528 ± 0.051 (− 26.36)	0.303 ± 0.021 (− 57.74)
	Lactic acid	0.743 ± 0.060	0.771 ± 0.831* (+ 3.77)	0.836 ± 0.020 (+ 12.52)	1.02 ± 0.06 (+ 37.28)	0.922 ± 0.020 (+ 24.09)	1.18 ± 0.12 (+ 58.82)
Gill	Total carbohydrates	13.69 ± 0.45	12.12 ± 0.46 (− 11.47)	9.98 ± 0.55 (− 27.10)	8.07 ± 0.41 (− 41.05)	9.57 ± 0.40 (− 30.09)	7.49 ± 0.36 (− 45.29)
	Pyruvate	0.538 ± 0.040	0.418 ± 0.030 (− 22.30)	0.370 ± 0.101 (− 31.23)	0.191 ± 0.020 (− 64.49)	0.289 ± 0.071 (− 46.28)	0.151 ± 0.020 (− 71.93)
	Lactic acid	0.157 ± 0.010	0.175 ± 0.005 (+ 11.46)	0.202 ± 0.007 (+ 28.66)	0.238 ± 0.006 (+ 51.59)	0.219 ± 0.006 (+ 39.49)	0.266 ± 0.025 (+ 69.43)

Values are mean ± SD of 6 individual observations; Values in parentheses are per cent changes over control; Values are significantly different at $P < 0.05$; * Not significant.

hatchability and teratogenesis[4] in animals. They cause disruption of nerve impulses and transmission by inhibiting acetylcholinesterase (AChE)[5]. This paper reports some studies on the physiological and biochemical effects of benthiocarb, an organocarbamate on glycolytic pathway in the tissues of teleost, *Sarotherodon mossambicus*.

MATERIALS AND METHODS

Freshwater fish, *S. mossambicus* (10 ± 2 g), collected from local freshwater ponds were stored in large cement tanks at room temperature (27 ± 1°C) with 12 h natural photoperiod. The fish were fed *ad libitum* with groundnut cake and starved for 24 h prior to experimentation[6]. A technical grade (98%) benthiocarb [S-(4-chlorobenzyl)-N, N-diethyl thiocarbamate], obtained from the Environmental Protection Agency, USA was used. LC_{50} (9.28 ppm) was determined[7] for 48 h and approximately 1/3rd of the LC_{50} (3 ppm) was selected for sublethal treatment. Three batches of six animals each exposed to sublethal concentration (SLC) (3 ppm) for 1, 2 and 10 days and another two batches of six animals each exposed to lethal concentration (LC) (9.28 ppm) for 1 and 2 days were used. The fish were then sacrificed and the liver, brain, muscle and gill were separated and blood collected in cold to determine the O_2 consumption[8], blood glucose[9], total carbohydrates[10], pyruvate[11], lactate[12], glycogen phosphorylase[13], aldolase[14], lactate dehydrogenase (LDH)[15], glucose 6-phosphate dehydrogenase (G-6-PDH)[16] and proteins[17].

RESULTS AND DISCUSSION

The modulations in the glycolysis pathway of teleost fish during benthiocarb toxicity are presented in tables 1–3. Oxygen consumption decreased significantly with increase in exposure period indicating a damage to the respiratory system. The blood glucose level was not altered (table 4) appreciably in fish exposed to SLC for 1 day whereas a significant decrease was noticed in fish exposed for 2 and 10 days. Similar changes were seen in fish exposed to LC. The decrease in blood glucose level could either be due to its increased uptake by the tissues or its decreased mobilization from glycogen reserves as a consequence of benthiocarb exposure. The benthiocarb probably affects the release and action of hormones (such as glucagon and epinephrine) involved in glycogenolysis and glucose metabolism[18].

The decreased levels of tissue carbohydrate and

Table 2 *Changes in activity levels of phosphorylase 'a' (μ mol of Pi/mg protein/h) and aldolase (μ mol of FDP cleaved/mg protein/h) in selected tissues of fish after exposing to sublethal and lethal concentrations of benthiocarb*

			Sublethal concentration			Lethal concentration	
Tissues	Parameter	Control	1 day	2 day	10 day	1 day	2 day
Brain	Phosphorylase 'a'	22.98 ± 2.01	24.61 ± 1.08 (+ 7.09)	27.32 ± 1 06 (+ 18.89)	30.51 ± 1.42 (+ 32.77)	25.19 ± 1.08 (+ 9.62)	28.49 ± 0.91 (+ 23.98)
	Aldolase	0.491 ± 0.002	0.535 ± 0.003 (+ 8.96)	0.613 ± 0.002 (+ 24.85)	0.691 ± 0.002 (+ 40.73)	0.567 ± 0.002 (+ 15.48)	0.662 ± 0.001 (+ 35.83)
Liver	Phosphorylase 'a'	31.70 ± 2.47	34.69 ± 1.73 (+ 9.43)	39.64 ± 2.37 (+ 25.05)	45.38 ± 2.64 (+ 43.15)	36.57 ± 1.59 (+ 15.36)	40.73 ± 2.73 (+ 28.49)
	Aldolase	0.933 ± 0.001	1.08 ± 0.01 (+ 16.76)	1.30 ± 0.002 (+ 39.34)	1.72 ± 0.01 (+ 84.35)	1.18 ± 0.01 (+ 26.47)	1.47 ± 0.01 (+ 57.56)
Muscle	Phosphorylase 'a'	12.67 ± 1.72	13.48 ± 1.22 (+ 6.39)	15.61 ± 1.39 (+ 23.20)	17.53 ± 1.13 (+ 38.38)	14.04 ± 1.18 (+ 10.81)	16.26 ± 1.10 (+ 28.33)
	Aldolase	0.428 ± 0.001	0.484 ± 0.001 (+ 13.08)	0.559 ± 0.002 (+ 30.61)	0.687 ± 0.002 (+ 60.51)	0.508 ± 0.001 (+ 18.69)	0.632 ± 0.002 (+ 47.66)
Gill	Phosphorylase 'a'	17.59 ± 1.01	19.17 ± 1.06 (+ 8.98)	21.65 ± 1.37 (+ 23.08)	23.75 ± 1.16 (+ 35.02)	19.61 ± 1.01 (+ 11.48)	22.48 ± 1.22 (+ 27.80)
	Aldolase	0.480 ± 0.001	0.534 ± 0.002 (+ 11.25)	0.618 ± 0.002 (+ 28.75)	0.701 ± 0.001 (+ 46.04)	0.557 ± 0.002 (+ 16.04)	0.678 ± 0.002 (+ 41.25)

Values are mean ± SD of 6 individual observations; Values in parentheses are per cent changes over control; Values are significantly different at $P < 0.05$.

Table 3 *Changes in LDH and G-6-PDH activity levels (μ mol of formazan/mg protein/h) in selected tissues of fish after exposing to sublethal and lethal concentrations of benthiocarb*

			Sublethal concentration			Lethal concentration	
Tissues	Parameter	Control	1 day	2 day	10 day	1 day	2 day
Brain	LDH	0.583 ± 0.041	0.548 ± 0.022 (− 7.72)	0.478 ± 0.031 (− 18.01)	0.357 ± 0.011 (− 38.77)	0.438 ± 0.021 (− 24.87)	0.293 ± 0.012 (− 49.74)
	G-6-PDH	0.187 ± 0.011	0.193 ± 0.210* (+ 3.21)	0.204 ± 0.012 (+ 9.09)	0.213 ± 0.020 (+ 13.96)	0.195 ± 0 031* (+ 4.28)	0.212 ± 0.011 (+ 13.37)
Liver	LDH	0.484 ± 0.030	0.391 ± 0.012 (− 18.21)	0.314 ± 0.021 (− 35.12)	0.214 ± 0.010 (− 55.79)	0 328 ± 0.023 (− 32.23)	0.164 ± 0.011 (− 66.12)
	G-6-PDH	0.275 ± 0.021	0.294 ± 0.034* (+ 6.52)	0.316 ± 0.02 (+ 14.49)	0.336 ± 0.021 (+ 21.74)	0.306 ± 0 031 (+ 10.87)	0.335 ± 0.022 (+ 21.38)
Muscle	LDH	0.109 ± 0.003	0.092 ± 0.004 (− 15.59)	0.072 ± 0.004 (− 33.94)	0.055 ± 0.004 (− 49.54)	0.074 ± 0.006 (− 32.11)	0.058 ± 0.002 (− 46.79)
	G-6-PDH	0.090 ± 0.003	0.094 ± 0.023* (+ 4 44)	0.098 ± 0.005 (+ 8.89)	0.103 ± 0.007 (+ 14.44)	0.093 ± 0.036* (+ 3.33)	0.101 ± 0.006 (+ 12.22)
Gill	LDH	0.087 ± 0.004	0.072 ± 0.003 (− 17.01)	0.067 ± 0.004 (− 22.99)	0.044 ± 0.001 (− 49.43)	0.069 ± 0.003 (− 20.69)	0.042 ± 0.005 (− 51.72)
	G-6-PDH	0.047 ± 0.001	0.048 ± 0.061* (+ 2.13)	0.049 ± 0.069* (− 4.26)	0.051 ± 0.002 (+ 8.51)	0.048 ± 0.056* (+ 2.13)	0.051 ± 0.002 (+ 8.51)

Values are mean ± SD of 6 individual observations; Values in parentheses are per cent changes over control; Values are significantly different at $P < 0.05$; * Not significant.

pyruvate (table 1) could be due to their decreased synthesis as a consequence of toxic stress[19]. A significant decrease in pyruvate content is strange despite the elevated aldolase activity. It is possible that pyruvate is converted to lactate[20]. Wilson and Cascarano[21] reported that decreased oxygen uptake (due to hypoxic conditions) might be due to low levels of pyruvate which probably diverts the metabolic pathway at the phosphoenol pyruvate level. The increased tissue lactate levels are possibly due to increased formation from pyruvate as a result of anaerobiosis. Lactate accumulation alters the permeability properties of cell resulting in disturbances in osmoregulation leading to degeneration of tissues[22].

An increase in the specific activities of phosphorylase, aldolase and G-6-PDH and a decrease in NAD^+-LDH were observed in all tissues of the fish as a consequence of benthiocarb exposure. The increase in phosphorylase 'b' could be due to the increased conversion of phosphorylase 'b' to phosphorylase 'a' as a result of activation of adenylate cyclase[20]. Kallicheran and Gibson[23] reported that pesticides stimulate secretion of epinephrine which

Table 4 *Changes in blood glucose (mg/ml) levels of fish after exposing to sublethal and lethal concentrations of benthiocarb*

	Sublethal concentration			Lethal concentration	
Control	1 day	2 day	10 day	1 day	2 day
6.44 ± 0.81	5.47 ± 0.81 (− 15.06)	4.65 ± 0.82 (− 27.79)	3.81 ± 0.76 (− 40.84)	4 69 ± 0.98 (− 27.17)	3.61 ± 0.95 (− 43.94)

Values are mean ± SD of 6 observations; Values in parentheses are per cent changes over control; Values are significantly different at $P < 0.05$.

in turn activates adenylate cyclase resulting in increased formation of C-AMP and leading to elevation of phosphorylase activity. Increased aldolase activity indicates increased breakdown of hexoses into trioses, suggesting the possible need for greater energy by the tissues during toxic stress. During toxic conditions large quantities of glyceraldehyde-3-phosphates and FAD are formed in the cells ensuring greater energy[24]. Decrease in NAD^+-LDH activity seen in the present study due to herbicide exposure indicates poor clearance of lactate resulting in lactate accumulation and pyruvate depletion in all the tissue of benthiocarb exposed fish. The prevalence of anaerobiosis consequent to decreased oxygen consumption results in lactate accumulation[25].

Anaerobiosis causes oxidation of glucose through HMP shunt as seen from the significant increase in G-6-PDH activity in the experimental tissues. This increased production of $NADPH_2$ is a compensatory source of energy to meet the energy demand caused by reduction in oxidation of glucose through citric acid cycle. During pesticide stress, the increased oxidation of glucose will be through HMP shunt, mediated by the G-6-PDH due to anaerobiosis[26]. The present study indicates that benthiocarb enhances glucose oxidation through anaerobic glycolysis and HMP shunt as a consequence of anaerobiosis as evidenced from the reduced oxygen uptake by fish.

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