Research Article

Glycolipids as Potential Energy Molecules during Starvation in Climbing Perch, *Anabas testudineus* (Bloch)

Padmavathi Godavarthy and Y. Sunila Kumari

Department of Zoology, Osmania University College for Women, Koti, Hyderabad 500095, India

Correspondence should be addressed to Padmavathi Godavarthy; anjanipadmavathi@gmail.com

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Glycolipids are membrane lipids which act as cellular markers and also provide energy for the cells. The present study is an attempt to understand whether glycolipids can act as energy sources during fasting. To achieve this, we selected and subjected *Anabas testudineus* to short-term (15 days) and long-term (60 days) laboratory starvation. We estimated glycolipids biochemically using a standard protocol in six different tissues. Results showed a selective decline in glycolipid concentration in certain tissues, and also an increase was observed in some tissues. Short-term fasting led to a decline in glycolipids in tissues such as brain ($P < 0.05$), accessory respiratory organ ($P < 0.001$), pectoral and lateral line muscle. Liver and kidney ($P < 0.002$) reported an increase. Long term starvation also resulted in a decline in tissues such as liver ($P < 0.001$), kidney ($P < 0.001$), brain, and accessory respiratory organ. Muscle tissue, that is, both the pectoral ($P < 0.002$) and lateral line muscle ($P < 0.05$), showed an increase in the glycolipid fraction. This selective decline in glycolipid content of certain tissues suggests a possible utilization of these lipids during starvation and the significant upsurge observed in certain tissues suggests a simultaneous synthesis occurring along the degradation, probably reducing the oxidative stress created by ROS (reactive oxygen species).

1. Introduction

Fish, during their life time, are capable of withstanding prolonged periods of natural starvation caused due to migration, reproduction and also during fish farming [1–3]. There are several instances where certain fish species have been reported to survive without food for several months or even for years as reported in case of silver eels which go without food for even four years [4, 5]. When the animal is in a state of food deprivation there are lines of evidence wherein the animal differentially utilizes proteins, carbohydrates, and lipids. Utilization of energy fuels is not uniform in all fish but is species dependent. In fish like *Acipenser oxyrinchus* and *Oncorhynchus mykiss*, parallel to glycogen mobilization, lipids are utilized initially and proteins are utilized as the last reserve [6]. In certain other fish species, liver glycogen stores are preserved and either protein is degraded *via* gluconeogenesis, or protein and lipid may be mobilized as energy substrates [6–8]. Apart from these regular energy substrates, other energy molecules such as glycolipids, which are

membrane lipids which have glucose and galactose attached to the lipid unit [9], may also be considered as potential sources of energy by the starving animal, when required. There are evidences where membrane lipids more specifically phospholipids are being mobilized during starvation [10]. However, utilization of glycolipids as potential energy sources during starvation, in *Anabas testudineus*, has not been studied so far. In this regard, we propose that glycolipids can act as efficient energy molecules, because of their composition; that is, glycolipids are lipid molecules, with sugars attached to them. When a glucose molecule is bonded to lipid unit it is called glucocerebroside and when galactose is attached it is called galactocerebroside [9]. These lipids can be enzymatically broken down to yield glucose or galactose and the entry of galactose into the glycolytic pathway is one of the feeder pathways in glycolysis, by which energy can be obtained. Based upon these evidences, we hypothesize that glycolipids may also be considered as efficient energy sources for the starving animal and the present study is an attempt in this direction.

2. Material and Method

Anabas testudineus samples weighing 20–25 gm ($SEM \pm 0.33$) were obtained from Kolleru Lake of Eluru, Andhra Pradesh, India. Care was taken to ensure quick transport to the laboratory. Overcrowding was avoided during packing to minimize the mortality rate. They were carefully transferred into Durex storage tank with capacity of 5000 liters, made of corrosive resistant polypropylene material. Fish which were injured or dead were removed from the tank from time to time. A mild dosage of $KMnO_4$ was used to avoid infection. They were given boiled egg, rice bran meal, and commercial fish feed *ad libitum*. Any leftover feed and fecal matter were removed daily. Water in the tank was changed every day. Fish were brought to the laboratory and kept in plastic tubs of dimensions 24 inches wide and 13 inches deep, which have water capacity of 30 liters. The mouth of these tubs was covered with fine mesh and was appropriately placed such that they were properly ventilated and well aerated. Fish were kept in these tubs for 10 days for sufficient acclimatization. Experimentation was done thereafter. Fish measuring about 4.5 inches in length and weighing an average of 22 gms were selected carefully and were grouped together. Two types of experimental set-up were designed. In the first set-up, fish were allowed to starve for fifteen days and parallel control was also maintained. The control animals were fed regularly both in the morning at 9 am and in the evening at 7 pm. At the end of the experimental time, both experimental and control animals were sacrificed by concussion, and the tissues were removed for biochemical analysis. The second experimental set-up consisted of fish which were allowed to starve for two months (long term). Experimental group and a corresponding control group were maintained. Control group was fed regularly as in the short-term case. After experimental time, the animals were sacrificed, for the experimentation. Six animals in the control and six in the experimental group were killed and sampled as the same as the first set-up. The tissues selected for the experimentation were liver, kidney, brain, accessory respiratory organ pectoral muscle, and lateral line muscle. Fish were dissected and tissues were carefully removed from the body and placed on ice before being further processed. They were gently dried using a tissue paper, before being weighed. Each selected tissue was weighed using an electric balance, Dhona analytical 200 D. A 1% homogenate of each tissue was prepared using 2 mL of $2NH_2SO_4$ as the homogenizing mixture.

They were quickly transferred into appropriately labeled test tubes and glycolipids were estimated by the method described by Raghuramulu et al. [11].

Principle. Glycolipids were estimated by determining the hexose part, of the sugar.

For biological samples, hydrolysis was done with 2 mL of $2NH_2SO_4$ for 2 hrs. After the completion of hydrolysis 4 mL of chloroform was added and this mixture was centrifuged. From the top aqueous layer, 1 mL was taken out separately. To this 50 μ lit of 80% phenol was added, followed by 4 mL of concentrated H_2SO_4 (analytical grade). The orange color developed was measured at 480 nm. The concentration of the

galactose part of the lipid was calculated from the galactose standard. Values were then multiplied by 4.45 to estimate the quantity of glycolipids. The quantity of glycolipids was expressed as mg of galactose.

After the experimentation, a statistical analysis was done to test the significance of the results. Student's *t*-test was performed, using Microsoft Excel. Standard deviation, standard error of mean, and % variation were calculated. *P* value was calculated accordingly, to know the levels of significance.

3. Results

Short-term and long-term fasting of *Anabas testudineus*, under laboratory starvation, yielded mixed results. There was an overall decline of glycolipids in certain tissues, suggesting their utilization. For the first time, we report that glycolipids may also be utilized as potential energy sources during starvation in *Anabas testudineus*. Short-term starvation caused a decrease in glycolipids in tissues such as brain, accessory respiratory organ, and pectoral lateral line muscle. Liver and kidney however showed an increase in glycolipid concentration. Long-term starvation also resulted in a decline of glycolipid concentration in tissues such as liver, kidney, brain, and accessory respiratory organ, while pectoral and lateral line muscle however showed an increase. We attribute the decrease in the glycolipid concentration to its utilization and the increase may be attributed to its simultaneous synthesis occurring along with the degradation.

Short-Term Starvation. Glycolipid fraction generally showed an increase in tissue such as liver and kidney, whereas the other tissues showed a significant decline, during short term starvation. The increase in liver tissue was found to be 0.09% (NS) and the rise in kidney tissue was 137.4% ($P < 0.02$). The other tissues showed a general decline. Brain tissue showed a decrease of 51.5% ($P < 0.05$), and the decline in accessory respiratory organ was found to be 65.3% ($P < 0.001$). Both pectoral and lateral line muscle showed a reduction. The decrease in pectoral muscle was 4.1% (NS) and lateral line muscle showed a decline of 10.4% (NS) (Table 1, Figures 1, 2, 3, 4, 5, 6, and 7).

Long-Term Starvation. Long-term starvation showed a significant decline in the glycolipid fraction in tissues such as liver, kidney, brain, and accessory respiratory organ of *Anabas*, while the pectoral and lateral line muscle showed an increase. The decrease in liver tissue was 59.4% ($P < 0.001$). Kidney showed a reduction of 67.4% ($P < 0.001$). Brain and accessory respiratory organ showed a decline but the result was not statistically significant. The decrease in brain was 30% (NS). Accessory respiratory organ showed a decrease of 17.1% (NS). Pectoral muscle showed an increase of 95.1% ($P < 0.02$) and the increase in lateral line muscle was 55.5% ($P < 0.05$) (Table 1, Figures 1, 2, 3, 4, 5, 6, and 7).

4. Discussion

The present starvation study, on *Anabas testudineus*, resulted in a significant decline in glycolipid concentration in certain

TABLE 1: Glycolipid levels in different tissues during short-term and long-term starvation in *A. testudineus*.

Tissue	Short-term starvation		Long-term starvation	
	Control	Experimental	Control	Experimental
Liver	303.6 ± 13.84	331.5 ^{NS} ± 20.14	438.6 ± 16.6	177.9*** ± 12.2
	% variation = +0.0918		% variation = −59.439	
	Values expressed as mg of galactose			
Kidney	14.83 ± 2.75	35.21** ± 5.30	91.10 ± 6.89	29.66*** ± 2.11
	% variation = +137.430		% variation = −67.437	
Brain	28.72 ± 4.84	13.905** ± 3.44	31.78 ± 4.34	22.24 ^{NS} ± 2.72
	% variation = −51.59		% variation = −30.00	
Accessory respiratory organ	48.20 ± 3.58	16.68*** ± 3.13	24.721 ± 2.98	20.483 ^{NS} ± 2.77
	% variation = −65.38		% variation = −17.14	
Pectoral muscle	44.01 ± 2.52	42.18 ^{NS} ± 3.54	21.71 ± 2.77	42.38* ± 4.76
	% variation = −4.16		% variation = +95.12	
Lateral line muscle	39.86 ± 1.85	35.68 ^{NS} ± 1.67	14.30 ± 2.56	22.24* ± 2.46
	% variation = −10.475		% variation = +55.576	

Each value is mean of SE ± of $n = 6$ individual observations.
 $P < 0.02^*$, $P < 0.05^{**}$, and $P < 0.001^{***}$. NS: not significant

FIGURE 1

tissues (brain, accessory respiratory organ, and pectoral and lateral line muscle) and contrastingly also showed an elevation in certain tissues (liver and kidney), during short-term starvation. Long-term food deprivation also yielded mixed results with tissues such as liver, kidney, brain, and accessory respiratory organ, showing a considerable decline in glycolipid content and considerable increase in pectoral and lateral line muscle. The decline of glycolipids in certain tissues, during short-term and long-term starvation, may be attributed to their utilization as they contain glucose/galactose unit attached to their lipid chain, which may be broken down, as galactose, by enzymes called ceramidases and cerebrosidases and thus the galactose released may be converted into galactose-1-phosphate at the expense of ATP by galactose kinase. The galactose-1-phosphate through a series of reactions is converted into glucose-1-phosphate and

FIGURE 2

FIGURE 3

enters the glycolytic cycle [12], thus providing energy, for the starving animal, and so it may be advantageous to use these membrane lipids along with other energy fuels. The rise of glycolipids observed in certain tissues may be attributed

FIGURE 4

FIGURE 6

FIGURE 5

ST: short-term starvation
LT: long-term starvation

FIGURE 7

to their simultaneous synthesis occurring along with the degradation as liver, kidney, and muscle are all gluconeogenic organs [13] and the glucose formed *via* gluconeogenesis may be utilized to be incorporated into the glycolipid fraction. These increased levels of glycolipids may be of an advantage for the starving animal, as these lipids may be helpful to scavenge the excess of ROS (reactive oxygen species), which may have been created and accumulated during the course of starvation to aid in autophagic degradation of cellular components including the membrane lipids as suggested by these authors [10, 14, 15].

In conclusion, we propose and suggest for the first time that glycolipids may also be degraded and utilized by starving *Anabas testudineus*, to satisfy the energy demands during the crisis of starvation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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