CORRELATION BETWEEN CHANGES IN GLYCOLIPID CONTENT AND SUBSTRATE OXIDATION IN SKELETAL MUSCLES OF RATS WITH ALLOXAN DIABETES

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Carbohydrate-containing compounds of membranes are specific markers for recognition of molecules and cells [4, 12], hormone receptors [7], and they also perform regulatory and adaptive roles [2, 4]. The ability of gangliosides to restore the sensitivity of muscle fibers to electrical excitation has been demonstrated [11]. Glycolipids of tissue membranes, especially of the brain, have been studied in more detail than extraneuronal tissues [2]. Yet some aspects of their functional role have virtually not been studied.

This paper gives the results of a study of the glycolipid content of skeletal muscles during the development of alloxan diabetes in rats, and also of oxidation of carbohydrate-lipid substrates in tissue slices.

EXPERIMENTAL METHOD

Male Wistar rats were used. Experimental diabetes was induced by subcutaneous injection of alloxan in a dose of 50-150 mg/kg. The blood sugar was determined by the orthotoluidine method. After decapitation of the rats the femoral muscles were detached, washed free from blood in the cold, and part of the tissue was used for isolation of lipids, part for cutting into sections to study oxidation of [1,6-^{14}C]-D-glucose and of [1-^{14}C]palmitate. Lipids were isolated by the method in [8], fractionated [1], and their content of sialic acids [9] and carbohydrates [1] was determined. Tissue slices were incubated in Drebs–Ringer buffer, pH 7.4, at 37°C in the small containers of a Warburg apparatus for 1 h, and [^{14}C]palmitate in the form of a complex with serum albumin and with [^{14}C]glucose (10^{-6} M), was added to the incubation medium. Metabolic $^{14}CO_2$ was collected in 10% KOH and radioactivity was measured on an LS-230 radiometer (Beckman, USA) with ZhS-8 scintillation fluid. Activity of endogenous neuraminidase was determined from the quantity of sialic acids secreted into the incubation medium.

EXPERIMENTAL RESULTS

As Table 1 shows, a definite relationship was found between elevation of the blood sugar level and the lowering of the ganglioside level, and also oxidation of [^{14}C]glucose against the background of activation of conversion of [^{14}C]palmitate into $^{14}CO_2$. When the blood sugar reached 800 ± 16 mg% the muscle ganglioside level was reduced by half and oxidation of glucose to $^{14}CO_2$ was inhibited by 80%; under these conditions fatty acid utilization for energy purposes was adaptively increased (by 76%). Membrane gangliosides, it must be assumed, participate in the transport and utilization of glucose, and for that reason the marked decrease in their content in diabetes is one factor disturbing carbohydrate oxidation. To confirm this hypothesis, activity of endogenous neuraminidase was studied in the period of hyperglycemia and an increase in secretion of sialic acid from the tissue into the incubation medium was found in the case of diabetic rats, evidence of intensification of hydrolytic degradation of structural glucoconjugates. Under these conditions maximal hydrolysis was observed in the monosialoganglioside fraction, the content of which was reduced by 30-35% (Table 2). The fall observed in the sialic acid level in patients with diabetes mellitus can be linked with acti-

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TABLE 1. Content of Total Gangliosides and Oxidation of [1,6-^{14}C]-D-Glucose and [1-^{14}C]Palmitate in Skeletal Muscle during Development of Alloxan Diabetes in Rats (n = 4, M ± m)

Blood sugar level, mg%	$^{14}CO_2$, cpm/g tissue		
	gangliosides, μg sialic acid/g	[1,6-^{14}C]-D-glucose	[1-^{14}C] palmitate
85±7	137±9	1610±100	1200±60**
200±14	125±5	1120±70**	1450±85
380±15	108±4	700±30*	1860±110**
620±19	95±2**	480±14*	2400±135*
800±16	63±4*	340±12*	2120±170*

Legend. *P < 0.01, **P < 0.05-0.02.

TABLE 2. Content of Glycolipid Fractions (in μg/g) of Skeletal Muscles from Normal Rats and Rats with Alloxan Diabetes (n = 10, M ± m)

Experimental conditions	Polysialogangliosides	Disialogangliosides	Monosialogangliosides	Cerebrosides	Cerebroside sulfates
Control	14,5±0,7	25,0±1,2	84,5±4,3	225±2	216±4
Diabetes (blood sugar 800 ± 16 mg%)	11,3±0,6	20,9±1,1**	57,1±2,7*	263±3*	182±2*

Legend. Here and in Table 3: *P < 0.001, **P < 0.05-0.02.

TABLE 3. Action of Total Gangliosides on Oxidation of [1,6-^{14}C]-D-Glucose by Skeletal Muscle Tissue Slices (in cpm/g tissue) from Normal Rats and Rats with Alloxan Diabetes (n = 12, M ± m)

Experimental conditions	Without gangliosides	Quantity of gangliosides added, μg			
		100	200	500	1000
Control	1610±100	1920±140	2410±130	2200±120	2100±90
Diabetes	340±12	590±26*	810±36**	1150±54*	1320±72*

vation of the tissue sialidases [3]. It is difficult to explain the increase in the cerebroside content (by 16%) in diabetes, accompanying the fall in the level of their sulfoesters. This phenomenon can evidently also be connected with tissue hypoxia, which leads to a deficiency in their synthesis. There is evidence that sulfocerebrosides participate in ion transport and neuropeptide reception [2]. Unlike gangliosides, cerebrosides contain mainly galactose in their structure, glucose less frequently, and they do not contain sialic acids [2]. We found that gangliosides, when added to the incubation medium, intensify oxidation of [1,6-^{14}C]-D-glucose by tissue slices. This effect of gangliosides can be used to restore disturbed metabolism in skeletal muscle in alloxan diabetes (Table 3). As the results show, the total ganglioside fraction exhibits appreciable biochemical activity in stimulating glucose utilization. There is evidence that [^{3}H]ganglioside interacts with cell membranes with a quite high degree of affinity [5]. Preliminary administration of cerebral gangliosides for 10 days before alloxan injection has a prophylactic effect and inhibits the development of diabetes [10]. Treatment of membranes with glycosidase reduces specific binding of insulin with high-affinity receptors [6].

Analysis of the results and data in the literature indicate that tissue membrane glucoconjugates play an important functional and structural role in the regulation of the fundamental biochemical processes inside and outside the cell.

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SYMPATHOADRENAL ACTIVITY IN DOGS AGED 8-9 YEARS DURING PHYSICAL EXERTION FOLLOWING INJECTION OF PROSTAGLANDIN E_2

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According to data in the literature prostaglandins (PG), especially those of the E group, can exert a modulating action on steroid hormones [8] and catecholamines [3].

If PGE_2 can exert a prophylactic effect under extremal conditions [3], it is important to distinguish whether the properties of this substance can be manifested when functional neurosecretion in the hypothalamus is depressed with age [7], for example, and what effect of PGE_2 is observed in the case of pharmacologically inhibited activity of the hormones of adaptation. It is also interesting to determine the degree of functional resistance of dogs to physical exertion when their adrenocortical and sympathoadrenal activity is modified by PG and dexamethasone. The investigation described below was undertaken to shed light on these problems.

EXPERIMENTAL METHOD

Experiments were carried out on mornings during the fall and winter on 10 mongrel male dogs aged over 8-9 years, weighing 16-18 kg, and with an angiostomy fistula to the abdominal aorta. A needle was inserted through the fistula into the aorta under aseptic conditions, and blood samples for biochemical analysis were removed after 17, 20, 27, 32, 40, 52, 60, and 70 min of the experiment. The concentrations of catecholamines, 11-hydroxycorticosteroids (11-HCS), and serotonin in arterial blood were determined fluorometrically [2], ACTH was determined by a biological method [5], monoamine oxidase (MAO) spectrophotometrically [1], glucose by the standard orthotoluidine method, and [^{125}I]insulin by means of a radioimmunoassay kit (Hungary). Radioactivity of [^{125}I]insulin was measured on an automatic instrument of the No. 322 type (from the "Gamma" Combine, Hungary).

PGE_2 (Academy of Sciences of the Estonian SSR, Tallin) in a dose of 11.5×10^{-8} mole/kg was injected intra-arterially in the course of 40 sec immediately after withdrawal of the background (17th minute of the experiment) blood sample, with monitoring of activity of the cardiovascular and respiratory systems. The doses of PGE_2 and dexamethasone were worked out previously in the laboratory and agree with data in the literature [11, 15]. Dexamethasone ("Galenika," Yugoslavia) in a dose of 6.4×10^{-6} mole/kg was injected intraperitoneally 4 h before the experiment began. The degree of action of dexamethasone on activity of the hypo-

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