Original

Altered glycogen metabolism in the submandibular and parotid salivary glands of rats with streptozotocin-induced diabetes

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Abstract: Experimental animal models of diabetes induced either by alloxan or streptozotocin have been used to study aspects of the pathophysiology of this disease. The purpose of this study was to examine the metabolism of glycogen in the submandibular and parotid salivary glands of diabetic rats. Diabetes was induced by an intraperitoneal injection of streptozotocin. Eight weeks after the induction of diabetes, the animals were sacrificed and the submandibular and parotid salivary glands were removed. The glands were analyzed for glycogen concentration, and activities of glycogen synthase and phosphorylase. Although the diabetic rats consumed more food than controls, they had a lower body weight eight weeks after diabetes induction. Glycogen concentration in the submandibular and parotid glands increased by about 27% and 130%, respectively. Glycogen phosphorylase a in the submandibular gland of diabetic rats showed a reduction of between 75% and 68% compared with controls. In parotid glands, phosphorylase a was reduced by between 84% and 79% compared with controls. The increase in the activity of glycogen synthase a (active) varied from 64% to 130% for the submandibular glands and from 75% to 110% for the parotid compared with controls. These results suggest that the diabetic state influences glycogen metabolism in the submandibular and parotid salivary glands of rats. (J. Oral Sci. 47, 111-116, 2005)

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Introduction

According to a report from the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (1). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction or failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (1). Symptoms of hyperglycemia include polyuria, polydipsia, polyphagia and weight loss (1). Polyphagia is associated with increased mastication frequency, which, in turn, influences salivary gland activity (2,3). Oral health complications associated with diabetes include xerostomia, tooth loss, gingivitis, and soft tissue lesions of the tongue and oral mucosa (4-6). Experimental animal models of diabetes induced either by alloxan or streptozotocin have been used to study aspects of the pathophysiology of this disease. A reduction in parotid and submandibular salivary gland weight has been demonstrated in diabetic animals (7-10).

The polysaccharide glycogen is the main storage form of glucose in mammalian tissues, and glycogen is most abundant in liver and muscle. However, this polysaccharide plays an important role in other tissues, for instance, the salivary glands. The salivary glands are exocrine glands secreting a variety of proteins, glycoproteins and electrolytes important for the integrity of healthy oral tissues.

Evidence of a relationship between the mobilization of

glycogen and the secretion process in salivary glands has been provided by experiments using adrenergic and muscarinic-cholinergic agonists (11-14). The aim of the present investigation was to examine glycogen metabolism in submandibular and parotid salivary glands of rats with streptozotocin-induced diabetes.

Materials and Methods

Animals

Adult male Wistar rats (180 - 250 g) were obtained from the Central Animal House of the São Paulo School of Medicine, UNIFESP. All animals were maintained in individual plastic cages with free access to food (Purina chow) and water under a constant 12 h light/12 h dark cycle. Animals were allowed to acclimatize for a minimum of 7 days before the start of the experiment. Care and handling of the animals throughout the study were conducted in accordance with the instructions of the Brazilian College of Animal Care. At the beginning of the experimental period, the animals were randomly divided into three groups: (a) control (C) rats receiving no treatment, (b) rats receiving an injection of only citrate buffer, pH 4.5 (CIT), and (c) rats in which diabetes was induced with an injection of streptozotocin (STZ) dissolved in citrate buffer (D).

Animal treatment

Diabetes was induced by an intraperitoneal injection of STZ (80 mg/kg body weight) dissolved in 100 mM citrate buffer, pH 4.5, to overnight-fasted rats. Before STZ injection, the blood glucose level was monitored from the tail vein using a blood glucose meter (Accu-Chek, Advantage-Roche Diagnostics, Mannheim, Germany). Seventy-two hours after STZ injection, glucose solution (2.5% and 5%) was administered to the diabetic animals to prevent secondary hypoglycemia and hyperinsulinemia. Rats were considered diabetic when they had a blood glucose level above 14 mM. The food offered to the rats was weighed daily, to allow the mean daily consumption per animal to be calculated. The weight of the rats was monitored weekly. Eight weeks after diabetes induction and an overnight fast, the rats were anesthetized with an intraperitoneal injection of nembutal (50 mg/kg body weight) in the afternoon, between 1400 and 1600 h. After collecting blood from the abdominal aorta, the salivary glands were removed and clamped between aluminium tongs pre-cooled in dry ice. The frozen glands were then stored at - 80°C until analysis.

Analysis

Glycogen content was determined as described elsewhere (15). Briefly, the frozen glands were weighed and digested with 30% KOH solution, precipitated with 95% ethanol, purified by treatment with 5% trichloroacetic acid (TCA) solution, and the glycogen content was determined using anthrone reagent.

For the determination of glycogen phosphorylase (a and total) (16), the frozen glands were homogenized at 10% (w/v) with a solution containing 100 mM NaF, 20 mM EDTA, 0.5% glycogen and 50 mM glycylglycine buffer, pH 7.4. The supernatant obtained by centrifugation at $4,400 \times g$ for 30 min was used for assay of phosphorylase activity. Phosphorylase a was determined in a medium containing 100 mM glucose-1-phosphate, 2% glycogen, 0.3 mM NaF and 1 mM caffeine, pH 6.1. The reaction was stopped with 19% TCA solution, and the inorganic phosphate released was measured (17). Total phosphorylase was assayed after activation in the following medium: 100 mM glucose-1-phosphate, 0.3 mM NaF, 1 mM AMP, 2% glycogen, 3 mM ATP and 5 mM $MgSO_4$, pH 6.1. One unit of enzyme activity corresponds to the amount of enzyme that forms 1 μmol of the product per minute. Specific activity is expressed in U/mg protein.

For the determination of glycogen synthase (a and total) (18), glands were homogenized at 20% (w/v) in a medium containing 25 mM sucrose, 10 mM EDTA, 10 mM dithiothreitol and 100 mM imidazole, pH 7.4. After centrifugation at $5,000 \times g$ for 20 min, an aliquot of the supernatant was incubated at 37°C for 30 min in the presence of 250 mM glycylglycine, 50 mM EDTA, 100 mM Na_2SO_4, 50 mM dithiothreitol, 50 mM UDP-G and 1.2 mg glycogen, for assay of glycogen synthase a. The amount of UDP formed was measured by spectrophotometric assay at 520 nm after incubation in the presence of 10 mM phosphoenolpyruvate, 0.2 U pyruvate kinase and 0.1% DNFH for 30 min. Total glycogen synthase was determined in the same incubation medium as that for synthase a except that 100 mM Na_2SO_4 was replaced by 100 mM glucose-6-phosphate.

Protein was determined with the Folin-phenol reagent (19), using bovine serum albumin as a standard.

The data are presented as mean ± SD. Comparison of the data between groups was performed by using the non-parametric Kruskal-Wallis test. The level of significance was set at 5%.

Results

The results are shown in Tables 1-4. Diabetic rats consumed more food (between 4.6 and 10.5 g) than control animals from the second week onwards, and from the third week the mean body weight of the diabetic animals measured weekly was lower than that of the controls (Table 1). No difference in food consumption or mean body

weight was observed between the control and the citrate buffer groups.

Table 2 shows the blood glucose concentration and glycogen content in the submandibular and parotid salivary glands of control, citrate buffer and diabetic rats. The glycemia at the beginning of the experiment (I) was about the same for all groups. At the end of the experiment (F), the blood glucose level showed no difference between the

Table 1 Mean daily food consumption and mean weekly body weight of control rats, rats treated with 0.1 M citrate buffer, pH 4.5, and rats with streptozotocin-induced diabetes during the experimental period
Mean ± SD
The number of rats is shown in parentheses.

	C (10)		Ccitr (10)		D (14)	
Weeks	Food/day (g)	Weight (g)	Food/day (g)	Weight (g)	Food/day (g)	Weight (g)
0	---------------	193.9 ± 13.4	---------------	191.6 ± 7.4	---------------	188.3 ± 13.7
1	26.6 ± 3.9	204.6 ± 16.8	25.4 ± 4.5	208.0 ± 7.1	24.8 ± 6.3	208.2 ± 20.2
2	26.3 ± 2.7	251.8 ± 18.1	27.1 ± 1.7	244.7 ± 11.3	30.9 ± 4.4	245.6 ± 23.2
3	26.5 ± 2.9	274.8 ± 24.6	27.3 ± 1.4	267.0 ± 15.7	31.4 ± 3.9	243.6 ± 22.3
4	26.5 ± 2.4	304.9 ± 30.4	26.9 ± 1.7	299.7 ± 13.4	33.5 ± 5.9	236.5 ± 30.7
5	27.6 ± 3.0	314.5 ± 31.3	27.6 ± 1.7	321.1 ± 16.5	37.0 ± 5.5	242.0 ± 33.9
6	28.3 ± 2.7	338.7 ± 27.8	28.8 ± 1.1	339.6 ± 18.1	34.2 ± 5.6	144.7 ± 43.0
7	27.4 ± 3.1	353.2 ± 32.3	27.4 ± 4.2	357.4 ± 17.7	32.1 ± 5.1	249.2 ± 49.4
8	29.6 ± 4.3	372.5 ± 36.3	27.6 ± 1.3	372.9 ± 19.6	35.0 ± 7.0	249.0 ± 60.4

Table 2 Blood glucose level and glycogen content in the submandibular and parotid glands of control rats, rats treated with 0.1 M citrate buffer, pH 4.5, and rats with streptozotocin-induced diabetes
Mean ± SD
The number of rats is shown in parentheses. The asterisks indicate statistically significant differences ($P < 0.05$).

Groups	Blood Glucose (mg/100 ml)		Glycogen (μg/ mg tissue)	
	Initial	Final	SM	P
C (10)	89.20 ± 10.63	86.20 ± 7.81	0.273 ± 0.084	0.162 ± 0.045
Ccitr(10)	85.10 ± 9.80	84.60 ± 6.20	0.277 ± 0.068	0.220 ± 0.061
D (14)	89.42 ± 6.71	358.50 ± 76.90*	0.349 ± 0.113*	0.520 ± 0.152*

Table 3 Glycogen phosphorylase (a and total) activity in the submandibular and parotid glands of control rats, rats treated with 0.1 M citrate buffer, pH 4.5, and rats with streptozotocin-induced diabetes
Mean ± SD
The number of rats is shown in parentheses. The asterisks indicate statistically significant differences ($P < 0.05$).

Groups	Gland type	Phosphorylase (U / mg protein)	
		a	total
C (10)	SM	0.0246 ± 0.0037	0.0501 ± 0.0052
	P	0.0615 ± 0.0128	0.1207 ± 0.0230
Ccitr (10)	SM	0.0183 ± 0.0057	0.0419 ± 0.0081
	P	0.0569 ± 0.0217	0.1608 ± 0.0558
D (13)	SM	0.0059 ± 0.0016*	0.0082 ± 0.0022*
	P	0.0428 ± 0.0146*	0.1577 ± 0.0281*

Table 4 Glycogen synthase (a and total) activity in the submandibular and parotid glands of control rats, rats treated with 0.1 M citrate buffer, pH 4.5, and rats with streptozotocin-induced diabetes
Mean ± SD
The number of rats is shown in parentheses. The asterisks indicate statistically significant differences ($P < 0.05$).

Groups	Gland type	Glycogen synthase (mU/ mg protein)	
		a	Total
C (10)	SM	1.40 ± 0.80	3.90 ± 1.20
	P	1.20 ± 0.40	4.70 ± 1.30
Ccitr (10)	SM	1.00 ± 0.30	2.20 ± 0.80
	P	1.00 ± 0.30	4.20 ± 1.30
D (13)	SM	2.30 ± 1.20*	6.30 ± 1.50*
	P	2.10 ± 1.20*	7.40 ± 1.70*

control and citrate buffer groups. However, the value for the diabetic animals was higher than in the other two groups. The glycogen concentration in both types of salivary glands showed the same pattern of variation, that is diabetic animals had a higher concentration than in the other two groups.

Tables 3 and 4 show the results obtained for the enzymes glycogen phosphorylase and glycogen synthase. The activity of phosphorylase a in both types of salivary glands from diabetic rats was lower than that in glands from control rats (Table 3). Conversely, the activity of glycogen

synthase a in both types of salivary glands from diabetic rats was higher than that from control rats. Similar results were obtained for total glycogen synthase activity.

Discussion

The results of this investigation show that the metabolism of glycogen in submandibular and parotid salivary glands is influenced by the diabetic state. One characteristic of the diabetic state is polyphagia. In fact, we observed that the diabetic rats consumed more food, even though they had a lower mean body weight than the control group. It has been reported that parotid glands in rats receiving a pelleted diet show an increase of cell number and synthetic capacity, probably due to the greater requirement for mastication (20,21). In this study it was expected that this characteristic would influence glycogen metabolism in salivary glands of diabetic animals, since the frequency of mastication would influence glandular activity according to diet consistency (2,3). The animals were always sacrificed between 1400 and 1600 h to minimize the influence of circadian rhythm. The initial blood glucose level was about the same in all the groups studied (control = 4.95 mM; citrate buffer = 4.72 mM; diabetic = 4.96 mM). However, at the time of sacrifice (60 days after the induction of diabetes), the blood glucose level in the control and citrate buffer groups was about the same as the initial level (4.78 and 4.70 mM respectively), while the diabetic rats had a blood glucose level of 19.92 mM. The glycogen concentrations in the submandibular and parotid salivary glands were higher in the diabetic rats than in the control and citrate buffer groups, indicating an accumulation of the polysaccharide in both types of glands in diabetic animals. This result is different from what has been reported for other tissues. The glycogen level in the liver of diabetic rats is lower than that in control animals (22,23). It has been reported that the glycogen concentration in rat liver varies with the state of feeding or fasting (24). However, the liver is the organ involved in blood glucose homeostasis, and shows active gluconeogenesis. The basal glycogen concentration in muscles from STZ diabetic rats is lower than that in control animals (25-28). However, a recent study has reported a slightly higher glycogen concentration in diabetic rats than in controls (29). Reports on glycogen metabolism in liver and muscle indicate differences in its regulation (30).

In our study, rats that had been diabetic for two months showed a higher glycogen level in submandibular and parotid salivary glands than that in control rats. For the submandibular gland, the increase in the glycogen level was about 28% and 26% in comparison with the control and citrate buffer groups, while for the parotid gland the corresponding increases were about 225% and 136%, respectively. Glycogen metabolism in salivary glands was found to be different from that in the liver of animals treated with excess vitamin A (15), and from muscle after exercise (31). In salivary glands, gluconeogenesis is not functional (32), so that glycogen formation depends only on glycogen synthase.

Glycogen metabolism is regulated by two enzymes, glycogen synthase and glycogen phosphorylase. Both enzymes are regulated by an allosteric and phosphorylation/dephosphorylation mechanism. Phosphorylation of glycogen synthase converts it to its inactive form, while phosphorylation of glycogen phosphorylase converts it to an active form. It has been reported that in skeletal muscle the binding of insulin to its receptor causes a cascade of chemical reactions, and an increased activity of glycogen synthase and hexokinase (33-35). The total glycogen synthase activity in hepatocytes from diabetic rats was markedly lower than in hepatocytes from healthy rats, while a tendency toward lower glycogen synthase a activity was also observed (36). Rats with STZ-induced diabetes are considered to be model of type 1 diabetes in which an insufficient amount of circulating insulin leads to a decrease in the activity of glycogen synthase and increased activity of glycogen phosphorylase. However, in our work with salivary glands, while we have found higher specific activity of glycogen synthase, glycogen phosphorylase had lower specific activity in diabetic rats than in controls, except for the total specific activity in the parotid gland, which was similar in all groups. Contrary to what is known for liver and muscle, no information is available on the control of glycogen synthesis and degradation in salivary glands, and so further studies on this issue will be necessary.

It is concluded that diabetes causes an accumulation of glycogen in the submandibular and parotid salivary glands of rats, with an increase in the activity of glycogen synthase and a reduction in the activity of glycogen phosphorylase.

Acknowledgments

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