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ACARBOSE IS A COMPETITIVE INHIBITOR OF MAMMALIAN LYSOSOMAL ACID α-D-GLUCOSIDASES

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ABSTRACT

Intraperitoneal injections (~400 mg/kg of body weight) of acarbose, an inhibitor of acid (1→4)-α-D-glucosidase, perturb the metabolism of glycogen in the liver, resulting in excess storage of lysosomal glycogen. The metabolism of skeletal muscle glycogen was unaffected, suggesting that acarbose either does not enter the tissue or that the muscle α-D-glucosidase is not inhibited. The hydrolysis of maltose and glycogen by the acid α-D-glucosidases from rat liver, rat skeletal muscle, and human placenta was inhibited competitively by acarbose. Thus, the lack of effect of acarbose upon the metabolism of muscle glycogen is due to its inability to enter the tissue.

INTRODUCTION

The genetic lack of the lysosomal enzyme (1→4)-α-D-glucosidase (EC 3.2.1.3) results in Pompe's disease (glycogenosis type II), with accumulation of glycogen within the lysosomes of almost all tissues[1,2]. It is now well established that, in the normal liver, a portion of the cellular glycogen is associated with the lysosomes[3–6] and is metabolised by acid α-D-glucosidase. This glycogen may be distinguished from that in the cytosol by size, structure, and incorporation of radioactive precursors of glycogen synthesis[3–11].

Acarbose (**1**) is a pseudotetrasaccharide of microbial origin that is a competitive inhibitor of α-D-glucosidases of intestinal mucosa[12,13] and human placenta[14]. Intraperitoneal (i.p.) injections of acarbose can induce hepatic glycogenosis[5,6,15]. The portion of hepatic glycogen that is normally found in the lysosomal compartment is not degraded because of enzyme inhibition, and accumulates[5,6,15]. There is also accumulation of lysosomal glycogen in kidney, adrenal, and spleen tissue, smooth muscle, and occasionally in skeletal muscle, but not in cardiac muscle or nervous tissue[16]. The investigation of the effects of acarbose upon the structure and metabolism of the liver glycogen of normal rats[5] and rats deficient in phosphorylase

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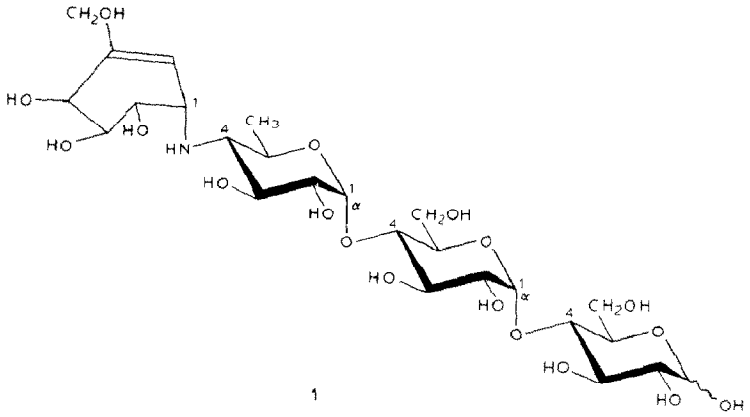

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kinase[6] led to the postulation of a sensitive feedback mechanism which controls the metabolism of liver glycogen[6].

A proportion of the glycogen in skeletal muscle is located intralysosomally, suggesting that its metabolism is compartmentalised in a manner similar to that in the liver[17]. Thus, it was of interest to attempt to perturb the metabolism of muscle glycogen, using acarbose, and to investigate the effect of the inhibitor upon purified acid α-D-glucosidases.

EXPERIMENTAL

Acarbose was a gift from Professor R. Lullmann-Rauch (Department of Anatomy, University of Kiel, F.R.G.). Wistar rats were used. A solution of acarbose (370 mg/kg of body weight) in aqueous 0.9% NaCl was administered by i.p. injection into fed rats which were killed 24 h later.

Glycogen was isolated from the livers by a phenol–cold water procedure[3] with care being taken to minimise the rapid non-uniform post-mortem degradation[18]. Glycogen was isolated from skeletal muscle by extraction with mercuric chloride[19,20]. Placental glycogen was isolated by a modification of the phenol–cold water method[21]. Maltose, 4-methylumbelliferyl α-D-glucopyranoside, and oyster glycogen were obtained from Sigma. Radiolabelled substrates were obtained from Amersham International.

Glycogen fractions were obtained by centrifugation on sucrose density gradients and their average molecular weights were calculated as described[8]. Glycogen concentrations were measured in the presence of iodine–iodide and saturated calcium chloride[22]. Whole-tissue glycogen and glucose were determined by the method of Kemp and van Heijningen[23]. D-Glucose was assayed by the D-glucose oxidase procedure[24] and protein concentrations were measured by a Coomassie Blue binding assay[25].

Lysosomal glycogen was prepared by the rapid differential centrifugation procedure[3], followed by phenol–cold water extraction similar to that used to isolate

whole-liver glycogen[5]. Lysosomal and whole-tissue glycogen contents were determined on the same samples of tissue.

Acid (1→4)-α-D-glucosidase activity was assayed at pH 4.5 with 4-methylumbelliferyl α-D-glucopyranoside as substrate[5]. The 4-methylumbelliferone released was measured by fluorimetry (excitation 358 nm, emission 448 nm). Other lysosomal enzymes were assayed similarly. One unit of enzyme activity liberated one μmol of 4-methylumbelliferone per min at 37°.

The activities of glycogen phosphorylase and phosphorylase kinase were determined by the radiochemical method described by Malthus *et al.*[26], based upon that of Tan and Nuttall[27]. One unit of glycogen phosphorylase activity catalysed the incorporation of one μmol of D-glucose 1-phosphate into glycogen per min at 37°. Total glycogen phosphorylase activity was measured in the presence of AMP. Active glycogen phosphorylase activity was measured in the absence of AMP and in the presence of caffeine in order to suppress the activity of phosphorylase b. The activities of glycogen synthese were determined by a radiochemical method[28]. One unit of enzyme activity incorporated one μmol of D-glucose 6-phosphate into glycogen per min at 37°.

Acid (1→4)-α-D-glucosidase was purified from a homogenate of adult rat liver or skeletal muscle by the dextran adsorption method[29]. The human placental enzyme was purified by the same method, except that maltose was included in the eluting buffer[30].

The values of K_m and V_{max} were determined for each enzyme, using several substrates[31], and at pH 4.5. The substrates were maltose (0–5mM), and glycogen from rat liver (0–30 mg/mL), human placenta (0–30 mg/mL), and oyster (0–30 mg/mL). The reactions were performed as described[5], but substituting for 4-methylumbelliferyl α-D-glucopyranoside as substrate. Data were plotted as Lineweaver–Burk plots. In order to determine the value of K_I for acarbose on the activity of acid α-D-glucosidase, acarbose at a final concentration of either 25 or 50 μg/mL was included in the reaction mixtures. The K_I values were calculated from Lineweaver–Burk plots.

RESULTS

Effects of intraperitoneal injection of acarbose upon content, structure, and metabolism of skeletal muscle glycogen. — Whereas i.p. injections of acarbose had a major effect upon the metabolism of liver glycogen[5,6], the metabolism of muscle glycogen was largely unaffected. Muscle glycogen contents were unaltered (0.62 ±0.01 mg/100 mg of tissue wet-weight for treated animals *vs.* 0.59 ±0.03 mg/100 mg of tissue wet-weight for untreated animals) as was the molecular-weight profile of skeletal muscle glycogen (data not shown). Similarly, treatment with acarbose had little effect on the activity of acid (1→4)-α-D-glucosidase in muscle and the activities of other muscle lysosomal enzymes were unaffected (Table I). Administration of acarbose had no effect upon the activities of any of the other enzymes involved in the metabolism of glycogen in skeletal muscle (Table II).

TABLE I

ACTIVITIES[a] OF ENZYMES IN MUSCLE FOLLOWING TREATMENT WITH ACARBOSE

	α-D-Glucosidase		*Acid phosphatase*		*β-Glucuronidase*	
	(mU/g of tissue)	*(mU/mg of protein)*	*(mU/g of tissue)*	*(mU/mg of protein)*	*(mU/g of tissue)*	*(mU/mg of protein)*
Control	1.70 ±0.31	0.017 ±0.003	178.7 ±10.1	1.81 ±0.11	54.3 ±5.6	0.55 ±0.06
Acarbose-treated	1.55 ±0.025	0.015 ±0.002	175.1 ±9.4	1.79 ±0.27	53.7 ±6.4	0.54 ±0.11
% of control	91	90	98	99	99	98

[a]Determined upon skeletal muscle homogenates, using the appropriate 4-methylumbelliferyl substrate. Data are the mean of six samples. Errors are ±standard deviation.

TABLE II

ACTIVITIES[a] OF ENZYMES IN MUSCLE FOLLOWING TREATMENT WITH ACARBOSE

Enzyme	*Control*	*Acarbose-treated*
Glycogen phosphorylase: Total	117.9 ±21.6	121.9 ±10.1
a	23.0 ±4.4	25.0 ±4.4
% active	19.6 ±2.4	20.5 ±2.7
Phosphorylase kinase	17.2 ±4.1	17.7 ±3.5
Glycogen synthase: Total	10.1 ±2.1	9.8 ±1.2
a	2.4 ±0.7	2.6 ±0.3
% active	23.9 ±5.1	26.9 ±3.6

[a]Determined using skeletal muscle homogenates (see Experimental) and expressed as units/g of tissue wet-weight. Data are the mean of six samples. Errors are ±standard deviation.

TABLE III

SUMMARY OF KINETIC DATA FOR MAMMALIAN (1→4)-α-D-GLUCOSIDASES

Substrate	*Kinetic parameter*[a]	*Enzyme source*		
		Rat liver	*Rat muscle*	*Human placenta*
Maltose	K_m (mM)	5.9	5.3	10.1
	V_{max}	31.3	10.9	47.4
	K_i (μM)	28.8	31.2	43.2
Oyster glycogen	K_m (mg/mL)	21.7	22.4	21.4
	V_{max}	19.2	6.9	39.9
	K_i (μM)	39.5	43.9	55.2
Placental glycogen	K_m (mg/mL)	19.6	21.8	22.9
	V_{max}	11.8	4.8	31.6
	K_i (μM)	37.4	36.3	52.7
Rat liver glycogen	K_m (mg/mL)	20.7	23.6	18.6
	V_{max}	5.8	3.1	21.3
	K_i (μM)	41.3	42.1	51.8

[a]The units of V_{max} are mg of maltose hydrolysed/h and mg of glucose liberated/h from glycogen. The values of K_i were determined from Lineweaver–Burk plots and are the average of two concentrations.

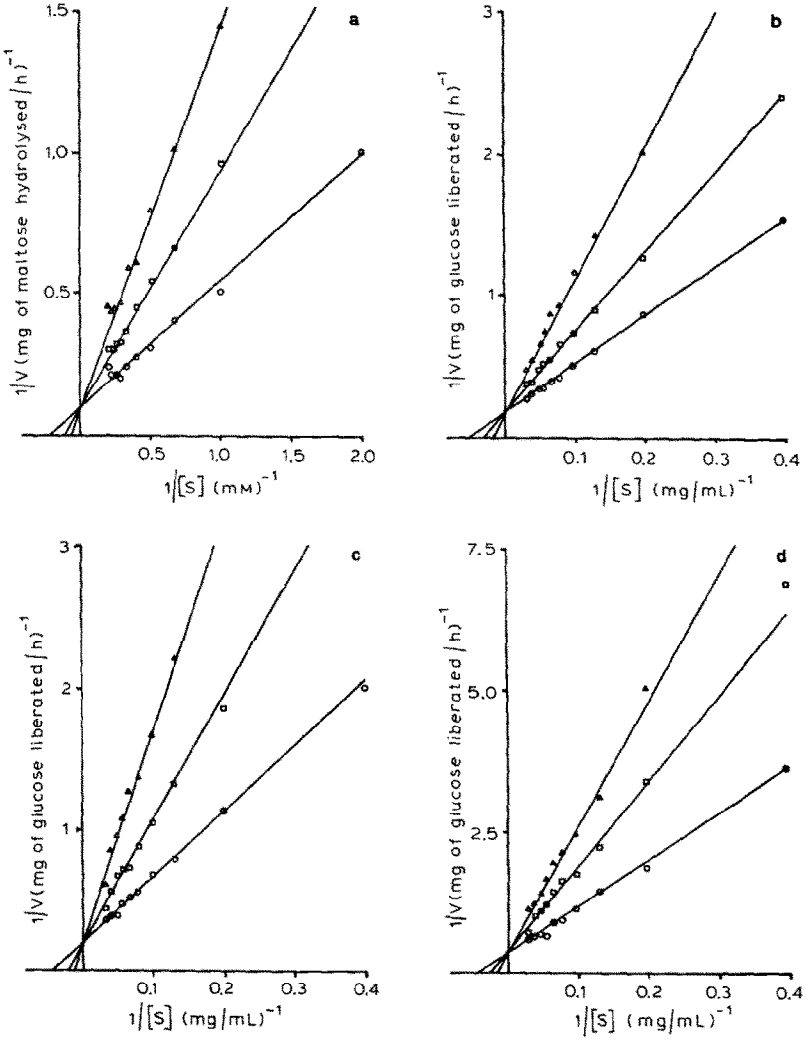

Fig. 1. Lineweaver–Burk plots for rat skeletal muscle acid (1→4)-α-D-glucosidase. Purified rat skeletal muscle acid α-D-glucosidase was incubated with various substrates for 15 min, and the amount of D-glucose liberated was determined by a D-glucose oxidase method[24]. The effect of acarbose was tested at final concentrations of 0 (○), 25 (□), or 50 μg/mL (△): (a) maltose, (b) oyster glycogen, (c) human placental glycogen, (d) rat liver glycogen. Data are the average of four determinations.

Inhibition of purified acid α-D-glucosidases by acarbose. — Three mammalian lysosomal acid α-D-glucosidases were purified and the inhibition of their activities by acarbose was studied. The Lineweaver–Burk plots for the rat skeletal muscle enzyme are shown in Fig. 1, and the value of K_I for each enzyme is listed in Table III. Acarbose competitively inhibited the hydrolysis of each of the substrates (maltose and glycogen from oyster, placenta, and liver) by each enzyme. For the liver

and muscle enzymes, the value of K_I for the hydrolysis of maltose was ~30μM and, for the hydrolysis of glycogen, ~40μM, irrespective of the source of the glycogen. The values of K_I were higher for the placental enzyme (40–60μM).

DISCUSSION

The effects of i.p. injections of acarbose on the metabolism of liver glycogen have been reported[5,6]. The drug causes a 25% decrease in the concentration of glycogen in the liver and increases the proportion of low-molecular-weight material[5]. Taking into account the reduced content of hepatic glycogen, acarbose lowered the absolute level of high-molecular-weight glycogen in the liver. The lysosome-enriched fraction obtained from the livers of acarbose-treated rats contains ~12% of the tissue glycogen, rather more than that from the untreated rat[5]. This result indicates that acid (1→4)-α-D-glucosidase is inhibited. Indeed, this activity is lowered by 55% one day after treatment with acarbose[5], whereas the activities of the other lysosomal enzymes are lowered to a lesser extent[5]. Acarbose also causes an elevation in the levels of hepatic phosphorylase and phosphorylase kinase, although the percentage of phosphorylase in the active form remained constant[6]. The drug also affected the molecular weight distribution of the glycogen in liver lysosomes. Despite the overall decrease in cellular content of high-molecular-weight glycogen, the lysosomes contained a higher proportion of this material[5]. The half-life of acarbose after intravenous injection is ~20 min[32], so that the large effect upon the structure of the glycogen after a single i.p. injection indicates that the drug was concentrated within the lysosomal apparatus, as shown by the observations of Lullmann-Rauch[15]. Indeed, the effects are still apparent 5 days after injection[5,6], although their magnitude is decreased. Acarbose enters the liver lysosomal apparatus by endocytosis[15,16] and then inhibits acid α-D-glucosidase. Thus, the degradation of the glycogen in hepatic lysosomes is impaired, and induced storage results. A feedback pathway controlling these events has been proposed[5,6] and it is suggested that disturbance of the normal lysosomal function disturbs the overall metabolism of cellular carbohydrates. This situation has been found also in studies where the metabolism of glycogen in hepatic lysosomes is perturbed by antibodies to acid (1→4)-α-D-glucosidase[4] or by treatment with castanospermine[33].

Once the existence of glycogen inside skeletal muscle lysosomes was established[17], attempts were made to perturb its metabolism using acarbose. The content and metabolism of the skeletal muscle glycogen were not affected by administration of acarbose (Tables I and II). The inability of i.p.-injected acarbose to perturb the metabolism of muscle glycogen is consistent with other results[16,34]. Lullmann-Rauch[16] noted the acarbose-induced storage of lysosomal glycogen in smooth muscle but not in cardiac or skeletal muscle, except rarely in soleus muscle. Similarly, perfusion of rat hind-limb muscle with acarbose (up to 1000 μg/mL) had no effect on the level of glycogen, the uptake of glucose, the release of lactate, the

oxidation of lactate, or the uptake of oxygen[34]. However, another study indicated that the metabolism of glycogen in cardiac muscle can be perturbed by acarbose[35]. Dietary acarbose reduced the increase in cardiac muscle glycogen which occurs with a high-carbohydrate diet. Although it is likely that this effect was due to inhibition of intestinal α-D-glucosidases, resulting in lowered uptake of dietary carbohydrate, there were specific effects of acarbose upon cardiac muscle phosphorylase. Total cardiac phosphorylase was increased markedly, but the percentage in the active form remained unaltered[35]. This effect is similar to that reported for the effect of acarbose upon liver glycogen phosphorylase[6]. Thus, it appears that acarbose can perturb the metabolism of glycogen in cardiac muscle[35], although no induced storage of glycogen in the lysosomes of cardiac muscle was noted by Lullmann-Rauch[16].

The lack of effect of acarbose on skeletal muscle glycogen metabolism could be due to the inefficiency of endocytotic uptake of acarbose or its inability, once uptaken, to inhibit acid α-D-glucosidase and so induce the storage of glycogen. Therefore, in order to investigate the ability of acarbose to inhibit the activity of α-D-glucosidase, the action of three mammalian lysosomal α-D-glucosidases on maltose and glycogen was studied in the presence and absence of acarbose. Acarbose inhibited competitively the hydrolysis of each substrate by each enzyme. The values of K_I obtained (30–60μM) were similar to those for inhibition by acarbose of the hydrolysis of 4-methylumbelliferyl α-D-glucopyranoside by α-D-glucosidases from placental lysosomes[14] and intestinal mucosa[12,13]. The similar values of K_I for the liver and muscle enzymes indicate that they were inhibited to the same extent by acarbose.

Acarbose is a particularly effective inhibitor of acid α-D-glucosidases. Turanose is perhaps the best defined competitive inhibitor of acid α-D-glucosidases. The value of K_I for the inhibition of maltase activity by turanose is ~3mM for liver α-D-glucosidase[36] and ~2mM for muscle α-D-glucosidase[37,38]. The value of K_I for the inhibition of the hydrolysis of glycogen is 1.7mM for the liver enzyme[39] and ~1mM for the muscle enzyme[37,38]. The value of K_I for the inhibition of the hydrolysis of 4-methylumbelliferyl α-D-glucopyranoside is 1mM for the rat liver enzyme and 3mM for the human placental enzyme[30]. In comparison, the values of K_I for acarbose (30–60μM) indicate much more effective inhibition.

The effectiveness of acarbose as an inhibitor of liver acid α-D-glucosidase *in vitro* is reflected in the excessive storage of glycogen in liver lysosomes induced *in vivo* by the drug[5,6,15]. The inhibition of the skeletal muscle enzyme indicates that the reason the storage of glycogen in lysosomes in skeletal muscle was not induced by acarbose is its inability to enter the muscle, because of the low endocytotic activity of the tissue.

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