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In vivo D-chiroinositol activates skeletal muscle glycogen synthase and inactivates glycogen phosphorylase in rhesus monkeys

Heidi K. Ortmeyer, Noni L. Bodkin, Barbara C. Hansen, and Joseph Larner*

*Obesity and Diabetes Research Center, Department of Physiology, School of Medicine, University of Maryland at Baltimore, Baltimore, MD USA; *Department of Pharmacology, School of Medicine, University of Virginia, Charlottesville, VA USA*

Chiroinositol, a component of an inositol phosphoglycan, has been found to be reduced in the urine of humans and monkeys with non–insulin-dependent diabetes mellitus. To determine whether in vivo repletion of this inositol could improve the action of insulin on skeletal muscle glycogen synthase and glycogen phosphorylase, D-chiroinositol was administered intravenously to six monkeys during a euglycemic hyperinsulinemic clamp. Enzyme activities were determined in vastus lateralis which was freeze-clamped in situ at three time periods: immediately prior to initiation of the clamp, after steady-state glucose and insulin concentrations had been reached and the glucose disposal rate determined, and 30 min after the administration of D-chiroinositol which was superimposed on the steady-state clamp condition. D-chiroinositol increased the activity (independent and fractional velocity) of glycogen synthase ($P < 0.05$) and decreased the activity (independent and activity ratio) of phosphorylase ($P < 0.05$) compared with the samples during the clamp immediately prior to D-chiroinositol administration. Total activities of glycogen synthase and glycogen phosphorylase were not different between any of the conditions studied. We conclude that intravenous administration of D-chiroinositol enhances intracellular insulin action on muscle glycogen synthase and glycogen phosphorylase in rhesus monkeys. (J. Nutr. Biochem. 6: 499–503, 1995.)

Keywords: glycogen synthase; glycogen phosphorylase; glucose disposal rate; D-chiroinositol; rhesus monkey (Macaca mulatta); insulin action

Introduction

The covalent activation of skeletal muscle glycogen synthase by in vivo insulin has been shown to be reduced in insulin-resistant and non–insulin-dependent diabetic humans,[1–4] in first-degree relatives of subjects with non–insulin-dependent diabetes mellitus (NIDDM),[5] in spontaneously β-cell hyper-responsive, insulin-resistant and non–insulin-dependent diabetic rhesus monkeys compared with normal monkeys,[6] and in some calorie-restricted monkeys.[7] This defective action of insulin on glycogen synthase may be responsible for reduced insulin-mediated glucose disposal rates in insulin-resistant and diabetic subjects, and this defect may be responsible for insulin resistance and diabetes.[8]

D-chiroinositol has been shown to be a component of an inositol phosphoglycan which has been postulated to be a mediator of insulin action.[9] The D-chiroinositol-containing phosphoglycan has been shown to activate both glycogen synthase phosphatase and pyruvate dehydrogenase phosphatase in vitro.[10,11] The urinary excretion rate of chiroinositol in diabetic humans and monkeys has been shown to be reduced compared with normal control subjects.[12,13] In monkeys, the urinary excretion rate of chiroinositol was significantly related to whole-body insulin-mediated glu-

Address reprint requests to Dr. Heidi K. Ortmeyer, Obesity and Diabetes Research Center, 10 S. Pine St., Room 6-00, Baltimore, MD 21201, USA.
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cose disposal rates and to insulin action on muscle and adipose tissue glycogen synthase (positive relationships), and it was inversely related to insulin action on muscle glycogen phosphorylase.[13] We therefore sought to determine whether the in vivo administration of D-chiroinositol might enhance insulin action of skeletal muscle enzyme activity by increasing the activation state of glycogen synthase and/or by decreasing the activation state of glycogen phosphorylase.

D-chiroinositol was administered intravenously during the steady-state period of a euglycemic hyperinsulinemic clamp to test the effects of D-chiroinositol on the activities of the two enzymes. Six monkeys were studied, all of which had shown relatively low responses to in vivo insulin on muscle glycogen synthase fractional velocity in a previous study (≤12%).[6,14] We hypothesized that the administration of D-chiroinositol during a clamp would result in increased activation of glycogen synthase and inactivation of glycogen phosphorylase when compared with insulin stimulation alone.

Materials and Methods

Animals

Six rhesus monkeys (*Macaca mulatta*) were selected for study from the Obesity and Diabetes Research Center. Monkeys at this Center are housed in individual stainless steel cages and maintained under consistent laboratory conditions in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. The monkeys have ad libitum access to monkey chow (Purina Mills, Inc., St. Louis, MO, USA). The monkeys were not receiving medication of any kind.

Three of the monkeys were normoglycemic but had previously been shown to have low insulin-mediated changes in skeletal muscle glycogen synthase fractional velocity (<9%) (O-8, Y-7, and Z-7), and three of the monkeys had spontaneous non–insulin-dependent diabetes (W-4, F-8, and L-7) and had varied responses to insulin on muscle glycogen synthase fractional velocity during previous experiments (3 to 12%). In clinically normal rhesus monkeys, there appear to be some monkeys with a relatively high sensitivity to insulin action on muscle glycogen synthase (insulin-stimulated minus basal glycogen synthase fractional velocity ≥13%) and others with a relatively low sensitivity to insulin action on this enzyme (≤9%),[14] possibly identifying those monkeys with a predisposition to diabetes.

The age, weight, fasting plasma glucose concentration, glucose disappearance rate, fasting plasma insulin concentration, and whole-body insulin-mediated glucose disposal rate of the six monkeys are shown in *Table 1*.

Experimental design

All protocols were reviewed and approved by the Institutional Animal Care and Use Committee. Prior to the present study, all monkeys were characterized on the basis of the following: (a) fasting basal plasma glucose and insulin concentrations following a 16 hr fast, (b) an intravenous glucose tolerance test, and (c) tritiated water determination of percent body fat.

In the present experiments, the monkeys underwent a euglycemic hyperinsulinemic clamp with muscle biopsies before and during the clamp procedure as previously described[6] with the following modifications. After the second muscle biopsy was obtained (148 ± 12 min after onset of insulin infusion) (mean ± SE), 1 g/kg of D-chiroinositol was administered intravenously over a 3 min period. The D-chiroinositol was diluted in lactated Ringer's (200 mg/mL). Approximately 30 min after the administration of D-chiroinositol (33 ± 1 min; 207 ± 51 min after onset of insulin infusion), an additional muscle biopsy was obtained from the vastus lateralis muscle. The mean difference between the second and third muscle biopsy was 49 ± 2 min. Insulin and glucose were maintained at a steady state throughout this additional procedure. The steady-state plasma insulin concentration during the insulin administration alone was 27,030 ± 3,078 pmol/L, and between the D-chiroinositol administration and the third muscle biopsy it was 25,710 ± 2,808 pmol/L.

Glycogen synthase and glycogen phosphorylase activities (total and independent activities) were compared under basal insulin-stimulated and insulin-stimulated plus D-chiroinositol conditions. The assays for glycogen synthase, phosphorylase, and protein have been previously described.[6,15] Briefly, the independent activity of glycogen synthase was measured in the presence of 0.1 mM glucose 6-phosphate, and the total activity was measured in the presence of 10 mM glucose 6-phosphate. The fractional velocity of glycogen synthase is the ratio of the independent to the total activity. The independent activity of glycogen phosphorylase was measured in the absence of AMP, and the total activity was measured in the presence of 3 mM AMP. The activity ratio of glycogen phosphorylase is the ratio of the independent to the total activity. The intra-assay coefficients of variation of glycogen synthase, phosphorylase and protein were 5, 2, and 6%, respectively. Total catecholamines (epinephrine and norepinephrine) were determined in plasma[16] obtained during the basal fasting, euglycemic hyperinsulinemic clamp, and euglycemic hyperinsulinemic clamp plus D-chiroinositol periods.

In addition, four lean young non-insulin-resistant monkeys underwent the same procedure as just described except that lactated Ringer's without D-chiroinositol was administered after the second muscle biopsy. The monkeys were 7 years old and ranged

Table 1 In vivo characteristics of the monkeys (n = 6)

Monkey identification	O-8	Y-7	Z-7	W-4	F-8	L-7
Age (year)	5.5	6.6	7.6	16.4	12.1	17.8
Weight (kg)	10.4	10.9	11.7	13.3	13.3	16.8
FPG* (mmol/L)	3.53	4.03	3.14	13.22	14.00	12.04
KGlucose† (%/min)	4.13	3.03	3.35	1.30	1.41	0.80
FIRI‡ (pmol/L)	312	174	516	360	4596	1296
M§ (mg/kg of FFM/min)	10.753	10.618	5.948	3.074	0.375	0.000

*Fasting plasma glucose
†Glucose disappearance rate
‡Fasting plasma insulin
§Whole-body insulin-mediated glucose disposal rate. FFM, fat-free mass

from 7 to 10 kg in body weight, from 6 to 9% in body fat, from 2.74 to 3.64 mmol/L in fasting plasma glucose, from 162 to 270 pmol/L in fasting plasma insulin, and from 2.79 to 5.25%/min in glucose disappearance rates. The glucose disposal rates during the clamps ranged from 6.49 to 10.35 mg/kg of FFM/min. The second and third muscle biopsies were obtained at 108 ± 6 min and at 169 ± 6 min after the onset of the insulin infusion, respectively. The mean difference between the second and third muscle biopsy was 61 ± 2 min. The steady-state plasma insulin concentration during the euglycemic hyperinsulinemic clamp was 31,004 ± 842 pmol/L.

Statistics

Statistical significance was determined by Student's paired *t*-test. Data are expressed as mean ± SE.

Results

Glycogen synthase activity

The insulin-stimulated and insulin-stimulated plus D-chiroinositol glycogen synthase fractional velocities for each monkey are shown in *Figure 1*. The fractional velocity of glyogen synthase was significantly greater under the insulin-stimulated plus D-chiroinositol (D-CI) (25 ± 6%) condition compared with the insulin-stimulated condition alone (14 ± 2%) ($P < 0.05$). The glycogen synthase independent activity was also significantly greater under the D-CI condition (3.85 ± 0.60 nmol/min/mg of protein) compared with the insulin-stimulated condition (2.38 ± 0.44 nmol/min/mg of protein) ($P < 0.05$). The total activities of glycogen synthase were not significantly different between the D-CI and insulin-stimulated conditions (16.96 ± 2.65 and 17.41 ± 3.18 nmol/min/mg of protein, respectively).

The fractional velocity of glycogen synthase was not significantly different under the insulin-stimulated plus lactated Ringer's condition (control) (21 ± 4%) compared with the insulin-stimulated condition (27 ± 9%) ($P = 0.39$) in the four control monkeys. The glycogen synthase–independent activity was also not significantly different under the control (lactated Ringer's) condition (3.97 ± 1.05 nmol/min/mg of protein) compared with the insulin-stimulated condition (5.61 ± 2.05 nmol/min/mg of protein) ($P = 0.29$). The total activities of glycogen synthase were

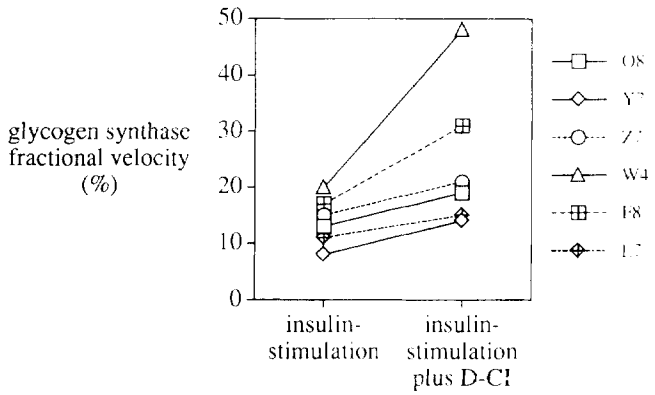

Figure 1 Skeletal muscle glycogen synthase fractional velocity under insulin-stimulated (euglycemic hyperinsulinemic clamp) and insulin-stimulated plus D-chiroinositol conditions in each of the 6 monkeys.

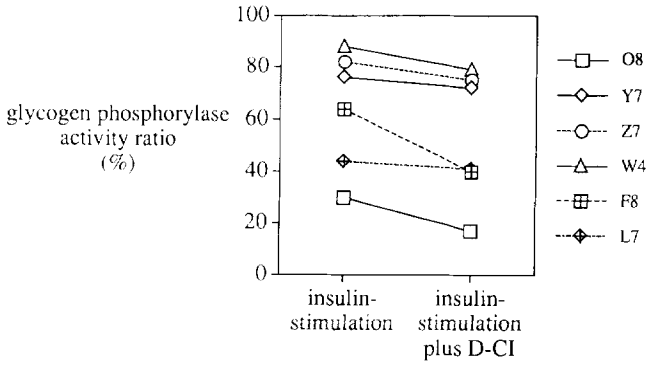

Figure 2 Skeletal muscle glycogen phosphorylase activity ratio under the same conditions as in *Figure 1*.

significantly different between the control condition and the insulin-stimulated condition (18.01 ± 2.70 and 19.32 ± 2.40 nmol/min/mg of protein, respectively) ($P < 0.05$).

Glycogen phosphorylase activity

The insulin-stimulated and insulin-stimulated plus D-chiroinositol glycogen phosphorylase activity ratios for each individual monkey are shown in *Figure 2*. The activity ratio of glycogen phosphorylase was significantly greater under the insulin-stimulated condition (64 ± 10%) compared with the D-CI condition (54 ± 11%, $P < 0.05$). The independent activity of phosphorylase was also significantly greater under the insulin-stimulated condition (1.86 ± 0.40 μmol/min/mg of protein) compared with the D-CI condition (1.43 ± 0.35 μmol/min/mg of protein, $P < 0.005$). The total activities of phosphorylase were not significantly different between the insulin-stimulated condition and the D-CI condition (2.97 ± 0.44 and 2.63 ± 0.26 μmol/min/mg of protein, respectively).

The activity ratio of glycogen phosphorylase was not significantly different under the insulin-stimulated condition (31 ± 6%) compared with the control (lactated Ringer's) condition (39 ± 4%) ($P = 0.17$) in the four control monkeys. The glycogen phosphorylase–independent activity was also not significantly different under the insulin-stimulated condition (0.78 ± 0.10 nmol/min/mg of protein) compared with the control condition (0.94 ± 0.15 nmol/min/mg of protein) ($P = 0.19$). The total activities of glycogen phosphorylase were not significantly different between the insulin-stimulated and control conditions (2.61 ± 0.22 and 2.40 ± 0.21 nmol/min/mg of protein, respectively) ($P = 0.38$).

Total catecholamines

In order to determine whether the effect of D-chiroinositol administration on muscle glycogen synthase and phosphorylase activity was due in part to a change in plasma catecholamine concentrations, total catecholamine concentrations were measured during each of the three conditions in four of the monkeys given D-chiroinositol. There were no differences in total catecholamine concentrations between the insulin-stimulated condition (7,068 ± 2,052 pmol/L) and the insulin-stimulated plus D-chiroinositol condition (7,330 ± 1,998 pmol/L).

Whole-body insulin-mediated glucose disposal rates

The whole body insulin-mediated glucose disposal rate during the last 30 min of the steady-state glucose and insulin infusion period (5.13 ± 1.98 mg/kg of FFM/min) did not differ from the glucose disposal rate during the 30 min period between the time of D-chiroinositol administration and the third muscle biopsy (4.45 ± 1.83 mg/kg of FFM/min).

Discussion

Previous experiments in rats with elevated glucose had demonstrated that intragastrically administered D-chiroinositol decreased plasma glucose, and further, that in normal rats given a glucose load, administration of D-chiroinositol increased glucose disappearance rate.[17]

In obese insulin-resistant rhesus monkeys, intravenously administered D-chiroinositol increased the rates of disappearance of both plasma glucose and insulin.[17] D-chiroinositol added to a meal resulted in significantly lower postprandial plasma glucose concentrations in obese insulin-resistant rhesus monkeys.[18]

It has been previously suggested that D-chiroinositol might be acting by virtue of its incorporation into a mediator precursor, processed to D-chiroinositol–containing mediator, which then acts at insulin-sensitive sites (muscle and liver) to decrease plasma glucose.[17] In a prior report, we have demonstrated that inositol phosphoglycan insulin mediators prepared from beef liver act in vivo in rats in nanomolar amounts equivalent to those of insulin to decrease plasma glucose in a dose-dependent manner.[19] To test further the hypothesis that a D-chiroinositol–containing inositol phosphoglycan may be important in defective insulin action, in the present experiments free D-chiroinositol was administered to monkeys under euglycemic hyperinsulinemic clamp conditions and muscle biopsies assayed for classical insulin-sensitive enzyme changes, namely the activation state of glycogen synthase and glycogen phosphorylase. Interestingly, even at steady-state maximally effective insulin infusion rates, D-chiroinositol further activated glycogen synthase above the activity during maximal insulin, and D-chiroinositol decreased glycogen phosphorylase activity. These effects demonstrate that under these conditions, insulin action on glycogen synthase is rate-limited and can be further enhanced, possibly by mediator generated in muscle, as previously suggested.[12]

It is unlikely that the increase in glycogen synthase activity (fractional activity and independent activity) after D-chiroinositol administration compared with the activity of glycogen synthase under maximal insulin-stimulated conditions alone was due to the additional duration of the insulin infusion. First, in the four lean young rhesus monkeys given vehicle without D-chiroinositol, there were no differences in glycogen synthase activity (fractional velocity or independent activity) or in glycogen phosphorylase activity (activity ratio or independent activity) between the second and third muscle biopsy during a euglycemic hyperinsulinemic clamp. Second, total catecholamines were not lower 30 min after D-chiroinositol administration (the time of the third muscle biopsy) compared with the time of the second muscle biopsy. Third, steady-state plasma insulin concentrations were not higher during the 30 min after D-chiroinositol administration compared with the time before the D-chiroinositol administration.

D-chiroinositol administration resulted in the greatest increase in glycogen synthase activity (fractional velocity and independent activity) in two of the three diabetic monkeys. This may have occurred because the diabetic monkeys have the lowest concentrations of chiroinositol in the muscle tissue and therefore respond more to repletion of this inositol.[12,13]

Whole-body insulin-mediated glucose disposal rates were not increased by D-chiroinositol in this study. This may be explained by the fact that D-chiroinositol was administered as a single bolus and not continually throughout the last phase of the euglycemic hyperinsulinemic clamp. Although a single bolus of D-chiroinositol was able to enhance insulin action on muscle glycogen synthase and glycogen phosphorylase within 30 min, a single bolus may not be enough to increase the steady-state glucose disposal rate.

The mechanism by which the insulinomimetic effects of D-chiroinositol are achieved has not yet been identified. One hypothesis is that the D-chiroinositol becomes incorporated into an inositol phosphoglycan, which is important in insulin action.

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