**N-acetylcysteine and taurine prevent hyperglycemia-induced insulin resistance in vivo: possible role of oxidative stress**

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Submitted 12 August 2002; accepted in final form 23 May 2003

**Abstract**

Haber, C. Andrew, Tony K. T. Lam, Zhiwen Yu, Neelhar Gupta, Tracy Goh, Elena Bogdanovic, Adria Giacca, and I. George Fantus. N-acetylcysteine and taurine prevent hyperglycemia-induced insulin resistance in vivo: possible role of oxidative stress. *Am J Physiol Endocrinol Metab* 285: E744–E753, 2003. First published June 10, 2003; 10.1152/ajpendo.00355.2002.—Exposure to high concentrations of glucose and insulin results in insulin resistance of metabolic target tissues, a characteristic feature of type 2 diabetes. High glucose has also been associated with oxidative stress, and increased levels of reactive oxygen species have been proposed to cause insulin resistance. To determine whether oxidative stress contributes to insulin resistance induced by hyperglycemia in vivo, nondiabetic rats were infused with glucose for 6 h to maintain a circulating glucose concentration of 15 mM with and without coinfusion of the antioxidant N-acetylcysteine (NAC), followed by a 2-h hyperinsulinemic-euglycemic clamp. High glucose (HG) induced a significant decrease in insulin-stimulated glucose uptake [tracer-determined disappearance rate (Rd), control 41.2 ± 1.7 vs. HG 32.4 ± 1.9 mg·kg⁻¹·min⁻¹, P < 0.05], which was prevented by NAC (HG + NAC 45.9 ± 3.5 mg·kg⁻¹·min⁻¹). Similar results were obtained with the antioxidant taurine. Neither NAC nor taurine alone altered Rd. HG caused a significant decrease in insulin-stimulated glucose uptake in soleus muscle protein carbonyl content, a marker of oxidative stress that was blocked by NAC, as well as elevated levels of malondialdehyde and 4-hydroxynonenal, markers of lipid peroxidation, which were reduced by taurine. In contrast to findings after long-term hyperglycemia, there was no membrane translocation of novel isoforms of protein kinase C in skeletal muscle after 6 h. These data support the concept that oxidative stress contributes to the pathogenesis of hyperglycemia-induced insulin resistance.

**INSULIN RESISTANCE** is one of the earliest detectable predictors of type 2 diabetes (47, 51, 39) and, along with relative insulin deficiency (39, 56), strongly contributes to the development of overt hyperglycemia. Hyperglycemia is in large part responsible for a host of complications found in diabetic subjects (15, 83, 86) and can worsen insulin resistance (11, 38, 72, 73, 75). The effect of hyperglycemia per se to induce insulin resistance in vivo was first demonstrated by Rossetti et al. (75) in the partially pancreatectomized rat model, which is characterized by moderate fasting hyperglycemia, glucose intolerance, and normal fasting insulin levels. In that study, phlorizin was used to normalize plasma glucose without affecting insulin secretion. Use of a hyperinsulinemic-euglycemic clamp revealed that the decreased insulin-stimulated glucose utilization was completely normalized in the phlorizin-treated rats, indicating a direct role of glucose in the induction of insulin resistance. Several mechanisms have been proposed to mediate hyperglycemia-induced insulin resistance, including the hexosamine biosynthetic pathway (4, 31, 67, 74, 87) and protein kinase C (55, 68, 78). Oxidative stress, which is present in diabetic subjects (6, 24, 82) and has been suggested to contribute to the complications of diabetes associated with chronic hyperglycemia (6, 24), has recently been suggested to induce insulin resistance (64, 66). There is evidence to support a link between elevated plasma glucose and oxidative stress. Experimental induction of hyperglycemia resulted in elevated markers of oxidative stress (16, 36). In type 2 diabetic subjects, plasma malondialdehyde and oxidized LDL were increased, whereas total plasma antioxidant capacity was decreased during postprandial hyperglycemia (~16 mM for 2 h) after a carbohydrate meal (17, 18).

There is also evidence that oxidative stress can cause insulin resistance. Low levels of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), can be produced in the presence of hyperglycemia (28, 33, 37). In vitro studies using a variety of cell lines have demonstrated that micromolar concentrations of H₂O₂ can inhibit insulin signaling and glucose transport (10, 29, 76, 85). In human subjects, the efficacy of using antioxidant supplementation to improve insulin sensitivity remains unclear. Several studies of small numbers of diabetic subjects have shown that intravenous vitamin C (61), oral vitamin E (62, 63), or lipoic acid (34, 35) administration could improve insulin-mediated glucose utilization. However, in all these studies, the antioxidants were able to effect only a partial improvement of insulin sensitivity.

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The etiology of the insulin resistance in type 2 diabetes is multifactorial. Thus, whether antioxidants improve insulin sensitivity by correcting the hyperglycemia-induced component of insulin resistance has not been addressed. Recently, the antioxidant N-acetylcysteine (NAC) has been shown to exert a protective effect on the β-cell of diabetic db/db mice (40), Zucker diabetic fatty rats (81), and alloxan-induced diabetic CD1 mice (32). In these studies, NAC supplementation prevented hyperglycemia or improved glucose intolerance associated with the preservation of glucose-induced insulin secretion. Insulin sensitivity was measured in only one study, in the db/db mouse model of diabetes may be due to the complex etiology of the insulin resistance and associated abnormalities, e.g., leptin resistance and dyslipidemia (42).

The objective of this study was to ascertain whether oxidative stress participated in the pathogenesis of the insulin resistance induced by hyperglycemia in nondiabetic rats. To accomplish this, a previously established model of hyperglycemia-induced insulin resistance, namely, the rat infused with glucose for 6 h, was used (54). Insulin action was measured using the hyperinsulinemic-euglycemic glucose clamp technique, an established sensitive method to measure insulin sensitivity in vivo. Coinfusion of two different antioxidants, NAC and taurine, with glucose was tested to determine whether hyperglycemia-induced insulin resistance could be prevented. At the same time, soleus muscle protein carbonyl content and circulating levels of malondialdehyde and 4-hydroxynonenal, markers of oxidative stress, were measured in the absence and presence of high glucose with and without antioxidant coinfusion.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River Breeding Laboratories, St Constant, QC, Canada) weighing 250–350 g were housed individually. Rats were subjected to a standard 12:12-h light-dark cycle and were given free access to food and water. The Faculty Advisory Committee on Animal Services at the University of Toronto approved all procedures.

Surgery. Rats were anesthetized with ketamine HCl (85 mg/kg), xylazine (1 mg/kg), and acepromazine maleate (1 mg/kg). Indwelling catheters were inserted into the right internal jugular vein and left carotid artery, tunneled subcutaneously to the back of the neck, and exteriorized. Polyethylene catheters (PE-50; Cay Adams, Boston, MA) extended 0.5 cm with a segment of Silastic tubing (0.02 in. ID; Dow Corning, Midland, MI) were used for vascular catheterization. Catheters were filled with heparinized saline and flushed every second day to ensure patency. The jugular catheter was used for infusions and the carotid catheter was used for blood sampling. Rats were allowed to recover for 3–5 days before the experiment.

Preclamp infusions. All studies were performed in awake, unstressed rats fasted overnight. The animals were infused for 6 h with 1) saline (30 μl/min) (n = 5), 2) a variable infusion of 50% dextrose to maintain plasma glucose concentrations at ~15 mM (n = 6), 3) 50% dextrose, as above, + NAC (0.35 mg·kg⁻¹·min⁻¹; n = 5), or 4) saline + NAC (n = 5). NAC is often used for the treatment of acetaminophen overdose, and the standard dose used in those instances is not known to cause significant adverse effects (5, 70, 77). The rate of infusion of NAC in our experiment was just slightly higher than that used for the treatment of acetaminophen overdose in humans, although the total dose delivered over the 8-h experiment was approximately one-half. Because side effects of NAC are known to be dose dependent (5), and in preliminary studies identical results were obtained in the experiments to be described with or without an initial bolus dose, it was decided to forego the initial bolus dose (150 mg/kg). Blood samples were taken at 0, 2, 4, and 6 h for determination of plasma insulin and C-peptide levels.

A similar protocol was used in a second set of experiments, which tested the efficacy of the antioxidant taurine (2-aminoethanesulfonic acid) (1, 30). Taurine, similar to NAC, has been used intravenously in animal models to reduce lung injury (22), as well as in human subjects to decrease reperfusion lipid peroxidation-associated injury during myocardial ischemia reperfusion (53). Animals were infused with 1) saline, 2) 50% dextrose + taurine (0.35 mg·kg⁻¹·min⁻¹; n = 5), and 4) saline + taurine (n = 6).

After the 6-h infusion period, a subset of animals that did not undergo the euglycemic clamp were used to obtain soleus muscle samples for analysis of oxidative protein damage and for evidence of translocation of PKC isoenzymes. Animals were placed under surgical anesthesia with a titrated dose of pentobarbital sodium. Soleus muscle was removed and flash-frozen with aluminum clamps cooled in liquid nitrogen. Muscle samples were stored at −70°C until further analysis.

Hyperinsulinemic euglycemic clamp. Insulin sensitivity was assessed using a 2-h hyperinsulinemic euglycemic clamp immediately after the 6-h infusion period during which infusion of NAC was continued. Regular porcine insulin (180 mU/kg bolus and 18 mU·kg⁻¹·min⁻¹) was infused at 6 h (time 0) to maximally stimulate glucose uptake and to suppress endogenous glucose production. Clamp experiments were performed in combination with [3-H]glucose infusion (40 μCi bolus and 0.4 μCi/min) for determination of glucose production. Blood samples (30 μl) were collected every 5 min during the 2-h clamp for the immediate determination of plasma glucose. A 50% dextrose solution was infused at a variable rate to maintain euglycemia. Additional blood samples (100 μl) were taken at 90, 100, 110, and 120 min for determination of [3-H]glucose specific activity and at 60 and 120 min for plasma insulin levels. To avoid changes due to volume depletion, red blood cells were collected and rein infused in equal volumes of normal saline.

Assays. Plasma glucose was measured by the glucose oxidase method by use of a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Insulin was measured by radioimmunoassay with a kit (Linco) for rat insulin with 100% cross-reactivity with porcine insulin. Plasma [3-H]glucose radioactivity was measured in duplicate after deproteinization using Ba(OH)₂ and ZnSO₄ and evaporation to dryness. An aliquot of the tracer infusate was also run with the plasma sample.

Determination of protein oxidation. Muscle tissue was homogenized at 4°C in buffer (50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 0.5 mM sodium orthovanadate, 0.1% 2-mercaptoethanol, 1% Triton X-100, 50 mM NaF, 5 mM sodium pyrophosphate, 10 mM sodium glycerophosphate, 0.1 mM PMSF, and 1 μg/ml each of aprotinin, pepstatin, and leupeptin), and cell debris was removed by centrifugation.
Protein concentrations were determined using a standardized assay kit (Bio-Rad) with bovine serum albumin as the standard (14). Oxidative protein damage, assessed by the formation of carbonyl groups, was measured essentially as described by Levine et al. (46). One milligram of protein was precipitated by addition of 20% TCA and centrifuged (8,500 g) for 3 min, and the supernatant was discarded.

Protein pellets were washed with PBS (phosphate-buffered saline) and incubated with and without 2,4-dinitrophenyhydrazine (DNPH), 10 mM in 2 M HCl, and were allowed to stand at room temperature for 1 h, with vortexing every 15 min. After incubation, protein was reprecipitated using 20% TCA, and the pellet was obtained by centrifugation. The pellets were washed 3 times with ethanol-ethyl acetate (1:1) to remove free DNPH; the samples were allowed to stand for 10 min each time before the supernatant was discarded. The pellet was then dissolved in guanidine solution (6 M with 20 mM potassium phosphate, adjusted to pH 2.3 with trifluoroacetic acid) for 1 h at 37°C. Insoluble material was removed by centrifugation. Carbonyl content was calculated from the sample absorbance at 365 nm after subtraction of HCl-treated blanks by use of a molar absorption coefficient of 22,000 M/cm.

Malondialdehyde and 4-hydroxynonenal analysis. Samples were analyzed for malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) by a modification of a previously described method (49). Briefly, plasma aldehydes and 100 pmol of benzaldehyde-ring-D5 (internal standard) were derivatized by pentafluorobenzyl hydroxylamine hydrochloride (PFBA-HCl) to form the aldehydes-PFBA derivative. Plasma protein was precipitated by methanol, and aldehydes were separated by hexane extraction. The hydroxyl group of 4-HNE was further derivatized by 50 μl of N,N-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) at 60°C for 15 min. Finally, 50 μl of hexane were added to each sample, and 1 μl was analyzed by gas chromatography-mass spectrometry (GC-MS).

The Hewlett-Packard 5973N MSD GC-MS was equipped with an HP-5 ms capillary column (30 m length, 0.25 mm ID, 0.25 μm film thickness), and the mass spectrometry was performed in negative-ion chemical ionization with methane as the reagent gas.

Determination of membrane PKC. Activation of PKCs is associated with translocation of the enzyme from the cytoplasm to cell membranes, and usually, but not always, with a corresponding decrease in cytosolic content of the enzyme (9, 44, 58). Therefore, activation of novel PKCs was assessed by quantification of the enzyme content in the membrane fractions of soleus muscle samples.

Samples were homogenized in 4 volumes of STE buffer [320 mM sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM EGTA, 5 mM NaF, 10 mM β-mercaptoethanol, 40 μM leupeptin, 1 mM PMSF, 50 mM NaF, and 15 μg/ml aprotinin] and then centrifuged at 100,000 g for 10 min. The supernatant was then centrifuged at 100,000 g for 60 min. The pellet was resuspended in STE buffer containing 1% Triton X-100. Protein concentration was determined by the Lowry method, with bovine serum albumin as a standard.

The solubilized membrane fractions were added to 1 volume of 10% SDS, 13% glycerol, 300 mM Tris-HCl, 130 mM dithiothreitol, and 0.2% bromophenol blue (pH 6.8) and boiled for 5 min. Twenty micrograms of protein were separated by SDS-PAGE and transferred electrophoretically to a nitrocellulose membrane (Schleicher and Schuell). The membranes were blocked with 5% nonfat milk in TBST buffer [150 mM NaCl, 10 mM Tris-HCl (pH 7.4), and 0.1% Tween-20] for ≥1 h. Affinity-purified isofrom-specific polyclonal an-ti-PKC antibodies (Sigma) were diluted 1:1,000 in the blocking buffer, and membranes were incubated for 12 h at 4°C. The membranes were washed and incubated with horseradish-peroxidase-linked goat anti-rabbit IgG (Amersham, Baie d’Urfe, QC, Canada), diluted in 5% nonfat milk, for 1 h at room temperature. Membranes were again washed and then detected by an enhanced chemiluminescence method (Zymed Laboratories, San Francisco, CA) with exposure to Hyperfilm.

Insulin degradation. The reduced form of NAC contains an active sulfhydryl group that can bind to free S-H, forming a disulfide bond, or compete for and disrupt disulfide bonds, thus acting as a reducing agent (52). Because the structure of the intact insulin molecule is dependent on the presence of 3 disulfide bonds, the effect of NAC on immunoreactive insulin was determined. Insulin, 100 nmol, was added to DMEM in the presence of 0–10 mM NAC and incubated for 0–6 h at 37°C. Aliquots of medium were taken at 1-h intervals and frozen at −70°C, and insulin concentration was determined by RIA (Linco). For the in vivo infusion studies, 1 ml of whole blood was obtained from rats by following the protocols described above after 3–6 h of infusion with and without NAC (before the clamp). Insulin (100 nmol) was added to the blood samples, which were then incubated for 4 h at 37°C. Aliquots were taken at 0, 15, 30, 60, 120, and 240 min and centrifuged, and serum was frozen at −70°C for later determination of insulin by RIA, as described above.

Statistical analysis and calculations. Hepatic glucose production and glucose disappearance rates were calculated using steady-state equations (79). All values are expressed as means ± SE. Statistical significance was determined by analysis of variance (ANOVA) for repeated measurements followed by Tukey’s t-tests. Differences were considered significant at P < 0.05.

RESULTS

There were no differences between groups in the means of the body weights recorded on the day of the experiment. The average fasting plasma glucose values just before the infusions were 7.1 ± 0.2, 6.5 ± 0.2, 6.8 ± 0.3, and 6.9 ± 0.2 mM in the saline (n = 5), HG (n = 6), HG + NAC (n = 5), and NAC only (n = 5) groups [P = nonsignificant (NS); Fig. 1]. Fasting plasma insulin concentrations were similar in all groups (mean ± SE: SAL = 6.07 ± 0.34, HG = 6.12 ± 0.39, HG + NAC = 6.20 ± 0.42, NAC = 6.28 ± 0.41). Although plasma glucose values were similar, the NAC groups showed a lower insulin response compared to saline and HG (Fig. 2). The insulin responses were not significantly different in saline and HG. The glucose disappearance rate in the saline group was 0.18 ± 0.04 mg/min/kg, whereas in the HG group it was 0.16 ± 0.02 mg/min/kg (P = 0.04). The liver glucose disposal rate in the saline group was 0.27 ± 0.04 mg/min/kg, whereas in the HG group it was 0.20 ± 0.02 mg/min/kg (P = 0.02). The HG + NAC group showed a trend toward a reduced glucose disposal rate compared to the HG group (0.19 ± 0.02 mg/min/kg; P = 0.08). The NAC group showed a significant reduction in the hepatic glucose disposal rate compared to the HG group (0.16 ± 0.02 mg/min/kg; P = 0.01).

Fig. 1. Effect of high glucose (HG) and N-acetylcysteine (NAC) on glucose levels. Rats were infused with saline (SAL), glucose to achieve a concentration of 15 mM (HG), HG + NAC, or NAC alone for 6 h (0–360 min) followed by a hyperinsulinemic-euglycemic clamp over 2 h (360–480 min), as described in MATERIALS AND METHODS. Plasma glucose was measured at the times indicated.
values for the saline, HG, HG + NAC, and NAC only groups were 92.2 ± 14.4, 68.8 ± 6.0, 64.6 ± 5.0, and 87.6 ± 8.4 pmol/l (P = NS; Fig. 2).

During the 6-h infusion period, plasma glucose was elevated to ~15 mM in both the HG and HG + NAC groups (P < 0.001 vs. saline and NAC only groups), and there were no significant differences between these two groups (Fig. 1). Additionally, the total amount of glucose delivered during the infusion period to maintain the 15 mM concentration was essentially identical between the HG and HG + NAC groups (13,294 ± 935 and 13,277 ± 766 mg/kg, respectively).

Plasma insulin was measured every 2 h during the infusion period, and as expected, it remained constant in the saline and NAC only groups (119 ± 27 and 111 ± 18 pmol/l). In response to the glucose infusion, plasma insulin levels were increased in the HG group (1,357 ± 188 pmol/l; P < 0.001 vs. all other groups) and HG + NAC group (695 ± 106 pmol/l; P < 0.001 vs. all other groups). There was a difference noted between the HG and HG + NAC groups for plasma insulin (P < 0.001; Fig. 2). C-peptide levels after 6 h of infusion were 226 ± 0.6, 2,550 ± 135, 1,670 ± 523, and 336 ± 163 pmol/l for the saline, HG, HG + NAC, and NAC only groups. C-peptide levels for the HG and HG + NAC groups were significantly greater than for control (P < 0.01) and different from each other (P < 0.05). Infusion of NAC alone did not alter C-peptide (P = NS).

During the hyperinsulinemic-euglycemic clamp, steady-state plasma glucose and insulin values were similar in all four groups (Figs. 1 and 2). Glucose infusion rate (GINF) for the HG group (31.1 ± 0.6 mg·kg⁻¹·min⁻¹) was ~20% lower than that of the saline group (37.8 ± 2.8 mg·kg⁻¹·min⁻¹; P < 0.05). This decrease in GINF was completely prevented by coinfusion of NAC (41.8 ± 3.0 mg·kg⁻¹·min⁻¹). GINF in the NAC only group (42.9 ± 4.5 mg·kg⁻¹·min⁻¹) was not different from saline. The rate of disappearance (Rd) of glucose is equal to the GINF plus endogenous glucose production (Rd) and, during hyperinsulinemic clamp conditions, reflects primarily glucose utilization by muscle (19). Six hours of glucose infusion (HG) induced a significant decrease in Rd (32.4 ± 1.9 mg·kg⁻¹·min⁻¹) compared with saline (41.2 ± 1.7 mg·kg⁻¹·min⁻¹) and NAC only (42.3 ± 3.8 mg·kg⁻¹·min⁻¹; P < 0.05, HG vs. all other groups). Coinfusion of NAC with glucose completely prevented this decrease (45.9 ± 3.5 mg·kg⁻¹·min⁻¹; Fig. 3). There were no significant differences in EGP (endogenous Rd) (mg·kg⁻¹·min⁻¹; saline = 1.6 ± 0.8; HG = −0.6 ± 1.4; HG + NAC = 2.9 ± 0.6; NAC only = 2.2 ± 1.8, P = NS; Fig. 3B). EGP was almost completely suppressed in each group because of the high insulin levels present during the clamp.

The efficacy of the antioxidant NAC to inhibit the hyperglycemia-induced insulin resistance suggested a potential role for oxidative stress in mediating the action of high glucose. To determine whether high glucose combined with the stimulated endogenous hyperinsulinemia caused oxidative stress in the major insulin target tissue, skeletal muscle, protein oxidation as assessed by DNP binding to carbonyl groups in soleus muscle was measured. The presence of protein carbonyl groups has been demonstrated to reflect elevated levels of ROS (8, 71). Protein carbonyls were raised ~5-fold (P < 0.001) after 6 h of glucose infusion compared with the saline-infused group. This increase was completely prevented by coinfusion of NAC in the HG + NAC group (Fig. 4).
Although these data suggested an essential role for oxidative stress in hyperglycemia-induced insulin resistance, NAC may have had other actions. It is a known precursor of glutathione (GSH), and GSH may act as a cofactor in a number of enzyme reactions. Most relevant to hyperglycemia is the glutathione pathway, which is responsible for metabolism of reactive triose phosphate-derived 3-carbon intermediates such as methylglyoxal (6). Because methylglyoxal has been implicated in the formation of advanced glycation end products and diabetes complications (7, 84), the reduction of this dicarbonyl intermediate by NAC could not be excluded as an alternate possibility. We therefore utilized a second antioxidant, taurine, to determine whether hyperglycemia-induced insulin resistance was prevented. Taurine was chosen not only because it has been used as an antioxidant in a variety of settings (see review in Ref. 30), but also because, whereas NAC can prevent the high glucose-induced increase in 4-hydroxynonenal and malondialdehyde by coinfusion of taurine (Table 2).

To determine that HG infusion did promote oxidative stress and that taurine infusion, under these conditions, acted as an antioxidant, the lipid oxidative stress markers MDA and 4-HNE (28, 49) were assayed. Both were significantly elevated by 6 h of HG infusion and were normalized (MDA) or markedly reduced (4-HNE) by coinfusion of taurine (Table 2).

Table 2. Taurine prevents the high glucose-induced increase in 4-hydroxynonenal and malondialdehyde

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<th>SAL</th>
<th>HG</th>
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<tr>
<td>4-Hydroxynonenal (nM)</td>
<td>94 ± 12</td>
<td>1151 ± 463*</td>
<td>147 ± 12</td>
<td>117 ± 15</td>
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<tr>
<td>Malondialdehyde (nM)</td>
<td>86 ± 12</td>
<td>840 ± 165†</td>
<td>78 ± 7</td>
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Values are means ± SE in nM obtained at the end of 6-h infusions. Abbreviations are as in Table 1. Values of n for SAL, HG, HG + TAU, and TAU are 5, 6, 5, and 6. *P < 0.05, HG vs. SAL or TAU; †P < 0.01, HG vs. all other groups.
METHODS. There was no effect of HG or HG insulin resistance. Consistent with previous results both in vivo (11, 38, 75) and in vitro (23, 50, 57) to cause residual insulin after 1 h, in fact, there was a tendency to detect a greater amount of B rats after 3 h of incubation at 37 °C in the presence of NAC, but it was not statistically significant.

DISCUSSION

Chronic hyperglycemia has been well documented both in vivo (11, 38, 75) and in vitro (23, 50, 57) to cause insulin resistance. Consistent with previous results (54), we found that, as assessed by the hyperinsulinemic euglycemic clamp, even a relatively short-term exposure to high glucose, 6 h, in vivo, resulted in a reduced sensitivity to insulin of ~25%. Under the clamp conditions employed, hepatic glucose production was completely suppressed, and insulin-stimulated glucose disposal was predominantly into muscle, indicating that insulin resistance was induced in skeletal muscle. The role of the associated endogenous hyperinsulinemia was not directly assessed here, but previous studies suggest that increased cellular entry of glucose is necessary to induce insulin resistance (50, 72). Thus perfusion of rat hindlimb with 12 mM glucose for 5 h decreased maximum insulin-stimulated glucose uptake by 54%, and addition of high insulin (20 U/ml) to the infusion augmented this inhibition to 67% (72). However, insulin alone did not induce a defect.

The mechanism by which hyperglycemia causes insulin resistance is not completely understood. Exposure to an elevated glucose concentration of a number of tissues that take up glucose independently of insulin has been shown to result in increased cellular glucose entry and metabolism (15, 44, 80) and to be associated with oxidative stress (6, 15, 16, 28, 33). The latter has been documented by measurement of ROS, e.g., H2O2 (33, 37), intracellular concentrations of GSH (reduced glutathione), levels of antioxidant enzymes (glutathione peroxidase, glutathione reductase, and superoxide dismutase), and resultant oxidative changes to protein, lipid, and DNA (6, 36, 66, 82). There is likely more than one mechanism by which increased glucose generates ROS. Although glucose autoxidation has been well documented and in the presence of redox cycling metal ions (Fe2+/3+, Cu1+/2+) can cause protein oxidation, this requires long-term exposure to high glucose. On the other hand, glucose metabolism results in the formation of reactive 3-carbon metabolites such as glyoxal and methylglyoxal, which, by more rapid nonenzymatic protein glycation, may generate ROS and protein carbonyls (6, 33, 37, 84). Another source of ROS may be the mitochondria. In endothelial cells, exposure to high glucose was found to cause elevated ROS, which was inhibited by blocking mitochondrial complex III and IV of the electron transport chain (15). It was postulated that the oxidative stress generated was reflected in the

Fig. 5. Effect of NAC on immunoreactive insulin in vitro and in vivo. A: insulin, 100 nM, was added to DMEM in the presence of the indicated concentrations of NAC and incubated for 0–6 h at 37°C. Aliquots were removed at 1-h intervals and insulin assayed. B: insulin, 100 nM, was added to 1 ml of whole blood taken from rats that had been infused as described in Fig. 1 for 180–360 min and then incubated at 37°C for a further 4 h. Aliquots were removed at the indicated times, and insulin was assayed. High concentrations of NAC added in vitro rapidly and significantly decreased immunoreactive insulin (A), but infusion of NAC as described in vivo had no effect to decrease insulin in whole blood (B).
cytosol and inhibited glyceraldehyde-3-phosphate dehydrogenase. In the present study, evidence of oxidative stress after 6 h of hyperglycemia was observed in skeletal muscle, an insulin target tissue, manifested by a markedly increased level of protein carbonyls. Furthermore, this was inhibited by confi...dation by the HG infusion, manifested by significantly elevated circulating levels of MDA and the more specific 4-HNE, which was inhibited by the antioxidant taurine. Importantly, the coinfusion of NAC or taurine with HG also inhibited the insulin resistance. Consistent with these observations, we have recently found that exposure of rat adipocytes in primary culture to high levels of glucose combined with insulin resulted in the increased generation of ROS, which was prevented by preincubation with NAC (48). These data suggest, but do not prove, that oxidative stress caused, or at least contributed to, the insulin resistance induced by high glucose.

There is previously published evidence suggesting that oxidative stress may play such a role. First, direct exposure of cultured cells, 3T3-L1 adipocytes, and L6 muscle cells to H$_2$O$_2$ has been reported to cause insulin resistance (10, 29, 76, 85). In addition, several in vivo studies have reported a partial improvement of insulin resistance in subjects with type 2 diabetes mellitus by oral administration of vitamin E (63), intravenous administration of vitamin C (61), or intravenous GSH (65). These were all small studies; clearly, further work is required to firmly establish this relationship in both rodent models and human subjects.

The mechanism by which oxidative stress may contribute to insulin resistance is not clear. Exposure of cells to H$_2$O$_2$ will activate numerous signaling systems, including PKC (43), MAPK family enzymes (10, 27), and the small G protein Ras (60, 69). Of these, the PKCs have been previously implicated as mediators of insulin resistance (9, 12, 41, 68). However, we were not able to demonstrate membrane translocation of any novel PKC isoforms in the muscle tissue from the insulin-resistant rats. This may be due to the short duration of hyperglycemia, as some studies using longer (24–96 h) glucose infusion in normal rats (45) and studies of chronic hyperglycemia in diabetic rats (3) reported translocation of PKC-ε and of PKC-α, -β, -δ, and -ε, respectively, in soleus muscle. Although the lack of membrane translocation suggests that PKC is not involved in this short-term model, this possibility cannot be entirely dismissed, as translocation of conventional PKC isoforms was not evaluated and PKC activation in the absence of translocation has been reported (58).

The mechanism by which NAC inhibits the induction of insulin resistance appears to be its antioxidant properties (2, 20). However, NAC provides cysteine for and promotes the synthesis of GSH (20). GSH has been found to have cellular functions unrelated to its antioxidant activity, such as serving as a cofactor in the glyoxalase-catalyzed metabolism of methylglyoxal (6). However, the finding that taurine, which cannot be converted to cysteine and GSH, was also effective supports an antioxidant mechanism. The end product of the glyoxalase pathway is d-lactate (6, 7). In preliminary studies, measurements of circulating d-lactate in the control and HG- and HG + taurine-infused groups at the end of 6 h were all undetectable (<0.3 mM). Of interest, NAC has also been found to protect β-cells in culture and in vivo from “glucose toxicity,” preserving insulin synthesis and secretion (40, 81). Oxidative stress may thus play a role in the dual abnormalities of insulin action and insulin secretion found in type 2 diabetes mellitus.

Finally, we noted a somewhat decreased insulin concentration in the rats infused with glucose combined with NAC compared with those receiving glucose alone. Because a previous report demonstrated that high concentrations of a-SH group containing antioxidants such as NAC and lipoic acid could decrease immunoreactive insulin (25), presumably by disrupting disulfide bonds, we determined whether this might have occurred during the infusions of NAC. In contrast to in vitro results, which showed a rapid and significant effect of >1 mM NAC on insulin concentrations, there was no effect of the NAC present in the circulation of the infused rats (Fig. 5). This was consistent with a much lower estimated concentration of NAC achieved with this infusion protocol. On the basis of clearance values determined in humans (0.11 to 0.207 1·h$^{-1}$·kg$^{-1}$), and with the assumption that clearance rates in rats would be similar or even higher, the maximum concentration at steady state during our infusions was estimated to range between 0.66 and 1.25 mM (13, 20, 59). Furthermore, in contrast to insulin, NAC in vitro did not decrease immunoreactive C-peptide (data not shown). Taken together, the data suggest that the lower insulin concentration in the presence of NAC reflects the state of increased insulin sensitivity observed in this group.

In summary, this study demonstrates that NAC and taurine prevent short-term hyperglycemia-induced insulin resistance in vivo. This model of insulin resistance is associated with increased oxidative stress, which is concomitantly inhibited by both antioxidants. The data support the hypothesis that oxidative stress contributes to the pathogenesis of insulin resistance.

We thank Mehrdad Yazdanpanah, Neelam Khaper, and Dr. Peter Liu for help with the assays of MDA and 4-HNE.

DISCLOSURES

This work was supported by a grant (MOP-38009) from the Canadian Institutes for Health Research to I. G. Fantus and from the Canadian Diabetes Association to A. Giacca. Z. Yu was supported by a postdoctoral fellowship from the Canadian Diabetes Association. C. A. Haber was supported in part by a University of Toronto Open Studentship and an Ontario Graduate Studentship in Science and Technology.

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