β-Blockade, but Not Normoglycemia or Hyperinsulinemia, Markedly Diminishes Stress-Induced Hyperglycemia in Diabetic Dogs

Shirya Rashid, Zhi Qing Shi, Masataka Niwa, Julian M.R. Mathoo, MayLiza Vandelangeryt, Debra Bilinski, Gary F. Lewis, and Mladen Vranic

Stress-induced hyperglycemia can lead to significant deterioration in glycemic control in individuals with diabetes. Previously, we have shown in normal dogs that, after intracerebroventricular (ICV) administration of carbachol (a model of moderate stress), increases in both the metabolic clearance rate (MCR) of glucose and endogenous glucose production (GP) occur. However, in hyperglycemic diabetic dogs subjected to the same stress, the MCR of glucose does not increase and glycemia therefore markedly deteriorates because of stimulation of GP. Our aims were to determine the following: *1*) whether insulin-induced acute normalization of glycemia, with or without β-blockade, would correct glucose clearance and prevent the hyperglycemic effect of stress, and *2*) whether hyperinsulinemia per se could correct these abnormalities. Stress was induced by ICV carbachol in 27 experiments in five alloxan-administered diabetic dogs subjected to the following protocols in random order: *1*) basal insulin infusion (BI) to restore normoglycemia; *2*) basal insulin infusion with β-blockade (BI+block); *3*) normoglycemic-hyperinsulinemic clamp with threefold elevation of insulin above basal (3× BI); and *4*) normoglycemic-hyperinsulinemic clamp with fivefold elevation of insulin above basal (5× BI). The BI+block protocol fully prevented stress-induced hyperglycemia, both by increasing MCR (ΔMCR at peak: 0.72 ± 0.25 ml · kg^{-1} · min^{-1} vs. no change in BI, $P < 0.05$) and by diminishing the stress-induced increment in GP observed in BI (ΔGP at peak: 3.72 ± 0.09 μmol · kg^{-1} · min^{-1} for BI+block vs. 14.10 ± 0.31 μmol · kg^{-1} · min^{-1} for BI, $P < 0.0001$). In contrast, 3× BI and 5× BI treatments with normoglycemic-hyperinsulinemic clamps proportionately increased basal MCR at baseline, but paradoxically were not associated with an increase in MCR in response to stress, which induced a twofold increase in GP. Thus, in alloxan-administered diabetic dogs, stress increased GP but not MCR, despite normalization of glycemia with basal or high insulin. In contrast, β-adrenergic blockade almost completely restored the metabolic response to stress to normal and prevented marked hyperglycemia, both by limiting the rise in GP and by increasing glucose MCR. We conclude that acute normalization of glycemia with basal insulin or hyperinsulinemia does not prevent hyperglycemic effects of stress unless accompanied by β-blockade, and we speculate that short-term β-blockade may be a useful treatment modality under some stress conditions in patients with diabetes. *Diabetes* 49:253–262, 2000

The increased demand for glucose by the brain, heart, and other tissues during stress necessitates adaptive alterations in glucose metabolism. In many forms of stress, the rate of glucose production rises above the rate of glucose clearance, resulting in hyperglycemia. It is well recognized that stress induces a greater derangement in glucose regulation in patients with diabetes than in nondiabetic individuals. Consequently, in diabetes there can be an exaggerated hyperglycemic response to stress, which has been found to increase the risk of ensuing complications that range from infections (1) to fluid and electrolyte imbalances and, in extreme cases, ketoacidosis (2).

Several experimental models have been used to examine the effect of hormonal changes on glucoregulation during stress (3–6). Peripheral epinephrine infusion has been used in many studies to replicate part of the hormonal and glucoregulatory responses during moderate to severe stress (7,8). However, studies utilizing this model of stress do not replicate the central activation of neural mechanisms, including the stimulation of the hypothalamo-pituitary-adrenal and sympathetic axes, characteristic of the stress response. Among the centrally administered bioactive agents that induce a stress response, the hormonal and glucoregulatory responses to the acetylcholine analogue, carbachol, have been characterized in both diabetic and nondiabetic conditions (9,10). The increases in counterregulatory hormones induced by carbachol are similar to those observed in the clinical setting and during exercise, and carbachol-induced stress thus provides a general model of acute moderate stress.

In a previous study (9), we found that stress induced with intracerebroventricular (ICV) carbachol produced only a minimal 5% change in glycemia in normal dogs, since the increment in glucose clearance nearly matched the incre-

From the Departments of Physiology (S.R., Z.Q.S., M.N., J.M.R.M., M.Va., D.B., M.Vr.), Medicine (G.F.L., M.Vr.), and Surgery (Z.Q.S.), Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada.

Address correspondence and reprint requests to Mladen Vranic, MD, DSc, FRCP, FRSC, Professor of Physiology and Medicine, University of Toronto, Room 3358, Medical Sciences Bldg., 1 King's College Cir., Toronto, ON, Canada M5S 1A8. E-mail: mladen.vranic@utoronto.ca.

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ANOVA, analysis of variance; BI, basal insulin infusion; BI+block, basal insulin infusion and β-adrenergic blockade; CV, coefficient of variation; FFA, free fatty acid; 5× BI, fivefold elevation of insulin above basal; GINF, glucose infusion rate; GP, rate of endogenous glucose production; GU, rate of glucose uptake/utilization; ICV, intracerebroventricular; ID, inside diameter; MCR, metabolic clearance rate; SA, 3-[^{3}H] plasma glucose specific activity; 3× BI, threefold elevation of insulin above basal.

ment in glucose production. This finding is consistent with some types of stress characterized by increased metabolic demand in normal individuals (e.g., sepsis, burn, and trauma) (11). In contrast, ICV carbachol produced an exaggerated sixfold-greater hyperglycemic response in insulin-infused, but persistently hyperglycemic, alloxan-administered diabetic dogs in comparison with normal dogs, despite similar glucose production rates in both groups (9). The main cause of the augmented hyperglycemia was that the increase in glucose clearance observed in normal dogs during carbachol stress was abolished in the diabetic dogs. Since glucose clearance failed to increase despite 40% higher peripheral venous insulin levels in the insulin-infused diabetic dogs, insulin resistance and/or relative insulin deficiency during stress may have contributed to the attenuated glucose clearance. The question remained whether acute restoration of normoglycemia before induction of stress or higher levels of insulin replacement would correct the defect in glucose clearance seen in response to stress in the diabetic dogs.

Elevated levels of catecholamines can aggravate underlying insulin resistance during stress in diabetes and can antagonize factors that increase glucose clearance, primarily through β-adrenergic–mediated effects at muscle and adipose tissue. Catecholamines may exert their β-adrenergic inhibitory effects on glucose clearance directly at muscle and adipose tissue, or indirectly through β-adrenergic stimulation of adipocyte lipolysis (12). In fact, after ICV carbachol administration, stress-induced increases in plasma free fatty acids (FFAs), glycerol, and lactate are markedly augmented in diabetic dogs (9), suggesting excessive catecholamine-induced β-adrenergic stimulation of lipolysis and muscle glycogenolysis. Excessive lipolysis and muscle glycogenolysis can antagonize glucose utilization (13–16).

We hypothesized that β-adrenergic effects of catecholamines in insulin-deficient states may play an important role in the failure of glucose clearance to rise during stress. Accordingly, the objectives of the present studies were to determine whether the normal stress-induced rise in glucose clearance could be restored in alloxan-administered diabetic dogs by the following: *1*) acute normalization of glycemia with a basal insulin infusion (BI); *2*) blockade of catecholamine β-adrenergic effects; and *3*) hyperinsulinemia per se.

RESEARCH DESIGN AND METHODS

Protocol for diabetic dogs. Five male mongrel dogs (22–34 kg) were selected for experiments. After completion of a 3-week conditioning period, the dogs were injected with alloxan (Sigma Chemicals, St. Louis, MO) intravenously (65 mg/kg) to induce diabetes. After a minimum of 2 weeks of recovery from the administration of alloxan, surgery was performed with the administration of general anesthetic (nitrous oxide and fluothane) for stereotaxic placement of an ICV stainless 24-gauge guide cannula into the third ventricle of the brain and for placement of vascular infusion and sampling catheters (9). The portal vein was cannulated via a branch of the splenic vein using a Silastic catheter (1.0-mm inside diameter [ID]) (Dow Corning, Midland, MI) for insulin infusion. A Silastic catheter (1.0-mm ID) was inserted into the aortic arch for blood sampling. Three Silastic catheters (0.76-mm ID) were placed in the superior vena cava via a jugular vein for infusion of tracer, glucose, and propranolol. All catheters were tunneled subcutaneously and exteriorized at the back of the neck. The catheters were filled with heparin (1,000 U/ml, Hepalean; Organon, Toronto) and were kept patent by flushing them every 4–5 days with saline. The dogs were fed once daily with a diet consisting of dry chow (25% protein, 9% fat, 4% fiber, and 12% moisture; Purina Mills, St. Louis, MO) mixed with canned meat (Dr. Ballard-Champion, 6.5% protein, 2.0% fat, 3.0% fiber, and 76.0% moisture; Friskies, Don Mills, ON, Canada) to maintain a constant body weight. At the time of feeding, the diabetic dogs were treated with subcutaneous injections of intermediate-acting isophane porcine insulin suspension (NPH; Eli Lilly, Indianapolis, IN) and short-acting (regular) porcine insulin (Eli Lilly) to maintain morning blood glucose between 8 and 14 mmol/l (moderate postabsorptive hyperglycemia). Therefore, these partially insulin-deficient alloxan-administered diabetic dogs represent a model of chronic suboptimally controlled type 2 diabetes (17).

The dogs were given at least 2 weeks to recover from surgery before the first experiment was performed and a minimum of 1 week was allowed between successive randomly performed experimental protocols. Before the experiments, the hematocrit levels (>0.35) of the dogs were monitored to ensure that only healthy dogs were used for experimentation. The NPH and regular insulin dose was adjusted before the start of experiments to ensure enough residual insulin to prevent ketogenesis, yet still ensure sufficient insulinopenia to allow for plasma glucose levels of an average of 16 mmol/l the following morning. To ensure that the diabetic dogs were in the postabsorptive state, food was withdrawn 16–18 h before each experiment. The last NPH and regular insulin injections (NPH, 20 U; regular, 10 U) were administered ~24 h before the start of the experiment. The mean morning plasma glucose for all experiments was 16 ± 1 mmol/l.

In all protocols, hyperglycemia was acutely normalized over a time span of 1.4 ± 0.2 h on the morning of the study with a peripheral infusion of regular insulin (prepared in a saline solvent containing ~3% vol/vol of the dog's own plasma), which was initially begun at a high rate (11.7 ± 3.9 mU · kg^{-1} · min^{-1}), and then gradually reduced as normoglycemia was achieved. The insulin was then infused at a fixed dose according to the particular protocol. In all protocols, plasma glucose was maintained at a constant level of ~5 mmol/l (normoglycemia) for ~3 h after normalization of blood glucose and before the baseline sampling period began. Within that period, a priming bolus (25 μCi) and constant infusion (0.25 μCi/min) of high-performance liquid chromatography–purified 3-[^{3}H]glucose tracer (New England Nuclear, Boston, MA) were begun for assessment of glucose turnover. Each experiment consisted of a tracer equilibration period (–160 to –40 min), a baseline sampling period (–40 to 0 min), and a stress period (0–180 min).

Four experimental protocols were performed in random order. Protocols 1 and 2 compared the effects with or without β-blockade on stress response at matched basal insulin concentrations. Protocols 3 and 4 compared the effects of two different levels of hyperinsulinemia on the glucoregulatory response to stress. The four protocols were as follows: *1*) insulin infusion at a basal rate (0.59 ± 0.09 mU · kg^{-1} · min^{-1}) to achieve normoglycemia ([BI]; n = 7 experiments); *2*) basal rate insulin infusion (0.59 ± 0.06 mU · kg^{-1} · min^{-1}) with β-adrenergic blockade (BI+block; n = 7). In this experiment, propranolol (Ciba-Geigy Canada, Mississauga, ON, Canada) was started 60 min before carbachol administration and continued throughout the experiment at a constant rate (5 μg · kg^{-1} · min^{-1}) to provide extensive adrenergic blockade; *3*) high rate (threefold elevation of insulin above basal [3× basal]) insulin infusion (1.7 ± 0.19 mU · kg^{-1} · min^{-1}; 3× BI; n = 8); and *4*) higher rate (fivefold elevation of insulin above basal [5× basal]) insulin infusion (3.3 ± 0.48 mU · kg^{-1} · min^{-1}; 5× BI; n = 5). For protocols 3 and 4, a 20% dextrose solution was infused and the rate of the dextrose infusion adjusted every 5 min according to blood glucose measurements to maintain normoglycemia. At time 0 in all protocols, stress was induced with a 5-μg ICV injection of carbachol through a 24-gauge injection cannula connected to a 100-μl microsyringe (Hamilton, Reno, NV) via polyethylene tubing (Intramedic PE 50; Clay Adams, Palisades Park, NJ). The guide cannula retaining screw was loosened to allow the injection needle to pierce the septum and extend to ~1 mm beyond the guide cannula.

Arterial blood samples were taken at times –40, –20, –30, –10, 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, and 180 min for determination of glucose and 3-[^{3}H] plasma glucose specific activity (SA) and at times –40, –20, 0, 10, 20, 40, 60, 120, and 180 min for hormone, metabolite, and catecholamine measurements. Blood samples for determination of plasma glucose, glucose SA, insulin, cortisol, lactate, and glycerol were collected in polypropylene tubes containing heparin (50 U/ml) and NaF (1–2 mg per tube). Samples for determination of plasma glucagon and FFA concentrations were collected in tubes containing 0.1 ml aprotinin (Trasylol; FBA Pharmaceuticals, New York) and 0.1 ml EDTA (BDH Chemicals, Toronto, Ontario, Canada). All blood was stored at 4°C and centrifuged within 60 min to separate plasma. Plasma samples were stored at –20°C, except the plasma for catecholamine assays, which was stored at –70°C.

Glucose turnover calculations. The rates of endogenous glucose production (GP) and glucose uptake/utilization (GU) were calculated with previously validated equations for non–steady-state conditions (18). Modified equations for GP and GU were used for the protocols with normoglycemic clamps to account for the added source of cold glucose infusion. In the normoglycemic clamp protocols, the glucose infusion rate (GINF) was subtracted from the total rate of glucose appearance to solve for the endogenous hepatic GP (19). The metabolic clearance rate (MCR) of glucose is a measurement of GU, partially corrected for the mass effect of glucose (MCR = GU/plasma glucose). The validity and meaning of this measurement have been reviewed (17). Data for SA and plasma glucose concentration were smoothed according to the Optical Segments program (20).

Laboratory methods. Plasma glucose concentrations were measured by a glucose oxidase method using a Beckman Glucose Analyzer II (Beckman, Fullerton,

CA). Glucose specific activity was derived from the plasma glucose concentration and 3-[^{3}H]glucose radioactivity, determined in duplicate assays as previously described (21). Insulin and glucagon (Diagnostic Products Corporation, Los Angeles) were measured by radioimmunoassays (coefficient of variation [CV] 16 and 13%, respectively). The catecholamines and cortisol were assessed using in-house radioenzymatic assays (CV = 21 and 12%, respectively). FFAs were measured by a microfluorometric procedure (22). Lactate and glycerol were determined with enzymatic microfluorometric methods (23).

Statistical analysis. Statistical analysis to assess differences between treatments was performed using one-way analysis of variance (ANOVA), corrected for the mean of the values immediately preceding the stress period (i.e., the baseline period). Statistical difference between and within the treatment protocols refers to the 40-min baseline period and the 180-min stress period, unless otherwise indicated. Changes in GP, GU, MCR, hormones (insulin, glucagon, norepinephrine, epinephrine, and cortisol), and metabolites (lactate, FFA, and glycerol) between protocols during stress were calculated with use of delta (Δ) values to account for differences in baseline levels. In all tests, significance was presumed at $P < 0.05$. Calculations were performed using the Statistical Analysis System (SAS Institute, Cary, NC). All data are expressed as means ± SE.

RESULTS

For clarity, data will be presented as two separate studies. Study 1 compared the effect of β-blockade on the glucoregulatory response to stress at matched basal insulin concentrations. In this study, we compared the BI protocol with the BI+block protocol. Study 2 compared the effect of hyperinsulinemia at two different levels on the glucoregulatory response to stress. In study 2, we compared the 3× BI and 5× BI infusion protocols.

Study 1: the effect of β-blockade on the glucoregulatory response to stress at matched basal insulin concentrations

Plasma glucose concentration and turnover. As can be seen in Fig. 1*A*, there was no difference in baseline plasma glucose between protocols (5.20 ± 0.09 vs. 5.32 ± 0.10 mmol/l in BI vs. BI+block, respectively). In the BI protocol, stress resulted in a marked and sustained hyperglycemic response (peak plasma glucose: 10.04 ± 0.47 mmol/l) that was twofold higher ($P < 0.0001$) than prestress levels. This rise in blood glucose during stress was much less ($P < 0.001$ vs. BI) in the BI+block protocol (peak plasma glucose: 6.36 ± 0.30 mmol/l, $P < 0.0001$).

Both basal GP (Fig. 1*B*) and GU (Fig. 1*C*) were higher in BI+block versus BI (GP: 15.32 ± 0.89 vs. 12.82 ± 0.67 μmol · kg^{-1} · min^{-1}, respectively, $P < 0.01$) (GU: 15.50 ± 0.83 vs. 10.82 ± 0.50 μmol · kg^{-1} · min^{-1}, respectively, $P < 0.0001$). In BI, the ICV injection of carbachol resulted in a rapid and significant

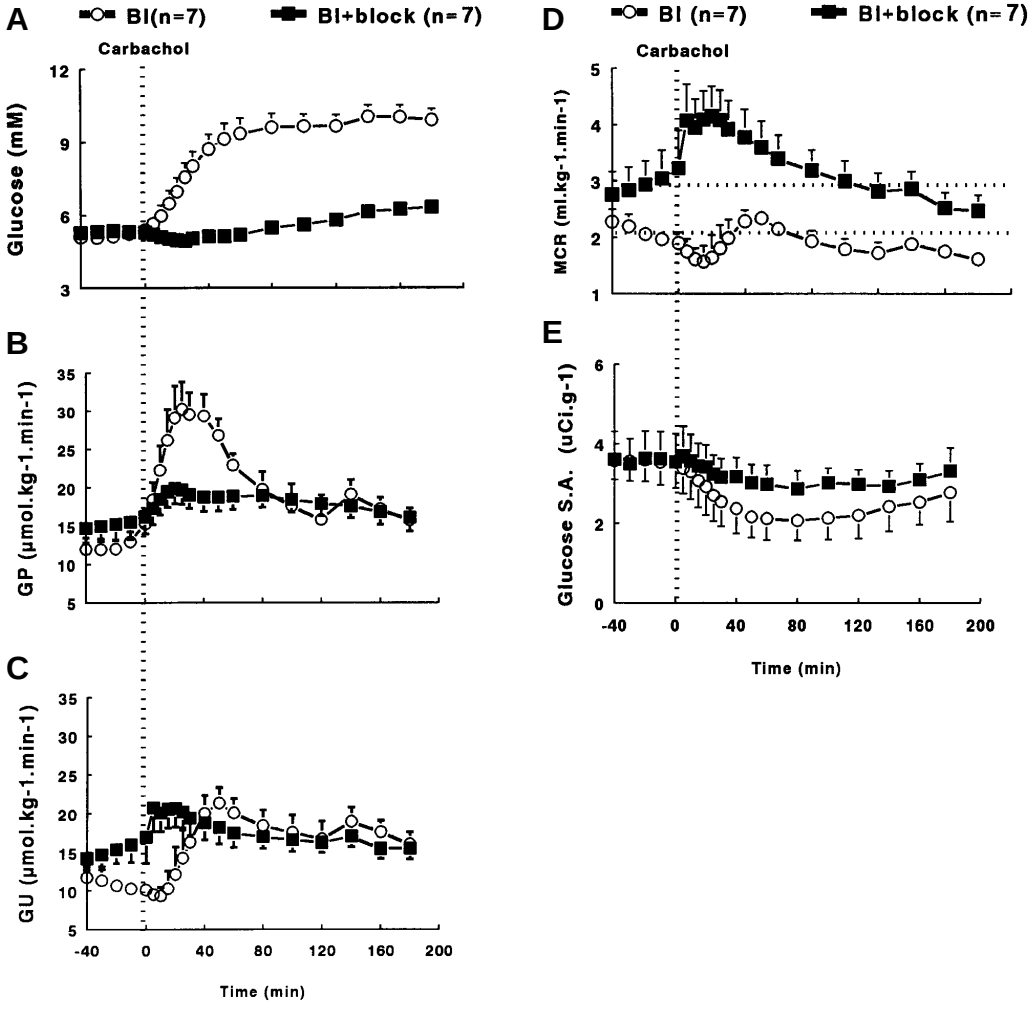

FIG. 1. Plasma glucose concentrations (*A*), GP (*B*), GU (*C*), MCR (*D*), and glucose specific activity (glucose SA) (*E*) in alloxan-administered diabetic dogs before and after stress induced by ICV carbachol injection (5 μg at time 0). Experiments were conducted in two protocols studied under acutely induced normoglycemia with insulin (0.6 ± 0.1 mU · kg^{-1} · min^{-1}, BI, ○) and with insulin (0.6 ± 0.1 mU · kg^{-1} · min^{-1}) plus the addition of the β-blocker, propranolol (5 μg · kg^{-1} · min^{-1}, BI+block, ■). Values are presented as means ± SE from seven experiments in each protocol.

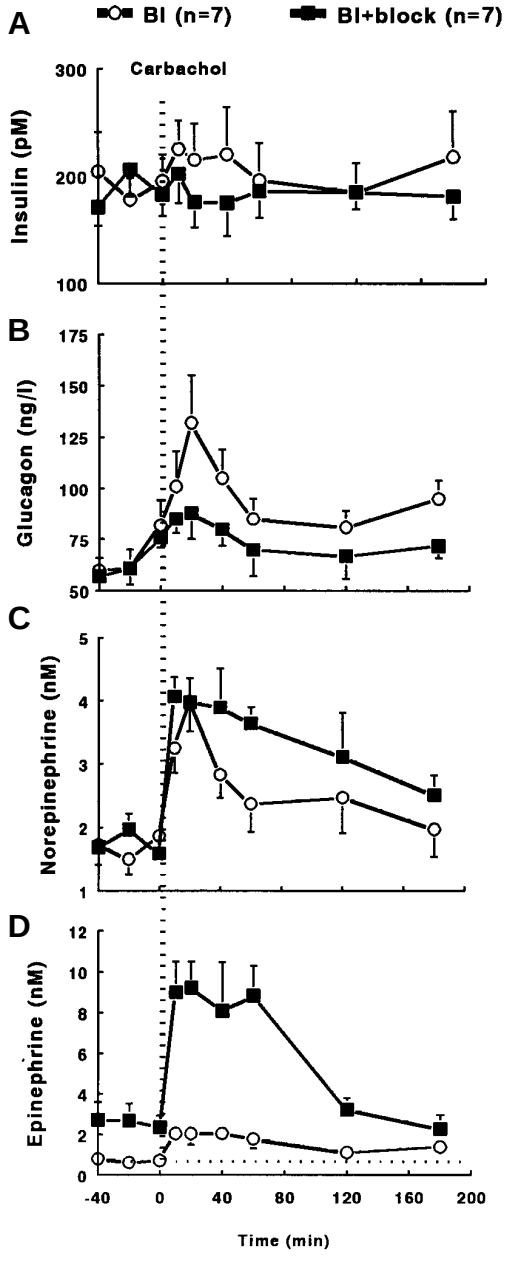

FIG. 2. Plasma concentrations of insulin (*A*), glucagon (*B*), norepinephrine (*C*), and epinephrine (*D*). Measurements were taken in alloxan-administered diabetic dogs at rest and after stress induced by ICV carbachol injection (5 µg at time 0). Experiments were conducted in two protocols studied under acutely induced normoglycemia with insulin (0.6 ± 0.1 mU · kg^{-1} · min^{-1}, BI, ○) and with insulin (0.6 ± 0.1 mU · kg^{-1} · min^{-1}) plus the addition of the β-blocker, propranolol (5µg · kg^{-1} · min^{-1}, BI+block, ■). Values are presented as means ± SE from seven experiments in each protocol.

($P < 0.0001$) increase in GP that peaked after 40 min of stress (ΔGP: 14.10 ± 0.31 µmol · kg^{-1} · min^{-1}). With BI+block, GP peaked after 20 min of stress and the peak (ΔGP: 3.72 ± 0.09 µmol · kg^{-1} · min^{-1}) was much lower ($P < 0.0001$) compared with BI. In BI, GU increased twofold (from 10.82 ± 0.0 to 21.35 ± 1.97 µmol · kg^{-1} · min^{-1}, $P < 0.0001$), proportionally to the twofold increment of plasma glucose. In BI+block, the GU increment was only 1.5-fold (from 15.50 ± 0.83 to 20.67 ± 2.41 µmol · kg^{-1} · min^{-1}), which was less ($P < 0.0001$) than that seen in BI.

Baseline glucose MCR (Fig. 1*D*) was higher ($P < 0.0001$) with BI+block (3.00 ± 0.18 ml · kg^{-1} · min^{-1}) compared with BI (2.08 ± 0.08 ml · kg^{-1} · min^{-1}). Despite normalization of glycemia, MCR after stress did not change from baseline with BI. However, there was a significant increase in MCR with BI+block (ΔMCR at peak: 0.72 ± 0.25 ml · kg^{-1} · min^{-1}; area under the curve: 19 ± 10 ml/kg over 40 min of stress, $P < 0.05$).

The peak rate of glucose SA (Fig. 1*E*) decline after stress was 0.5% per minute in BI and 0.25% per minute in BI+block. We have demonstrated previously that a moderate 0.4% per minute decline in SA does not lead to an underestimation of hepatic glucose (24). In BI and BI+block, the maximum rates of SA decline are close to or less than this validated rate.

Hormones. The level of insulin (Fig. 2*A*) required to achieve normoglycemia (basal insulin levels) was not different during the baseline period (–40 to 0 min) for the BI and BI+block (192 ± 24 and 187 ± 18 pmol/l, NS). Insulin levels remained constant throughout the experimental period (0–180 min) in both protocols (204 ± 13 in BI and 184 ± 9.4 pmol/l in BI+block) and were more than twofold higher than the basal insulin level (85 ± 13 pmol/l) reported in nondiabetic dogs in our previous stress study (9), most likely due to the increased insulin resistance in these diabetic dogs. Baseline glucagon levels (Fig. 2*B*) were not different between BI and BI+block (68 ± 6 and 65 ± 4 ng/l, NS). During stress, the rise of glucagon was significant in both protocols (BI: $P < 0.001$; BI+block: $P < 0.05$) and there was a greater sustained elevation of plasma glucagon in BI versus BI+block (132 ± 23 vs. 88 ± 13 ng/l, $P < 0.001$). No differences in baseline norepinephrine (Fig. 2*C*) were observed between the two groups (BI: 1.70 ± 0.17 and BI+block: 1.74 ± 0.11 nmol/l, NS). ICV injection of carbachol resulted in a rapid twofold elevation in plasma norepinephrine in both BI (peak: 3.99 ± 0.48 nmol/l) and BI+block (peak: 4.06 ± 0.31 nmol/l). However, the increment of norepinephrine was higher in BI+block throughout the experiment ($P < 0.05$). Baseline epinephrine levels (Fig. 2*D*) were higher in BI+block compared with BI (2.59 ± 0.41 vs. 0.72 ± 0.08 nmol/l, $P < 0.0001$). Plasma epinephrine levels rapidly increased in both protocols, reaching 2.05 ± 0.44 in BI and 9.20 ± 1.29 nmol/l in BI+block. As expected, the increments in both norepinephrine and epinephrine during stress were greater in the BI+block protocol compared with the BI protocol ($P < 0.01$ and $P < 0.0001$, respectively), which is consistent with the effect of adrenergic blockers in suppressing clearance of catecholamines (25). There was a sixfold increase in cortisol (not illustrated) in BI (from 31 ± 4 to 201 ± 22 nmol/l) compared with a fourfold increase in BI+block (from 52 ± 7 to 188 ± 28 nmol/l, NS between groups) during stress.

Metabolites. Baseline lactate levels (Fig. 3*A*) were lower in BI versus BI+block (0.36 ± 0.03 vs. 0.40 ± 0.03 mmol/l, respectively, $P < 0.01$), whereas both FFA (Fig. 3*B*) (1,807 ± 191 vs. 856 ± 145 µmol/l, respectively) and glycerol levels (Fig. 3*C*) (0.25 ± 0.03 vs. 0.12 ± 0.02 mmol/l, respectively) were greater in BI than BI+block ($P < 0.0001$ and $P < 0.001$, respectively). During stress, a rapid fourfold rise in lactate was observed with the BI protocol (peak: Δ 0.97 ± 0.03 mmol/l), but there was only a small rise in the BI+block protocol (peak: Δ0.15 ± 0.01 mmol/l, $P < 0.01$). The stress-induced rise (Δ) in FFA levels was very small with BI+block compared with BI (peak: Δ221 ± 28 vs. 1,295 ± 49 µmol/l, $P < 0.0001$). Changes in plasma glycerol (Δ) (peak: BI = Δ0.20 ± 0.01; BI+block =

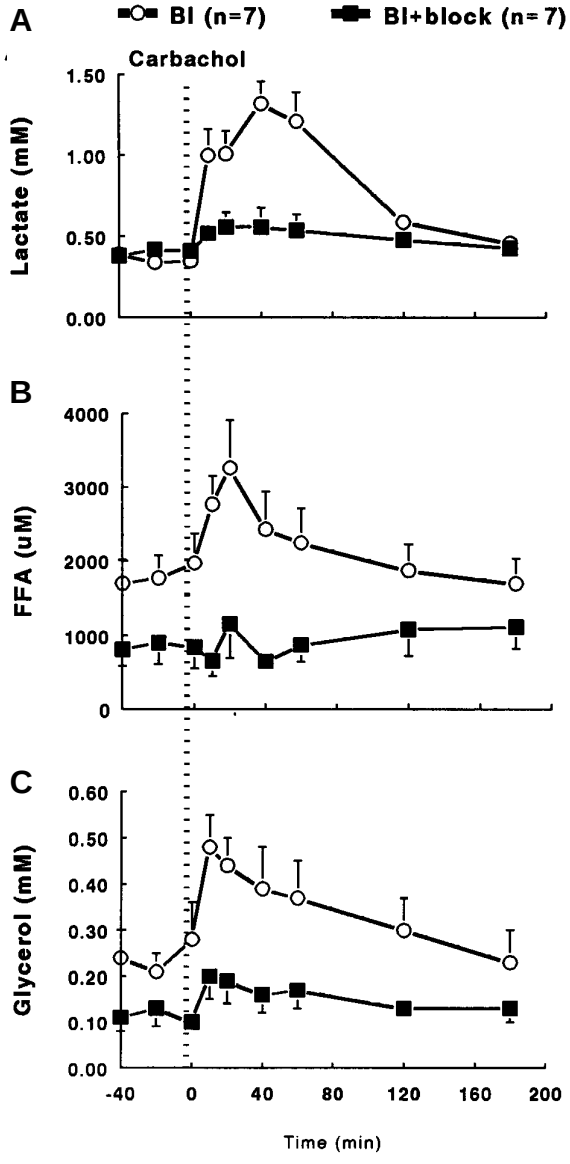

FIG. 3. Plasma concentrations of lactate (*A*), FFA (*B*), and glycerol (*C*) in alloxan-administered diabetic dogs before and after stress induced by ICV carbachol injection (5 µg at time 0). Experiments were conducted in two protocols studied under acutely induced normoglycemia with insulin (0.6 ± 0.1 mU · kg^{-1} · min^{-1}, BI, ○) and with insulin (0.6 ± 0.1 mU · kg^{-1} · min^{-1}) plus the addition of the β-blocker, propranolol (5 µg · kg^{-1} · min^{-1}, BI+block, ■). Values are presented as means ± SE from seven experiments in each protocol.

0.10 ± 0.01 mmol/l, $P < 0.01$) mirrored the changes in fatty acids, indicating that β-blockade suppressed basal and stress-induced lipolysis.

Study 2: the effect of hyperinsulinemia at two different levels on the glucoregulatory response to stress

Glucose turnover. Normoglycemia (Fig. 4*A*) was achieved during the basal period in both 3× BI and 5× BI as per experimental design (4.63 ± 0.07 and 5.33 ± 0.07 mmol/l, $P <$ 0.0001) and did not change during stress (NS). The exogenous GINF rate (Fig. 4B) required to maintain normoglycemia during the baseline period (–40 to 0 min) was higher in 5× BI compared with 3× BI (36.64 ± 0.94 vs. 18.21 ± 0.94µmol · kg^{-1} · min^{-1}, respectively, $P < 0.001$) and declined in both groups during stress (Δminimum: –15.77 ± 3.71 in 5× BI and –9.61 ± 2.56 µmol · kg^{-1} · min^{-1} in 3× BI, $P < 0.0001$ vs. baseline for both groups).

Baseline GP (Fig. 4*C*) was similar in the two protocols (10.49 ± 0.67 in 3× BI and 10.44 ± 1.28 µmol · kg^{-1} · min^{-1} in 5× BI, NS) and was slightly lower than the basal GP in the protocol with basal insulin (BI in study 1: 12.82 ± 0.67 µmol · kg^{-1} · min^{-1}, $P < 0.01$ vs. 3× BI). In both protocols, endogenous GP doubled after stress (to 20.79 ± 3.17 in 3× BI and 20.91 ± 4.28 µmol · kg^{-1} · min^{-1} in 5× BI, $P < 0.0001$).

Basal GU (Fig. 4*D*) reflected insulin levels (5× BI: 47.63 ± 1.83 µmol · kg^{-1} · min^{-1}; 3× BI: 28.59 ± 0.94 µmol · kg^{-1} · min^{-1}; BI: 10.82 ± 0.50 µmol · kg^{-1} · min^{-1}, $P < 0.0001$). In 3× BI, GU did not change during stress. However, in 5× BI, GU declined (–Δ9.52 ± 2.67 µmol · kg^{-1} · min^{-1}) ($P < 0.0001$: 5× BI vs. 3× BI).

Baseline glucose MCR (Fig. 4*E*) was ~1.5 times higher with 5× BI (9.03 ± 0.36 ml · kg^{-1} · min^{-1}) compared with 3× BI (6.31 ± 0.23 ml · kg^{-1} · min^{-1}, $P < 0.0001$), and ~4 times higher compared with BI (2.08 ± 0.08 ml · kg^{-1} · min^{-1}, $P < 0.0001$). Since normoglycemia was maintained, changes in MCR and GU after stress were the same.

The peak rate of glucose SA (Fig. 4*F*) change was 15% in 3× BI and 14% in 5× BI. We have demonstrated previously that a moderate 32% decline in specific activity does not lead to an underestimation of hepatic glucose production (24).

Hormones. Insulin levels (Fig. 5*A*) during the baseline period were by design 3× basal in 3× BI (538 ± 38 pmol/l) and 5× basal in 5× BI (888 ± 55 pmol/l). Baseline glucagon (Fig. 5*B*) levels were not different (3× BI = 58 ± 3 ng/l; 5× BI = 57 ± 6 ng/l, NS). During stress, there was a small rise in glucagon in 3× BI (peak: 76 ± 12 ng/l, $P < 0.01$) but not in 5× BI. Baseline norepinephrine (Fig. 5*C*) and epinephrine (Fig. 5*D*) levels were not different in 3× BI compared with 5× BI (norepinephrine: 1.33 ± 0.11 vs. 1.29 ± 0.12 nmol/l, respectively; epinephrine: 0.78 ± 0.07 vs. 0.85 ± 0.15 nmol/l, respectively). A significant ($P < 0.0001$) threefold elevation in plasma norepinephrine (peak: 3× BI = 3.48 ± 0.38 nmol/l; 5× BI = 3.79 ± 0.45 nmol/l) and a four- to sixfold increase in epinephrine, consistent with moderate stress, were observed in the protocols (peak: 3× BI = 3.31 ± 0.58, 5× BI = 4.5 ± 0.70 nmol/l). Interestingly, the stress-induced increments in both norepinephrine and epinephrine were significantly greater with higher insulin (5× BI) (norepinephrine: $P <$ 0.001, epinephrine: $P < 0.0001$). Cortisol levels (not illustrated) rose in 3× BI (from 46.90 ± 4.97 to 166.37 ± 14.34 nmol/l) compared with 5× BI (from 38.35 ± 5.52 to 159.47 ± 16.55 nmol/l) were not different between studies in either the baseline or stress periods.

Metabolites. Baseline lactate levels (Fig. 6*A*) were lower in 3× BI vs. 5× BI (0.38 ± 0.02 vs. 0.57 ± 0.04 mmol/l, $P <$ 0.0001), whereas baseline FFA levels (Fig. 6*B*) were greater in 3× BI (840 ± 73 vs. 605 ± 88 µmol/l, $P < 0.05$) and glycerol (Fig. 6*C*) was not different between the protocols (0.18 ± 0.02 in 3× BI vs. 0.19 ± 0.02 mmol/l in 5× BI, NS). During stress in the 3× BI protocol, a significant 4-fold rise in plasma lactate, a 2.5-fold increase in FFA, and a 2-fold increase in glycerol were observed (peak: Δ1.34 ± 0.07 mmol/l; Δ888 ± 31 µmol/l; and Δ0.19 ± 0.01mmol/l, respectively). The increases in FFA and lactate, but not glycerol, were greater in the 5× BI protocol (peak: Δ1,265 ± 34 µmol/l, $P < 0.01$; Δ1.52 ± 0.15 mmol/l, $P < 0.01$; and Δ0.25 ± 0.01 mmol/l, NS, respectively).

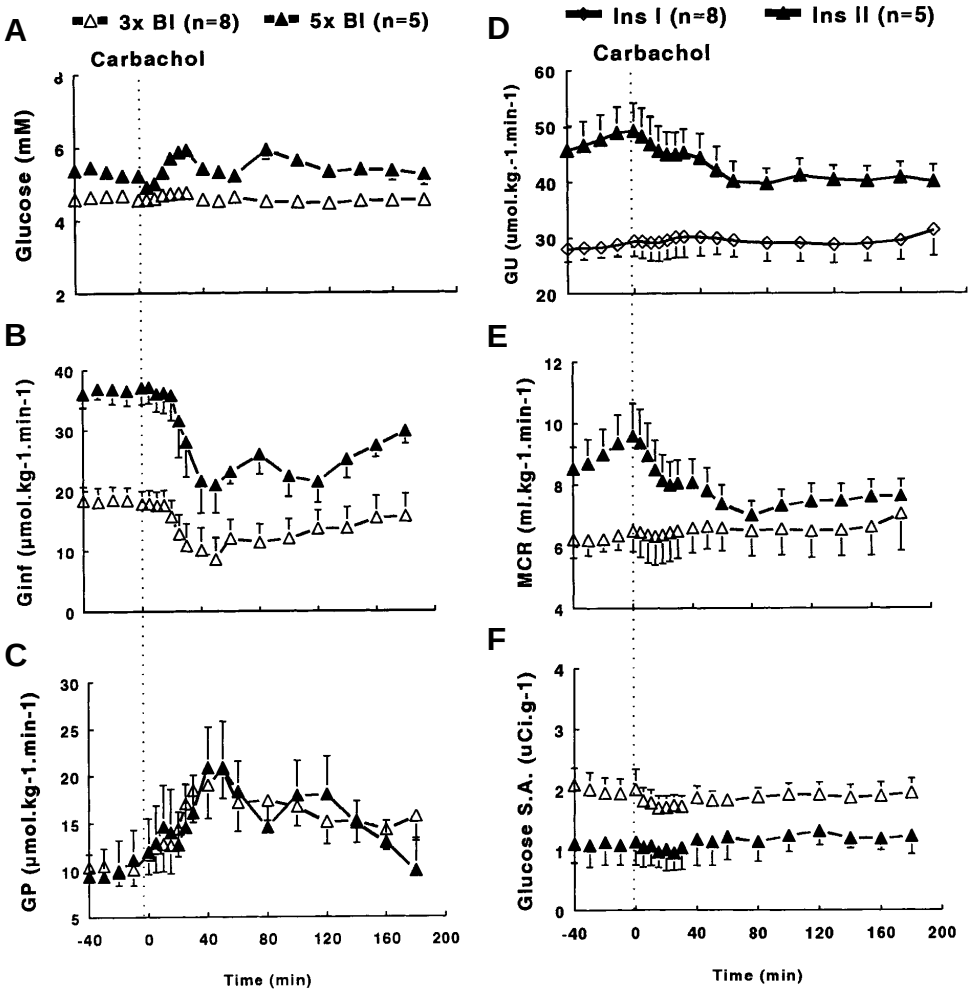

FIG. 4. Plasma glucose concentrations (*A*) and rates of exogenous GINF (*B*), endogenous GP (*C*), GU (*D*), MCR (*E*), and glucose specific activity (glucose SA) (*F*) in alloxan-administered diabetic dogs before and after stress induced by ICV carbachol injection (5 µg at time 0). Experiments were conducted in two protocols in alloxan-administered diabetic dogs during acutely induced normoglycemia with either 3× basal insulin (1.7 ± 0.2 mU · kg^{-1} · min^{-1}, 3× BI, △) or 5× basal insulin (3.3 ± 0.5 mU · kg^{-1} · min^{-1}, 5× BI, ▲). Values are represented as means ± SE from eight experiments in the 3× BI protocol and five experiments in the 5× BI protocol.

DISCUSSION

Study 1: the effect of β-blockade on the glucoregulatory response to stress at basal insulin concentration. The most striking finding of the present study was that β-adrenergic blockade almost completely prevented the hyperglycemic response to stress induced by ICV carbachol in alloxan-administered diabetic dogs. Improved glucoregulation after carbachol was due both to partial restoration of the normal stress-induced stimulation of glucose MCR and diminished GP. The increased MCR with β-blockade was an early effect observed during the first 40 min of stress.

The results of the present study can be compared with those of two of our group's previous studies (9,26), which examined the glucoregulatory response to ICV-carbachol stress in the dog model. In normal (nondiabetic) dogs (9), plasma glucose increased by only ~5% in response to ICV carbachol, due to an increase in MCR that almost perfectly matched the increase in glucose production. When the stress-induced increase in plasma insulin was prevented in normal dogs with a somatostatin-induced bihormonal (insulin and glucagon) pancreatic clamp (26), plasma glucose still did not rise appreciably, but the stress-induced increment in MCR was 30% less. This suggested that the stress-induced rise in MCR is largely, but not totally, independent of the rise in insulin. When normal dogs were studied with both a pancreatic clamp and β-blockade (26), plasma glucose did not rise and MCR increased twofold above that seen in normal dogs without a pancreatic clamp and β-blockade (9). This confirmed the importance of catecholamines in mediating the rise in glucose MCR in nondiabetic dogs in this model of stress but did not address their role in the diabetic state.

Alloxan-administered diabetic dogs were previously studied with use of the same stress model in a hyperglycemic state with low-dose (sub-basal) insulin infusion (9), and they demonstrated a hyperglycemic response to stress (plasma glucose increased from a value at baseline of ~9 to ~13 mmol/l). This occurred because, in contrast to normal (nondiabetic) dogs, their MCR did not rise following ICV carbachol. In contrast, in the present study, the MCR response to stress was restored almost to normal with β-blockade (i.e., the rise in MCR almost perfectly matched

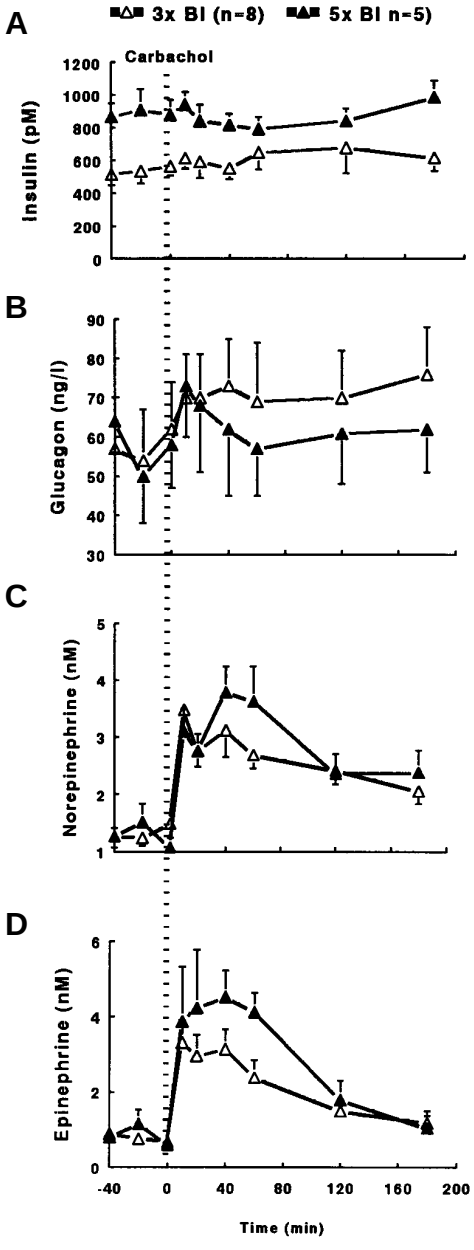

FIG. 5. Plasma concentrations of insulin (*A*), glucagon (*B*), norepinephrine (*C*), and epinephrine (*D*). Measurements were taken in alloxan-administered diabetic dogs before and after stress induced by ICV carbachol injection (5 µg at time 0). Experiments were conducted in two protocols in alloxan-administered diabetic dogs during acutely induced normoglycemia with either 3× basal insulin (1.7 ± 0.2 mU · kg^{-1} · min^{-1}, 3× BI, △) or 5× basal insulin (3.3 ± 0.5 mU · kg^{-1} · min^{-1}, 5× BI, ▲). Values are represented as means ± SE from eight experiments in the 3× BI protocol and five experiments in the 5× BI protocol.

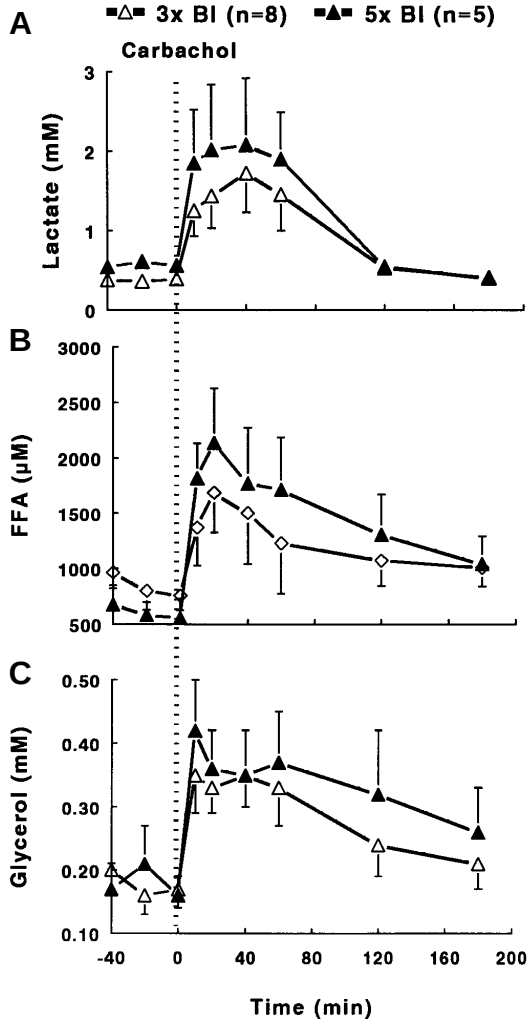

FIG. 6. Plasma concentrations of lactate (*A*), FFA (*B*), and glycerol (*C*) in alloxan-administered diabetic dogs before and after stress induced by ICV carbachol injection (5 µg at time 0). Experiments were conducted in two protocols in alloxan-administered diabetic dogs during acutely induced normoglycemia with either 3× basal insulin (1.7 ± 0.2 mU · kg^{-1} · min^{-1}, 3× BI, △) or 5× basal insulin (3.3 ± 0.5 mU · kg^{-1} · min^{-1}, 5× BI, ▲). Values are represented as means ± SE from eight experiments in the 3× BI protocol and five experiments in the 5× BI protocol.

the rise in glucose production) and the hyperglycemic response to stress was almost eliminated. In addition, it should be noted that in the present study, restoration of normoglycemia with BI alone without β-blockade did not restore the MCR or glucose response to stress.

As indicated, in both hyperglycemic and acutely normoglycemic insulin-infused diabetic dogs, plasma glucose increased because, unlike in normal dogs, MCR did not rise during stress. This is in contrast to a previous observation in alloxan-administered diabetic dogs during acute moderate exercise, in which normalization of glycemia, either with basal insulin or phlorizin, normalized the impaired exercise-induced increase in MCR (17). The plasma FFA as well as lactate and catecholamine levels in the exercising diabetic dogs were comparable to the levels observed during stress in the present study. It is conceivable that a prolonged training period before the exercise experiments normalized insulin sensitivity in diabetic dogs. The evidence for this is the normal basal requirement of insulin for maintaining normoglycemia in trained dogs, whereas in nontrained dogs that underwent stress, the basal insulin requirements were twice normal. With normal insulin sensitivity, but not with insulin resistance, the response of MCR to exercise is normal. It is tempting to speculate that glucoregulation would be less offset by stress in trained than untrained diabetic subjects. Alternatively, signaling mechanisms to increase GU may be different between stress and exercise.

The difference in MCR between dogs with or without β-blockade was probably not affected by a 4 mmol/l difference in glycemia. Part of the hyperglycemia-induced impairment in MCR has been attributed to a saturation of GU in insulin-independent tissues (mainly basal brain GU). A correction factor may be applied to MCR by subtracting an estimate of this component of GU from overall GU and then dividing by plasma glucose concentration. However, in a previous study, the percent difference between MCR in uncontrolled diabetic dogs and in normal dogs, in which there was a 20 mmol/l difference in glycemia, was the same whether or not such a factor was applied (17). Therefore, the conclusions about MCR in the present article should not be affected by the prevailing glucose concentrations, since the difference in glycemia was only 4 mmol/l.

The improvement in MCR responses observed in diabetic dogs given β-blockade occurred in the absence of a rise in insulin. The fact that in normal dogs MCR increments are largely insulin independent was clearly demonstrated by our previous experiments with bihormonal clamps (26). This finding is consistent with studies delineating the β-adrenoreceptor as mediating a direct insulin antagonistic effect of catecholamines on glucose uptake by isolated adipose and muscle tissues (27,28). β-Adrenergic stimulation has been shown to decrease insulin receptor binding and/or function (29,30), and to decrease the intrinsic activity of GLUT4 glucose transporters at the plasma membrane (31–33), thereby decreasing insulin-mediated glucose uptake by tissues (34,35). The increment in MCR observed with β-blockade in this study, therefore, was likely due to enhanced whole-body insulin-mediated glucose uptake, presumably at muscle and adipose tissue, independent of a rise in insulin. Hyperglycemia also downregulates glucose transporters acutely and chronically in the presence or absence of residual insulin (36,37) and restoration of normoglycemia reverses that effect (37), but the present study indicates that restoration or the normal increase in MCR with stress was due predominantly to the effect of the β-blockade per se rather than to normalization of hyperglycemia with basal insulin.

In this study, the effects of β-blockade on MCR during stress may have been mediated at least partly by indirect mechanisms as well. Stress was associated with a rapid rise in FFAs and glycerol in the BI but not in the BI+block studies, presumably due to rapid activation in β-adrenergic–mediated lipolysis. Glucose clearance can be impaired in the presence of elevated FFAs due to substrate competition between glucose and FFAs for oxidation, a process that was first described by Randle et al. (38) and has come to be known as the glucose-fatty acid cycle (15,16). Under most conditions, a change in FFA levels does not affect GU for 2–3 h (15).

Stress with ICV carbachol also stimulated a rise in plasma lactate, presumably due to enhanced muscle glycogenolysis during stress. Less muscle glycogenolysis with β-blockade, reflected by lower lactate levels, can decrease glucose-6-phosphate levels, thereby reducing the inhibition of hexokinase by glucose-6-phosphate, a process that could indirectly enhance glucose MCR (39). This mechanism may be more important or additive to that of the fatty acid–glucose cycle. Although β-blockade effects on blood flow were not measured, the effects of blood flow on muscle MCR would be expected to be minimal, since a recent study found no correlation between incremental muscle sympathetic nerve activity and leg blood flow or uptake during both hyperinsulinemic- and normoinsulinemic-normoglycemic clamps in humans (40).

The effect of β-blockade, in part, also reflects an increased effect of α-adrenergic stimulation, because during β-blockade, the levels of catecholamines are greatly increased and β-blockade may also unmask the α-adrenergic effects (41). α-Adrenergic blockade in diabetes during moderate exercise (42) and in normal dogs during carbachol stress (26) results in a decrease in GU and MCR, indicating that α-adrenergic stimulation tends to enhance both GU and MCR.

Evidence for extensive β-blockade in the BI+block protocol was demonstrated by the following: *1*) suppression of stress-induced lipolysis and lactate, which reflects reduced glycogenolysis, and *2*) marked increases in norepinephrine and epinephrine, presumably reflecting reduced clearance of catecholamines. In humans, at least, epinephrine and norepinephrine are cleared mainly through β-adrenergic receptors (25). With propranolol-induced β-blockade, the epinephrine levels were increased much more than norepinephrine during stress, an effect also observed in humans during high-intensity exercise-induced stress (43). Propranolol's inhibitory effect on catecholamine uptake may be selective for nonneuronal uptake mechanisms and may be less effective for norepinephrine uptake processes at sympathetic nerve terminals (44).

The augmented adrenergic response observed in diabetic dogs in the present study, compared with that observed in normal dogs studied previously (9), is due, in part, to increased levels of norepinephrine. Enhanced β-adrenergic catecholamine action observed in diabetes has been attributed to increased postreceptor response to β-adrenergic stimulation at the level of glycogen phosphorylase (45) and the adenyl cyclase/G-protein system (46). Additionally, diabetic animals have a downregulation of glucose transporters in muscle plasma membrane (47), which may make them more sensitive to the effect of catecholamines. Even when the catecholamine secretory response is reduced, as in a previous study during hypoglycemic stress, diabetic subjects can exhibit an enhanced β-adrenergic response to stress (48).

In addition to improving MCR responses during stress, β-blockade also markedly inhibited the stress-induced increase in GP by 70%. As in epinephrine infusion studies, the initial suppression probably reflects inhibition of glycogenolysis, whereas the latter suppression can be attributed to inhibition of gluconeogenesis (49). β-Blockade–mediated suppression of glucagon release would have had an additive inhibitory effect on hepatic glucose production. The changes in GU more or less paralleled the changes in MCR in the study with β-blockade, but they increased to a far greater extent than MCR in the basal insulin study. In the latter study, GU increased proportionally to the increment in plasma glucose that was brought about by a threefold increment in GP. It is important to note that the response of GU reflects the effects of hyperglycemia, whereas MCR is only marginally dependent on plasma glucose (17).

In a recent article, we studied the effect of β-blockade in normal and type 1 diabetic subjects during and after strenuous exercise (41). One can certainly consider 12–14 min of strenuous exercise a model of stress. In normal subjects, plasma glucose increased at the end of exercise and normalized within 100 min. In contrast, in patients with diabetes, the plasma glucose response to exercise was excessive

and remained elevated and unaltered at the end of the experimental period. β-Blockade normalized the response of plasma glucose to exercise in the diabetic patients because it normalized MCR during the exercise and postexercise periods. This would indicate a beneficial effect of β-blockade during exercise-induced stress in diabetes. The similarity of responses between the two models of stress could indicate that our experiments in dogs would be relevant to humans. We are not aware of any other systematic studies with respect to β-blockade and glucoregulation during stress in human diabetes.

Study 2: the effect of hyperinsulinemia at two different levels on the glucoregulatory response to stress. In this second study, we investigated whether the defect in glucose clearance in diabetic dogs can be overcome by hyperinsulinemia. Surprisingly, selective increases in peripheral plasma insulin three- and fivefold above basal, with maintenance of normoglycemia, did not improve the defective MCR response to stress. Interestingly, this occurred despite the fact that the hyperinsulinemia markedly increased basal MCR in proportion to the plasma insulin concentration. The ineffectiveness of physiological suprabasal elevations of insulin to improve MCR during stress was surprising, since insulin is well known to offset the inhibitory effects of catecholamines on MCR during stress. In previous studies, hyperinsulinemia during physical stress (intense exercise) was shown to prevent prolonged hyperglycemia in individuals with type 1 diabetes by restoring the normal increment in MCR (50). Insulin, through its direct action at insulin-sensitive tissues, particularly skeletal muscle and adipose tissue, rapidly stimulates glucose transport by inducing the translocation of glucose transporters from intracellular pool(s) to the plasma membrane (51,52) and/or by increasing glucose transporter intrinsic activity (53). Catecholamine-mediated increases in lipolysis at adipose tissue, and FFA oxidation and glycogenolysis at muscle, are processes that can inhibit glucose disposal during stress (12,15,39), whereas sufficient insulin levels and/or action can restrain these effects. The observation that even marked hyperinsulinemia, that greatly increased basal MCR, could not restore the MCR response to stress suggests that signaling mechanisms to increase GU in stress are different from those related to insulin or exercise.

Interestingly, a fivefold increase in insulin even decreased MCR during stress by 16%. We do not have a definite explanation for this paradoxical finding, but high physiological insulin levels have been shown to stimulate peripheral autonomic nervous outflow (54–56), thereby enhancing the stress response. Indeed, the stress-induced increments in catecholamines were greater in the 5× BI study than in the other studies, supporting this as a possibility. In addition to a direct antagonistic action of the catecholamines on MCR, the persistently high acute FFA response to stress in the hyperinsulinemic animals, which was even greater in the 5× BI group, may have played an important role in preventing the rise of MCR.

In summary, ICV carbachol-induced stress leads to marked hyperglycemia in diabetic dogs, because glucose MCR remains unchanged, despite a rapid increment in GP. This is in contrast to normal dogs, in which during the same stress, normoglycemia is maintained because the increment in GP is matched by an increment in the MCR of glucose. The defect of glucose MCR is not improved by acute antecedent normalization of hyperglycemia with basal or elevated insulin. Moreover, hyperglycemia after stress was fully prevented by β-blockade during BI because the increment in MCR was partially restored and increments of GP were in part suppressed. In patients with insulin-treated diabetes, stress can produce similar metabolic effects, leading to deterioration of their glycemic control (41), with adverse clinical sequelae. β-Blockade may dramatically ameliorate this deterioration. Consideration should be given to testing the administration of β-blockade in humans with diabetes during other stressful conditions, such as surgery, which are known to adversely affect glycemic control.

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REFERENCES

1. Pomposelli JJ, Baxter JK, Babineau TJ, Pomfret EA, Driscoll DF, Forse RA, Bistrian BR: Early postoperative glucose control predicts nosocomial infection rate in diabetic patients. *JPEN J Parenter Enteral Nutr* 22:77–81, 1998
2. Malchoff CD, Pohl SL, Kaiser DL, Carey RM: Determinants of glucose and ketoacid concentrations in acutely hyperglycemic diabetic patients. *Am J Med* 77:275–285, 1984
3. Wasserman DH, Lickley HL, Vranic M: Interactions between glucagon and other counterregulatory hormones during normoglycemic and hypoglycemic exercise in dogs. *J Clin Invest* 74:1404–1413, 1984
4. el-Tayeb KM, Brubaker PL, Vranic M, Lickley HL: Beta-endorphin modulation of the glucoregulatory effects of repeated epinephrine infusion in normal dogs. *Diabetes* 34:1293–1300, 1985
5. Frizzell RT, Jones EM, Davis SN, Biggers DW, Myers SR, Connolly CC, Neal DW, Jaspan JB, Cherrington AD: Counterregulation during hypoglycemia is directed by widespread brain regions. *Diabetes* 42:1253–1261, 1993
6. McGuinness OP, Burgin K, Moran C, Bracy D, Cherrington AD: Role of glucagon in the metabolic response to stress hormone infusion in the conscious dog. *Am J Physiol* 266:E438–E447, 1994
7. Rizza R, Haymond M, Cryer P, Gerich J: Differential effects of epinephrine on glucose production and disposal in man. *Am J Physiol* 237:E356–E362, 1979
8. Goldstein RE, Abumrad NN, Lacy DB, Wasserman DH, Cherrington AD: Effects of an acute increase in epinephrine and cortisol on carbohydrate metabolism during insulin deficiency. *Diabetes* 44:672–681, 1995
9. Miles PD, Yamatani K, Lickley HL, Vranic M: Mechanism of glucoregulatory responses to stress and their deficiency in diabetes. *Proc Natl Acad Sci U S A* 88:1296–1300, 1991
10. Miles PD, Yamatani K, Brown MR, Lickley HL, Vranic M: Intracerebroventricular administration of somatostatin octapeptide counteracts the hormonal and metabolic responses to stress in normal and diabetic dogs. *Metabolism* 43:1134–1143, 1994
11. Mizock BA: Alterations in carbohydrate metabolism during stress: a review of the literature. *Am J Med* 98:75–84, 1995
12. Attvall S, Eriksson VM, Fowelin J, von Schenck H, Lager I, Smith U: Early posthypoglycemic insulin resistance in man is mainly an effect of beta-adrenergic stimulation. *J Clin Invest* 80:437–442, 1987
13. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46:3–10, 1997 (Erratum published in *Diabetes* 46:536, 1997)
14. Boden G, Chen X, Ruiz J, Heifets M, Morris M, Badosa F: Insulin receptor down-regulation and impaired antilipolytic action of insulin in diabetic patients after pancreas/kidney transplantation. *J Clin Endocrinol Metab* 78:657–663, 1994
15. Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* 93:2438–2446, 1994
16. Boden G, Jadali F: Effects of lipid on basal carbohydrate metabolism in normal men. *Diabetes* 40:686–692, 1991

17. Fisher SJ, Lekas M, Shi ZQ, Bilinski D, Carvalho G, Giacca A, Vranic M: Insulin-independent acute restoration of euglycemia normalizes the impaired glucose clearance during exercise in diabetic dogs. *Diabetes* 46:1805–1812, 1997
18. Radziuk J, Norwich KH, Vranic M: Experimental validation of measurements of glucose turnover in nonsteady state. *Am J Physiol* 234:E84–E93, 1978
19. Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps: comparison of unlabeled and labeled exogenous glucose infusates. *Diabetes* 36:914–924, 1987
20. Bradley DC, Steil GM, Bergman RN: Quantitation of measurement error with optimal segments: basis for adaptive time course smoothing. *Am J Physiol* 264:E902–E911, 1993
21. Bjorkman O, Miles PD, Wasserman D, Lickley HL, Vranic M: Regulation of glucose turnover during exercise in pancreatectomized, totally insulin deficent dogs: effects of beta-adrenergic blockade. *J Clin Invest* 81:1759–1767, 1988
22. Miles JJ, Glasscock J, Aikens J, Gerich J, Haymond M: A microfluorometric method for the determination of free fatty acids in plasma. *J Lipid Res* 24:96–99, 1983
23. Lloyd B, Burrin J, Smythe P, Alberti KG: Enzymic fluorometric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol, and 3-hydroxybutyrate. *Clin Chem* 24:1724–1729, 1978
24. Fisher SJ, Shi ZQ, Lickley HLA, Efendic S, Vranic M, Giacca A: A moderate decline in specific activity does not lead to an underestimation of hepatic glucose production during a glucose clamp. *Metabolism* 45:587–593, 1996
25. Cryer PE, Rizza R, Haymond M, Gerich J: Epinephrine and norepinephrine are cleared through beta-adrenergic, but not alpha-adrenergic, mechanisms in man. *Metabolism* 1114–1118, 1980
26. Lekas M, Fisher SJ, Van Delangerit M, Vranic M, Shi ZQ: Glucose uptake during stress is enhanced by beta-blockade and is not mediated by insulin. *J Appl Physiol* 87:722–731, 1999
27. Hagstrom-Toft E, Arner P, Johansson U, Eriksson LS, Ungerstedt U, Bolinder J: Effect of insulin on human adipose tissue metabolism in situ: interactions with beta-adrenoceptors. *Diabetologia* 35:664–670, 1992
28. Chiasson JL, Shikama H, Chu DT, Exton JH: Inhibitory effect of epinephrine on insulin-stimulated glucose uptake by rat skeletal muscle. *J Clin Invest* 68:706–713, 1981
29. Pessin JE, Gitomer W, Oka Y, Oppenheimer CL, Czech MP: Beta-adrenergic regulation of insulin and epidermal growth factor receptors in rat adipocytes. *J Biol Chem* 258:7386–7394, 1983
30. Lonnroth P, Wesslau C, Stenstrom G, Tisell LE, Smith U: Reduced insulin binding to human fat cells following beta-adrenergic stimulation-experimental evidence and studies in patients with pheochromocytoma. *Diabetologia* 28: 901–906, 1985
31. Bonen A, Megeney LA, McCarthy SC, McDermott JC, Tan MH: Epinephrine administration stimulates GLUT4 translocation but reduces glucose transport in muscle. *Biochem Biophys Res Commun* 187:685–691, 1992
32. Nishimura H, Saltis J, Habberfield AD, Garty NB, Greenberg AS, Cushman SW, Londos C, Simpson IA: Phosphorylation state of the GLUT4 isoform of the glucose transporter in subfractions of the rat adipose cell: effects of insulin, adenosine, and isoproterenol. *Proc Natl Acad Sci U S A* 88:11500–11504, 1991
33. Joost H, Weber M, Cushman S, Simpson I: Insulin stimulated glucose transport in rat adipose cells: modulation of transporter activity by isoproterenol and adenosine. *J Biol Chem* 261:10033–10036, 1986
34. Young DA, Wallberg-Henriksson H, Cranshaw J, Chen M, Holloszy JO: Effect of catecholamines on glucose uptake and glycogenolysis in rat skeletal muscle. *Am J Physiol* 248:C406–C409, 1985
35. Wasserman DH, Lickley HL, Vranic M: Role of beta-adrenergic mechanisms during exercise in poorly controlled diabetes. *J Appl Physiol* 59:1282–1289, 1985
36. Dimitrakoudis D, Ramlal T, Rastogi S, Vranic M, Klip A: Glycaemia regulates the glucose transporter number in the plasma membrane of rat skeletal muscle. *Biochem J* 284:341–348, 1992
37. Mathoo JM, Shi ZQ, Klip A, Vranic M: Opposite effects of acute hypoglycemia and acute hyperglycemia on glucose transport and glucose transporters in perfused rat skeletal muscle. *Diabetes* 48:1281–1288, 1999
38. Randle PJ, Garland PB, Hales CN, Newsholme EA: The glucose-fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* i:785–789, 1963
39. James DE, Burleigh KM, Kraegen EW: In vivo glucose metabolism in individual tissues of the rat: interaction between epinephrine and insulin. *J Biol Chem* 261:6366–6374, 1986
40. Spraul M, Ravussin E, Baron AD: Lack of relationship between muscle sympathetic nerve activity and skeletal muscle vasodilation in response to insulin infusion. *Diabetologia* 39:91–96, 1996
41. Sigal RJ, Fisher SJ, Halter JB, Vranic M, Marliss EB: Glucoregulation during and after intense exercise: effects of beta-adrenergic blockade in subjects with type 1 diabetes mellitus. *J Clin Endocrinol Metab* 84:3961–3971, 1999
42. Simonson DC, Koivisto V, Sherwin RS, Ferrannini E, Hendler R, Juhlin-Dannfelt A, DeFronzo RA: Adrenergic blockade alters glucose kinetics during exercise in insulin-dependent diabetics. *J Clin Invest* 73:1648–1658, 1984
43. Purdon C, Brousson M, Nyveen SL, Miles PD, Halter JB, Vranic M, Marliss EB: The roles of insulin and catecholamines in the glucoregulatory response during intense exercise and early recovery in insulin-dependent diabetic and control subjects. *J Clin Endocrinol Metab* 76:566–573, 1993
44. Ziegler MG, Chernow B, Woodson LC, Coyle J, Cruess D, Lake CR: The effect of propranolol on catecholamine clearance. *Clin Pharmacol Ther* 40:116–119, 1986
45. Buczek-Thomas JA, Jaspers SR, Miller TB Jr: Adrenergic activation of glycogen phosphorylase in primary culture diabetic cardiomyocytes. *Am J Physiol* 262:H649–H653, 1992
46. Buczek-Thomas JA, Jaspers SR, Miller TB Jr: Post-receptor defect accounts for phosphorylase hypersensitivity in cultured diabetic cardiomyocytes. *Mol Cell Biochem* 117:63–70, 1992
47. Ramlal T, Rastogi S, Vranic M, Klip A: Decrease in glucose transporter number in skeletal muscle of mildly diabetic (streptozotocin-treated) rats. *Endocrinology* 125:890–897, 1989
48. Bolinder J, Sjoberg S, Arner P: Stimulation of adipose tissue lipolysis following insulin-induced hypoglycaemia: evidence of increased beta-adrenoceptor-mediated lipolytic response in IDDM. *Diabetologia* 39:845–853, 1996
49. Stevenson RW, Steiner KE, Connolly CC, Fuchs H, Alberti KG, Williams PE, Cherrington AD: Dose-related effects of epinephrine on glucose production in conscious dogs. *Am J Physiol* 260:E363–E370, 1991
50. Signal R, Purdon C, Fisher S, Halter JB, Vranic M, Marliss EB: Hyperinsulinemia prevents prolonged hyperglycemia after intense exercise in insulin-dependent diabetic subjects. *J Clin Endocrinol Metab* 79:1049–1057, 1994
51. Klip A, Ramlal T, Young DA, Holloszy JO: Insulin-induced translocation of glucose transporters in rat hindlimb muscles. *FEBS Lett* 224:224–230, 1987
52. Cushman SW, Warzala LJ: Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell: apparent translocation of intracellular transport systems to the plasma membrane. *J Biol Chem* 255:4758–4762, 1980
53. Sternlicht E, Barnard RJ, Grimditch GK: Mechanism of insulin action on glucose transport in rat skeletal muscle. *Am J Physiol* 254:E633–E638, 1988
54. Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg L: Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes* 30:219–225, 1981
55. Tack CJ, Lenders JW, Willemsen JJ, van Druten JA, Thien T, Lutterman JA, Smits P: Insulin stimulates epinephrine release under euglycemic conditions in humans. *Metabolism* 47:243–249, 1998
56. Paramore DS, Fanelli CG, Shah SD, Cryer PE: Forearm norepinephrine spillover during standing, hyperinsulinemia, and hypoglycemia. *Am J Physiol* 275:E872–E881, 1998