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

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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 m l ·k g⁻¹ ·m in⁻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 ·k g⁻¹ ·m in⁻¹ for BI+block vs. 14.10 ± 0.31 µmol ·k g⁻¹ ·m in⁻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.

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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 glucose metabolism 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 hypothalamic-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 increase in glucose clearance nearly matched the incre-
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 (B1); 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 fluorothane) 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, Port Huron, Michigan, 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 morning preinfusion 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 by a constant-rate insulin infusion (n = 7 experiments); plasma glucose was normalized by a constant-rate insulin infusion (n = 7 experiments). The question remained whether acute restoration of nor-

**Glucose turnover calculations.** The rates of endogenous glucose production (GP) and glucose uptake (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 and a measure of the efficiency and capacity of the metabolic processes involved in the clearance of glucose (20). 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,

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Glucose specific activity was derived from the plasma glucose concentration and 3[3H]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. 1A, 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. 1B) and GU (Fig. 1C) 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
Baseline glucose MCR (Fig. 1D) 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 (AMCR 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. 1E) 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. 2A) required to achieve normoglycemia (basal insulin levels) was not different during the baseline period (–40 to 0 min) for the BI and BI+block protocols. Baseline 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. 2B) 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. 2C) 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 increase of norepinephrine was higher in BI+block throughout the experiment (P < 0.05). Baseline epinephrine levels (Fig. 2D) were higher in BI+block compared with BI (2.59 ± 0.41 vs. 0.25 ± 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. 3A) 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. 3B) (1,807 ± 191 vs. 856 ± 145 µmol/l, respectively) and glycerol levels (Fig. 3C) (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 = Δ0.20 ± 0.01)
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. 4A) 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 ± 0.94 μmol·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. 4C) 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. 4D) 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. 4E) 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. 4F) 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. 5A) 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. 5B) levels were not different (3× BI = 56 ± 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. 5C) and epinephrine (Fig. 5D) 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. 6A) were lower in 3× BI with 5× BI (0.38 ± 0.02 vs. 0.57 ± 0.04 mmol/l, P < 0.0001), whereas baseline FFA levels (Fig. 6B) were greater in 3× BI (840 ± 73 vs. 605 ± 88 mmol/l, P < 0.05) and glycerol (Fig. 6C) 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 ± 0.01 mmol/l, respectively). The increases in FFA and lactate, but not glycerol, were greater in the 5× BI protocol (peak: Δ1.265 ± 0.34 mmol/l, P < 0.01; Δ1.52 ± 0.15 mmol/l, P < 0.01; and Δ0.25 ± 0.01 mmol/l, NS, respectively).
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
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 off-set by stress in trained than untrained diabetic subjects. Alternatively, signaling mechanisms to increase GU may be different between stress and exercise.

**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 3x basal insulin (1.7 ± 0.2 mU · kg⁻¹ · min⁻¹, 3x BI, △) or 5x basal insulin (3.3 ± 0.5 mU · kg⁻¹ · min⁻¹, 5x BI, ▲). Values are represented as means ± SE from eight experiments in the 3x BI protocol and five experiments in the 5x 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 3x basal insulin (1.7 ± 0.2 mU · kg⁻¹ · min⁻¹, 3x BI, △) or 5x basal insulin (3.3 ± 0.5 mU · kg⁻¹ · min⁻¹, 5x BI, ▲). Values are represented as means ± SE from eight experiments in the 3x BI protocol and five experiments in the 5x BI protocol.
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-adrenergic receptor 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 increase 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 gluco-regulation during stress in human diabetes.

**Study 2: the effect of hyperinsulinemia at two different levels on the gluco-regulatory 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 glycolysis 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 glucose utilization 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× B1 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× B1 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**

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