Glucose uptake during centrally induced stress is insulin independent and enhanced by adrenergic blockade

MICHAEL C. LEKAS, SIMON J. FISHER, BAN EL-BAHRANI, MAYLIZA VAN DELANGERYT, MLADEN VVRANIC, AND Z. QING SHI

Glucose uptake during centrally induced stress is insulin independent and enhanced by adrenergic blockade. J. Appl. Physiol. 87(2): 722–731, 1999.—Glucose utilization increases markedly in the normal dog during stress induced by the intracerebroventricular (ICV) injection of carbachol. To determine the extent to which insulin, glucagon, and selective (α/β)-adrenergic activation mediate the increase in glucose metabolic clearance rate (MCR) and glucose production (Rg), we used five groups of normal mongrel dogs: 1) pancreatic clamp (PC; n = 7) with peripheral somatostatin (0.8 µg·kg⁻¹·min⁻¹) and intraportal replacement of insulin (1.48 ± 0.24 pmol·kg⁻¹·min⁻¹) and glucagon (0.65 ng·kg⁻¹·min⁻¹) infusions; 2) PC plus combined (phentolamine)- and β-(propranolol)-blockade (7 and 5 µg·kg⁻¹·min⁻¹, respectively; α; n = 5); 3) PC plus α-blockade (α; n = 6); 4) PC plus β-blockade (β; n = 5); and 5) a carbachol control group without PC (Con; n = 10). During ICV carbachol stress (0–120 min), catecholamines, ACTH, and cortisol increased markedly in all groups. Baseline insulin and glucagon levels were maintained in all groups except Con, where glucagon rose 33%, and α, where insulin increased slightly but significantly. Stress increased (P < 0.05) plasma glucose in Con, PC, and α but decreased it in β and α + β. The MCR increment was greater (P < 0.05) in β and α + β than in Con, PC, and α. Rg increased (P < 0.05) in all groups but was attenuated in α + β. Stress-induced lipolysis was abolished in β (P < 0.05). The marked rise in lactate in Con, PC, and α was abolished in α + β. We conclude that the stress-induced increase in MCR is largely independent of changes in insulin, markedly augmented by β-blockade, and related, at least in part, to inhibition of lipolysis and glycogenolysis, and that Rg is augmented by glucagon and α- and β-catecholamine effects.

INCREASED ADRENERGIC TONE resulting from both sympathetic nervous system and adrenomedullary activation is an important part of the physiological stress response. Adrenergic activation, along with a surge of counterregulatory hormones, accounts for the increased glucose production (Rg) in stress (17, 23, 28, 50). Glucose utilization (Rg), however, may increase or decrease depending on the form of stress, level of insulin, and physical activity (50, 52). An acute model of moderate stress induced by the intracerebroventricular (ICV) injection of carbachol, an acetylcholine analog, has been used to study the impact of centrally induced stress on glucose homeostasis (10, 21). We observed a marked increase in tracer-determined Rg in this stress model in normal dogs (31). In addition, however, Rg and metabolic clearance rate (MCR) increased markedly and rapidly (31). The driving mechanism for the increase in MCR could not be determined because it took place in the presence of large surges in counterregulatory hormones.

To unravel the mechanism(s) of the intriguing increase in Rg, we wished to identify the roles of insulin and adrenergic activation. Although the stress model was associated only with a small increase in plasma insulin, it appears that even a small increment in insulin may be important in the control of Rg (29). Limited sensitivity of the insulin immunoassay could also underestimate the contribution of small changes in circulating insulin levels to glucose disposal. Furthermore, it cannot be excluded that some of the stress conditions, as in the case of exercise, may have a synergistic effect with insulin, thus increasing the effect of even small changes in plasma insulin. To identify the role of insulin in this stress model, we used a pancreatic bihormonal clamp that maintains stable insulin and glucagon levels by suppressing endogenous insulin and glucagon secretion with somatostatin and replacing the two hormones with exogenous infusions. It is possible that stress may activate an otherwise dormant neuroendocrine pathway in modulating glucose uptake. The notion that the increased glucose clearance could be linked to neurally mediated autonomic outflow was prompted by our observation that ICV injection of a somatostatin analog, ODT8-SS, before carbachol administration abolished both the stress-induced increment in Rg and that in epinephrine and norepinephrine (30). The role of the catecholamines in regulation of glucose metabolism appears to be multifaceted, depending on the metabolic milieu. Simulation of the adrenergic stress response with epinephrine infusion resulted in carbohydrate intolerance with inhibition of Rg and MCR (16, 36). However, catecholamines have been shown to increase both basal and insulin-mediated glucose uptake, chronically in the basal state in vivo (26) and acutely in the in vitro rat brown adipocyte (27) and acutely stimulated energy expenditure (6). Efferent sympathetic neural activity is believed to mediate an increase in glucose uptake in brown adipose tissue induced by electrical stimulation of the ventromedial hypothalamus (49).

We also wanted to determine contributions of glucagon and catecholamines to the rapid Rg response to centrally induced stress. During moderate exercise, Rg...
is governed primarily by the glucagon-to-insulin (glucagon/insulin) ratio (41, 53). In strenuous exercise, however, catecholamines rather than the glucagon/insulin ratio (42) become the main regulators of Ra. In this centrally induced model of stress, it is not known whether the increased Ra is driven by glucagon and/or catecholamines. To explore the overall role of the neural and adrenal catecholamines and to differentiate between the α- and β-adrenoceptor-mediated catecholamine effects in modulating Ra and Rd, we employed combined and selective α- and β-blockade in this study. α- and β-Adrenoceptors may have differential or even opposing effects on MCR. In addition, both α- and β-adrenergic effects may stimulate Ra through different second messengers. Selective inhibition of one adrenoceptor type with consequential changes in Ra would allow us to determine contributions of each adrenoceptor subtype.

The purposes of the present study were threefold. They were to determine in this stress model 1) the reason and extent to which the increment in Ra and MCR is dependent on rises in insulin; 2) the effects of adrenergic activation on the non-insulin-dependent portion of the stress-induced increase in MCR; and 3) the relative roles of glucagon and catecholamines in the increase in Ra. To achieve these purposes, it was imperative to use the bihormonal pancreatic clamp (PC), to minimize changes in insulin and glucagon secretion due to variations of catecholamine and α- and β-blockers.

MATERIALS AND METHODS

Experimental animals. In normal male mongrel dogs (body wt, 15–25 kg), under general anesthesia (fluothane and nitrous oxide) and assisted ventilation, an ICV stainless 22-gauge guide cannula was implanted into the third ventricle of the brain by using a canine stereotaxic apparatus. The patency of the cerebral cannula was verified by aspiration of a small volume of cerebrospinal fluid. For insulin and glucagon infusion, the portal vein was cannulated with a Silastic catheter (1.0 mm ID), which was inserted via a branch of the splenic vein. A Silastic catheter (1.0 mm ID) was placed into the aortic arch via a carotid artery for arterial blood sampling. Three Silastic catheters (0.76 mm ID) were inserted via an external jugular vein into the superior vena cava just above the right atrium for infusions. All catheters were tunneled subcutaneously and exteriorized from the back of the neck. The catheters were filled with a heparin solution (1,000 U/ml) and flushed weekly to maintain patency. The dogs were housed under controlled-temperature and -light conditions and fed once per day a mixed diet of 300–400 g of dry dog chow (Lab canine diet 5006; Purina Mills, St. Louis, MO) and 400–500 g of meat (Romar Pet Supply, Toronto, ON). Food intake, body weight, temperature, and hematocrit were monitored to ensure that only healthy dogs were used in the experiments. All procedures were performed in accordance with Canadian Council on Animal Care standards and were approved by the Animal Care Committee of the University of Toronto.

Experimental protocol. The dogs were allowed at least 2 wk to recover from surgery before the first experiment. A minimum period of 1 wk was allowed between successive experiments. Food was withdrawn 18 h before each experiment. Each experiment consisted of a tracer equilibration period (−160 to −40 min), a basal sampling period (−40 to 0 min), and a stress period (0–120 min). A priming (25 µCi) dose of [3-3H]glucose (New England Nuclear, Boston, MA) was given at −160 min, when a constant infusion of [3-3H]glucose (0.25 µCi/min) was initiated and continued throughout the study. Five groups were included in the present study. 1) A previously reported carbachol control group (31) was supplemented with three additional experiments (Con, n = 10). No differences were observed between present and former control subjects in values of hormones and metabolites [insulin, glucagon, norepinephrine, epinephrine, cortisol, free fatty acids (FFA), and glycerol], and glucose turnover (Ra, Rd) under both basal and stress conditions. 2) A group received a PC (n = 7) by means of a peripheral somatostatin infusion at a dose established to inhibit release of endogenous insulin and glucagon (11). Constant basal levels of insulin and glucagon were sustained by intraportal replacement infusions. 3) A group received a PC and combined adrenergic blockade (α + β; n = 5) through infusions of an α-blocker (phentolamine) and a β-blocker (propranolol). 4) A group received an α-blocker (phentolamine; 5 µg · kg⁻¹ · min⁻¹, Ciba-Geigy Canada, Mississauga, ON) infusion superimposed on a PC (α + β; n = 6). 5) Another group received a β-blocker (propranolol, 7 µg · kg⁻¹ · min⁻¹, Ciba-Geigy Canada) infusion and a PC (β; n = 5). Somatostatin (0.8 µg · kg⁻¹ · min⁻¹) infusion (in PC, α + β, α, and β) was started at −160 min. Simultaneous intraportal replacement infusions of insulin (1,200–2,100 pmol · kg⁻¹ · min⁻¹) and glucagon (at a fixed rate of 0.65 ng · kg⁻¹ · min⁻¹) were then begun. The rate of insulin infusion was adjusted so that plasma glucose levels monitored every 5–10 min were maintained at preequilibration euglycemia. The last adjustment of the insulin infusion was at least 30 min before the start of the basal sampling period. α- and β-Blockers were given at doses shown previously to attain sufficient α- or β-blockade (20, 22, 57). They were initiated at −20 min and continued throughout the experiment. At time 0, 5 µg of carbachol (carbamylcholine; Aldrich) in 50 µl of sterile water were injected ICV through a 24-gauge injection cannula connected to a 100-µl Hamilton microsyringe via polyethylene tubing (Intramedic PE 50; Clay Adams). Arterial blood pressure and heart rate were monitored by using a pressure transducer connected to the carotid catheter and recorded with a physiograph (Gilion).

Processing of blood samples. Blood samples were taken every 5–10 min for plasma glucose levels and every 10–20 min for determination of insulin, glucagon, cortisol, norepinephrine, epinephrine, lactate, glycerol, and FFA. Blood samples for determination of plasma glucose, glucose specific activity, insulin, cortisol, lactate, and glycerol were collected in tubes containing heparin (50 U/ml) and NaF (1–2 mg/tube). Samples for determination of plasma glucagon and FFA concentrations were collected in tubes containing 1:1 (vol/vol) aprotinin (Trasylol, 10,000 KIU/ml, Bayer) and EDTA (25 mg/ml, BDH Chemicals). For assays of norepinephrine and epinephrine, 1-ml blood samples were collected in polyethylene tubes containing 2.5 mg glutathione (Boehringer Mannheim) and 10 µl EGTA (Sigma Chemical). All blood samples were stored at 4°C and centrifuged within 60 min. The resultant plasma was stored at −20°C, except the plasma for catecholamine assays, which was deproteinized with 2 M HClO₄ and stored at −70°C. Plasma glucose was measured by a glucose oxidase method by using a Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma glucose specific activity was derived from plasma glucose concentration and [3-3H]glucose radioactivity determined in a liquid scintillation counter after deproteinization of the plasma samples with Ba(OH)₂ and ZnSO₄ and evaporation of the supernatant.
Insulin and glucagon were analyzed by radioimmunoassays. Catecholamines were measured by a radioenzymatic assay (45) and cortisol by a modified competitive binding assay. Plasma FFA were determined by a radiochemical technique (38). Lactate and glycerol were measured by enzymatic microfluorometric methods (24).

Calculations. Glucose concentration and specific activity data were systematically smoothed by using the optimal segments technique (13). Ra and Rd were calculated with an equation for non-steady-state turnover (46). Glucose MCR was calculated by dividing Rd by the prevailing plasma glucose concentration. MCR represents an estimate of Rd partially corrected for the mass action effect of glucose (33).

Statistical analyses. All values are expressed as means ± SE. Statistical analysis, to assess the stress-induced responses and differences both within and between the experimental groups, was performed by using ANOVA for unbalanced data (Statistical Analysis System; SAS Institute, Cary, NC), with the time effects of treatments involved in the experiments taken into account.

RESULTS

Insulin and glucagon levels. Basal insulin levels were slightly higher (P < 0.05) in the Con (75.6 ± 5.0 mU/mL) and β-blockade (79.2 ± 11.4 mU/mL) vs. the PC (57.0 ± 3.6 mU/mL) and α-blockade (59 ± 8.4 mU/mL) groups. Also, basal glucagon levels were greater in α+β than in the Con and α groups (186 ± 24 vs. 144 ± 9 and 134 ± 14 ng/l, respectively, P < 0.05). Because of such basal differences among groups, Δ values, representing changes from the basal period, were calculated (Fig. 1). A small rise in insulin levels seen in the Con animals during stress was obviated with the bihormonal PC (P < 0.05, PC vs. Con). Arterial plasma insulin levels were stable in PC (basal vs. stress period: 57 ± 3.6 vs. 58 ± 4.0 mU/mL), β (79.2 ± 11.4 vs. 82.8 ± 8.4 mU/mL), and α+β (69.6 ± 5.4 vs. 68.4 ± 4.5 mU/mL) groups, although the α group was associated with a small but significant increase in insulin levels during stress (59 ± 8.4 vs. 73 ± 6.6 mU/mL, P < 0.05). The increase in plasma glucagon due to stress observed in the Con group (144 ± 9 to 192 ± 19 ng/l, P < 0.01) was obviated by the PC in the PC (171 ± 16 to 172 ± 15 ng/l), α+β (186 ± 24 to 164 ± 13 ng/l), α (134 ± 14 to 154 ± 20 ng/l), and β (150 ± 12 to 146 ± 9 ng/l) groups (all P < 0.01 vs. Con).

Basal glucose turnover (Table 1). Basal Ra in the α+β and β groups was greater (P < 0.05) than in the Con, PC, and α groups. Similarly, basal Rd and MCR in the α+β and β groups were greater (P < 0.05) than in Con, PC, and α, respectively.

Glucose turnover during stress. During stress, plasma glucose levels increased slightly yet significantly in the Con, PC, and α groups (Fig. 2). However, in the α+β and β groups there was a small but significant fall. In all groups, maximal changes in plasma glucose during stress never exceeded 11% of the respective basal values. On induction of stress with ICV carbachol injection, Ra rapidly reached peak levels in the Con (Δ170 ± 45 μmol·kg⁻¹·min⁻¹), PC (Δ95.5 ± 10 μmol·kg⁻¹·min⁻¹), and α (ΔΔ111 ± 24 μmol·kg⁻¹·min⁻¹) groups within 15 min (Fig. 3A). However, the peak increment in Ra (Δ56 ± 14 μmol·kg⁻¹·min⁻¹) in the α+β group was substantially lower (P < 0.05) than those in the Con, PC, and α groups (Fig. 3A). In the β group, the peak of Ra was delayed and rose to Δ101 ± 31 μmol·kg⁻¹·min⁻¹ at 50 min of stress. Poststress Ra gradually reverted to respective baseline values in all groups except for α+β, in which the decline of Ra was much slower. Ra peaked within the first 20 min of stress in all groups except α+β, in which the maximum increment in Ra was reached at almost 40 min of stress (Fig. 3). The marked peak increment in Ra seen in Con (Δ109 ± 27 μmol·kg⁻¹·min⁻¹) was significantly attenuated in the PC (peak: Δ64 ± 15 μmol·kg⁻¹·min⁻¹), α (peak: Δ63 ± 15 μmol·kg⁻¹·min⁻¹), and α+β (peak: Δ56 ± 39 μmol·kg⁻¹·min⁻¹) groups. The stress-induced peak Ra reverted to respective baseline rates at 120 min in all groups except β, which displayed the greatest increment in Ra (peak: 129 ± 33 μmol·kg⁻¹·min⁻¹) and the slowest decline in poststress
Rd of all the other groups (P < 0.01, Fig. 3). When both insulin and glucagon levels were clamped, the peak increments in MCR in the PC (Δ1.03 ± 0.35 ml·kg⁻¹·min⁻¹) and α (Δ1.00 ± 0.23 ml·kg⁻¹·min⁻¹) groups were 30% lower (P < 0.05) than that observed in the Con group (Δ1.52 ± 0.18 ml·kg⁻¹·min⁻¹). In comparison with the PC group, in the α+β group the peak increment in MCR was significantly (P < 0.01) augmented (1.7-fold). The increment in MCR during stress with β peaked at Δ2.76 ± 0.72 ml·kg⁻¹·min⁻¹, which was much greater than in all other groups.

Another analysis of absolute values during the stress period was performed and compared with that of net changes (Δ values). It was found that both methods reveal that glucose production is greatest in Con (P < 0.05) and decreased with combined blockade (P < 0.05). Also, glucose clearance is greater (P < 0.05) in β and α+β than in all other groups.

Hormones and metabolites. On ICV administration of carbachol, arterial epinephrine and norepinephrine levels increased (P < 0.05) in all groups (Table 2). The rise in epinephrine was greater (P < 0.01) when β-adrenoceptor was blocked with α + β (4-fold) and β (4.4-fold) compared with other groups (1.7-fold in PC, 2.9-fold in α, and 2.8 fold in Con). Norepinephrine levels rose (P < 0.05) during the entire stress period from basal values in the Con (1.6-fold), PC (1.2-fold), and β (1.7-fold) groups, whereas the largest increment was observed when α-blockade was applied (1.9-fold in α and 2.8 fold in α+β). ICV carbachol resulted in rapid two- to threefold elevations (P < 0.01) in arterial plasma ACTH, an index of central mediation of the stress response, in all groups (Table 1). The stress response was also characterized by significant (P < 0.05) cortisol increments in the α (4.4-fold), β (5.7-fold), PC (1.5 fold), and Con (4-fold) groups (Table 1). The increment in the α+β group (66 ± 17 to 84 ± 10 nM) did not reach significance. The induction of stress was associated with increments of FFA in the Con (898 ± 70 to 1,178 ± 65 µeq/l, P < 0.05) and α (722 ± 91 to 1,064 ± 74 µeq/l, P < 0.05) groups (Fig. 4). The increment in FFA was blunted in the α+β (580 ± 47 to 681 ± 63 µeq/l) and PC (654 ± 44 to 746 ± 49 µeq/l) groups. During stress, there was a small rise in glycerol (PC, 88 ± 5 to 128 ± 7 µM P < 0.05) (Fig. 5). With α, the glycerol profile, parallel to that of FFA, increased from 87 ± 8 to 168 ± 12 µM (P < 0.05). However, β-blockade resulted in a fall (656 ± 64 to 440 ± 29 µeq/l, P < 0.05) in FFA levels and prevented a stress-induced rise in glycerol levels (91 ± 8 to 84 ± 4 µM). The observed increment of arterial glycerol in the Con group during stress (100 ± 6 to 187 ± 13.3 µM, P < 0.05) was reduced (P < 0.05) in the PC (88 ± 4.5 to 129 ± 7 µM, P < 0.05) and α+β (99 ± 16 to 125 ± 13 µM, P < 0.05) groups. Dramatic rises (2- to 3-fold, P < 0.01) in arterial lactate levels were observed during stress (0–120 min) in the PC, α, and Con groups (Fig. 6). However, most strikingly, β and α+β completely prevented any stress-induced increment in lactate levels, suggesting a reduction in glycogenolysis due to inhibition of β-adrenergic activities.

Hemodynamic data. β-Blockade increased (P < 0.05) basal systolic, diastolic, and mean arterial pressure above those in the other three groups (Table 3). Stress induced increases (P < 0.01) in systolic (152 ± 10 to 176 ± 5 mmHg), diastolic (126 ± 13 to 151 ± 5 mmHg), and mean arterial pressure (135 ± 12 to 159 ± 5 mmHg) in the α+β group. During stress, systolic, diastolic, and mean arterial pressure were greater (P < 0.05) in α+β and β groups than in the other two groups. α-Blockade during stress resulted in an increased heart rate (110 ± 11 to 193 ± 12 beats/min, P < 0.001).

**DISCUSSION**

Cholinergic mechanisms originating in the circumventricular neurons play a physiological role in the central regulation of glucose metabolism (21). ICV administration of carbachol, an acetylcholine analog, induces changes in neuroendocrine activity with increased re-
lease of counterregulatory hormones similarly to general stress (9, 30, 31). We hypothesize the involvement of a putative efferent pathway activated by the circumventricular hypothalamic neurons during cholinergic stimulation. Our present study aims at delineating the roles of insulin, glucagon, the catecholamines, and their receptors in the control of $R_a$, MCR, and $R_d$ during centrally induced stress. Owing to baseline variations associated with all five experimental approaches, Δ values, reflecting net changes from baseline values during stress, were used for statistical analyses and comparisons. The baseline variations were due to the effects of adrenergic blocker infusions started during the basal period. The elevated basal glucose production, uptake, and clearance rates with β- and α + β-blockade presumably reflect increased metabolic fuel reliance on glucose because of β-blockade-induced inhibition of lipolysis and muscle glycogenolysis. Thus, with the use of Δ values, the effects of each experimental condition on glucose metabolism during stress are separated from their baseline effects. Analyses with Δ values allow for quantitative assessment of the stress-
induced net changes in glucose turnover against the uneven basal background of each group. The effects of pancreatic hormonal clamping on glucose turnover during stress. In a previous study, R_d markedly increased during ICV carbachol stress in the normal dog, whereas plasma insulin increased only marginally (31). Because even a small increase in insulin could have a significant impact on whole glucose uptake during exercise, another form of stress (40), the use of a PC, was necessary to identify the contribution of insulin in this form of central stress. The stress-induced increases in glucose uptake and clearance observed in the Con group were only modestly reduced when stable insulin levels were maintained by the PC, indicating that the increases were largely insulin independent. The elevated lactate in both the Con and PC groups suggests increased glycolysis in skeletal muscle. Intracellular glucose derived from both enhanced glucose uptake and muscle glycogenolysis fuels glycolysis in excess of glucose oxidation, resulting in increased lac-

Table 2. Effects of ICV 5 μg of carbachol injected at time 0 on plasma concentrations of epinephrine, norepinephrine, ACTH and cortisol in control, PC-, PC + α-blockade, PC + β-blockade, and PC + α + β-infused normal dogs

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<td>α*</td>
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<td>101 ± 25</td>
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<tr>
<td>β††</td>
<td>20 ± 1</td>
<td>44 ± 17</td>
<td>63 ± 18</td>
<td>140 ± 31</td>
<td>182 ± 44</td>
<td>136 ± 41</td>
</tr>
<tr>
<td>α + β††</td>
<td>65 ± 11</td>
<td>65 ± 28</td>
<td>73 ± 28</td>
<td>113 ± 17</td>
<td>105 ± 20</td>
<td>67 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10, 7, 6, 5, and 5 dogs in the control, PC, α, β, and α + β groups, respectively. *Difference between basal and stress periods, P < 0.05. †Different from PC group during basal period (−40 to 0 min), P < 0.05. ‡Different from PC group during ICV-carbachol-induced stress period (0–120 min, P < 0.05).
tate release into the circulation. Although high FFA levels can limit $R_d$, a fourfold rise in FFA levels is required to decrease insulin-mediated glucose uptake by ~20% (51). Therefore, the limited increase in lipolysis, evidenced by FFA and glycerol levels, was unlikely to substantially decrease the stress-induced increment in glucose clearance in the PC group. Glycerol increased to a greater extent than FFA with PC, presumably reflecting augmented FFA reesterification. The elevated lactate levels and a trend in reesterification indicate that the increase in glucose clearance occurs in muscle and adipocytes (30). This model of stress appears to be similar to exercise, where increases in muscle glucose clearance are independent of insulin increments (14, 41).

The peak increase in $R_d$ in the PC group, in which glucagon was clamped at basal levels, amounted to one-half of that in the Con group, indicating that the rise in glucagon accounts for ~50% of $R_d$ during the initial phase of stress. Others have shown that acute (first 15 min) infusions of glucagon (48) increase $R_d$ primarily through enhanced glycogenolysis.

Effects of adrenergic blockade on $R_d$ and MCR during stress. In our previous experiments (30), ICV injection of a somatostatin analog before ICV carbachol both decreased the release of catecholamines and prevented the rise in glucose uptake. Although an increase in epinephrine directly decreases MCR in dogs and in humans in a dose-dependent fashion (16, 36), some in vitro studies indicate a dual effect of epinephrine, whereby it inhibits $R_d$ at low doses but stimulates $R_d$ at higher doses (5). Also, $\beta$-adrenergic stimulation with isoproterenol increased glucose uptake in rat epitrochlearis muscle incubated with albumin (60). Long-term exposure to norepinephrine resulted in stimulation of both basal and insulin-stimulated glucose uptake (26) in the rat.

The purpose of the adrenergic blockade during stress was twofold: to ascertain whether catecholamines stimulate or inhibit glucose uptake and to investigate the role of catecholamines in control of $R_d$. During moderate exercise, which has very similar increments of catecholamines as the type of stress in the present study, it is mainly glucagon-insulin interaction that controls $R_d$ (41, 53). Thus a bihormonal clamp was essential to assess the contribution of catecholamines without the confounding effect of glucagon. Our findings that MCR is enhanced in the $\alpha+\beta$ group above that in the PC group despite the same insulin levels are in agreement with those of others who have shown that epinephrine suppresses insulin-mediated glucose uptake (3). Total adrenergic blockade circumvented at least some inhibitory effects of adrenergic output on $R_d$, thus enhancing MCR.

The effect of catecholamines in suppressing glucose uptake from the circulation may be due, in part, to the stimulation of muscle glycogenolysis and accumulation of intracellular glucose-6-phosphate. Suppression of muscle glycogenolysis during $\beta$- and combined $\alpha$- and $\beta$-adrenergic blockade is associated with inhibition of lactate production despite increased MCR, suggesting that increased lactate was associated with increased glycogenolysis and not with MCR. Adrenergic blockade and “clamped” glucagon lead to lower glucose levels, $R_d$, and $R_d$. The full impact of adrenergic blockade on glucose extraction is revealed when glucose uptake is measured as MCR rather than $R_d$. Adrenergic blockade greatly enhanced glucose extraction (MCR) from the circulation despite the lower glycemia and lesser increments in glucose uptake.

With insulin and glucagon clamped, $\beta$-blockade alone augments stress-induced $R_d$ (2-fold) and MCR (2-fold) more than PC and combined adrenergic blockade. Epinephrine impairs glucose effectiveness and insulin
sensitivity (1) through activation of β-adrenoreceptors (23). Insulin resistance during stress is in part related to adrenergic stimulation of lipolysis and muscle glycogenolysis (8). Reductions in FFA levels induce greater $R_d$ acutely (32) and chronically (7). Suppression of glycogenolysis has been implicated in the enhanced MCR in exercising dogs in our previous studies (40, 54, 58). Therefore, the enhanced increment in MCR with β-blockade could be related, in part, to both the attenuation in the FFA-glucose cycle (34) and to a suppression of glycogenolysis, evidenced by diminished FFA, glycerol, and lactate levels. Such an effect of β-blockade has also been observed during insulin-induced hypoglycemia in dogs (20).

Disengaging the α-adrenoreceptors with α-blockade did not change the patterns of increments in $R_d$ and MCR established by the PC. Our result is supported by other studies, in which the α-adrenoreceptor had little or no impact on catecholamine-mediated antagonism of insulin action on MCR (38, 56). Also, α-blockade had no apparent effect on muscle glycogenolysis, as evidenced by lactate levels similar to those in the PC group. In one report it was indicated that α-adrenergic activation can increase MCR (35). The finding that β-blockade had a somewhat larger effect on enhancing MCR than did a double blockade suggests an α-adrenoreceptor-mediated stimulation of MCR, yet α-blockade alone did not affect MCR. We hypothesize that α-adrenergic stimulation of glucose uptake may become manifest under conditions of β-blockade and inhibited muscle glycogenolysis. This illustrates the necessity of studying both single and double blockades to gain in-depth insight into the regulatory role of catecholamines.

An analysis of the absolute values during the stress period was also performed, which resulted in qualitatively similar conclusions to those of Δ value comparisons. Both methods reveal that glucose production is greatest in the control group and decreased with combined blockade and that glucose clearance is greatest in the β- and α+β groups than in all other groups. Thus both analyses using absolute and Δ values resulted in the same conclusions.

α-Blockade raised heart rate without affecting blood pressure during stress, suggesting an enhanced $\beta_1$ cardiac effect in response to augmented sympathoadrenal output. The elevated systolic, diastolic, and mean arterial pressure observed with β-blockade during both basal and stress periods may reflect an acute suppression of vasodilative β-$\delta$-receptors and a compensatory sympathetic reflex that activates vasoconstrictive α-adrenoreceptors (19). With the combined blockade, the observed effects of β-blockade were largely obviated in the basal state and were slightly attenuated during stress, due to the vasodilative effect of α-blockade. Blood flow has also been shown to independently modulate insulin-mediated glucose uptake (2). We did not measure blood flow and therefore are limited in our conclusions in that respect. However, acute β-blockade is known to decrease cardiac output and increase peripheral resistance (19). The observed greater rates of glucose utilization during stress with β-blockade are thus not attributable to its cardiovascular actions.

Effect of adrenergic blockade on $R_a$ during stress. Our study outlined the roles of glucagon and catecholamines in control of $R_a$ during stress. In the early phase of stress, glucagon clamp (as seen with PC) suppressed Δ$R_a$ by 44%, but combined glucagon clamp and double adrenergic blockade suppressed Δ$R_a$ by 80% compared with the Con group. The difference between the two protocols indicates the contribution of the catecholamines, presumably via glycogenolysis (47). The greater increment in circulating catecholamine levels observed in the α+β than in the Con, PC, and either α or β groups is due to a reduction in catecholamine binding and clearance. Plasma catecholamines are only a rough indicator of sympathoadrenergic activation (4) and during blockade cannot be associated with changes in $R_a$. However, even in the absence of the adrenergic blockade, the peak levels of infused epinephrine lag behind the $R_a$ increments (47). It is surprising that there is a residual increase in $R_a$, despite the bimodal clamp and α-β-blockade. This may reflect activity of other stress hormones such as vasopressin, which increases in stress (30) and may stimulate $R_a$ (12). It is also conceivable that hepatic autoregulation is triggered by the small decline in glycemia. Cortisol is not an important factor in control of rapid changes in $R_a$, as it is a slow-acting hormone.

α-Adrenergic activation inhibits lipolysis (55), and the effect of α-blockade is clearly confirmed as stress-induced lipolysis was enhanced, as evidenced by an accentuated rise in FFA and glycerol. The effect of β-blockade is clearly confirmed by the complete inhibition of the stress-induced increments in lactate, glycerol, and fatty acids, all metabolites that are elevated with β-adrenergic stimulation. β-Adrenergic blockade delayed the initial peak of $R_a$. The finding that the $R_a$ increment is greatly diminished with combined adrenergic blockade and not with β-blockade alone suggests that the increment in $R_a$ is sustained, in part, by α-mediated $R_a$, a novel finding in the dog. Indeed, α-adrenergic-mediated $R_a$ has been shown in humans (37, 38) and in vitro with perfused liver and isolated hepatocytes of the rat (39), rabbit (59), and cat (25). Thus α- and β-receptors may represent two complementary backup systems in maintaining $R_a$. Either blockade could cause an increase in circulating catecholamines, which can potentiate the other nonblocked (backup) receptor subtype(s). This is how we interpret the fact that β-blockade alone delayed but did not diminish the $R_a$ peak and that α-blockade did not affect $R_a$, whereas the double blockade resulted in a substantial decrease in $R_a$. Failure of β-blockade per se to inhibit $R_a$ was also demonstrated in healthy human subjects during strenuous exercise (43). Our finding that α-blockade resulted in higher norepinephrine levels than observed in both the PC and β groups is in agreement with others who have shown that such blockade relieves presynaptic inhibition of norepinephrine release and reduces norepinephrine clearance (44). Indeed, α-adrenergic blockade not only reveals unop-
posed β-adrenergic receptor effects but may even augment them.

Conclusions. In the ICV carbamyl model of stress, the increment in MCR is largely independent of changes in insulin and is enhanced with combined adrenergic blockade. β-Blockade enhances stress-induced MCR, presumably through inhibition of lipolysis and muscle glycogenolysis, to a much greater extent than with combined blockade, and it unmasks the full impact of the insulin-independent neuroendocrine stimulation of glucose uptake in vivo. The effect of β-blockade in enhancing glucose utilization during stress does not appear to be dependent on its cardiovascular actions. The neuroendocrine mechanism responsible for the insulin-independent stress-induced increments in glucose uptake needs further identification. Both catecholamines and glucagon play an equal role in stimulating the early-phase increment in $R_\alpha$, whereas the increment in the later phase is mostly mediated by glucagon. However, a small remaining portion of the increment in $R_\alpha$ is not accounted for by either hormone. Combined α- and β-blockade, but not α- or β-blockade per se, diminished increments in $R_\alpha$ during stress. This suggests a complementary backup mechanism of the α- and β-adrenergic receptors in stimulation of glucose production during stress.

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