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

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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 increment in glucose metabolic clearance rate (MCR) and glucose production (Ra), we used five groups of normal mongrel dogs: 1) pancreatic clamp (PC; n = 7) with peripheral somatostatin (0.0 µg · kg⁻¹ · min⁻¹) and intraportal replacement of insulin (1.48 ± 0.84 pmol · kg⁻¹ · min⁻¹) and glucagon (0.65 ng · kg⁻¹ · min⁻¹) infusions; 2) PC plus combined (propranolol- and β) (propranolol)-blockade (5 µg · kg⁻¹ · min⁻¹, respectively; α + β; 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 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. Ra 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 Ra is augmented by glucagon and α- and β-catecholamine effects.

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is governed primarily by the glucagon-to-insulin (gluca-
gon/insulin) ratio (41, 53). In strenuous exercise, how-
ever, catecholamines rather than the glucagon/insulin
ratio (42) become the main regulators of R_a. In this
centrally induced model of stress, it is not known
whether the increased R_a 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 catechol-
amine effects in modulating R_a and R_g, 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 R_a through different
second messengers. Selective inhibition of one adreno-
captor type with consequential changes in R_a would
allow us to determine contributions of each adrenocep-
tor 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 R_g 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 R_a. 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 (fluenthane and
nitrous oxide) and assisted ventilation, an ICV stainless
22-gauge guide cannula was implanted into the third ven-
tricle of the brain by using a canine stereotaxic apparatus.
The patency of the cerebral cannula was verified by aspira-
tion 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 (Romer 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 mini-
mum period of 1 wk was allowed between successive exper-
iments. 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 previ-
ously reported carbachol control group (31) was supple-
mented with three additional experiments (Con, n = 10). No
differences were observed between present and former con-
tral subjects in values of hormones and metabolites [insulin,
glucagon, norepinephrine, epinephrine, cortisol, free fatty
acids (FFA), and glycerol], and glucose turnover (R_a, R_g)
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 infu-
sions. 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; n = 6). 5) Another group received a β-blocker (proprano-
lol, 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 can-
nula connected to a 100-µl Hamilton microsyringe via poly-
hylene 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, norepi-
 nephrine, 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 poly-
ethylene 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 spe-
cific activity was derived from plasma glucose concentration
and [3-3H]glucose radioactivity determined in a liquid scintil-
lation counter after deproteinization of the plasma samples
with Ba(OH)₂ and ZnSO₄ and evaporation of the supernatant
Insulin and glucagon levels. Basal insulin levels were slightly higher (P < 0.05) in the Con (75.6 ± 5.0 µM) and β-blockade (79.7 ± 11.5 µM) vs. the PC (57.0 ± 3.6 µM) and α-blockade (59 ± 8.4 µM) 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 by 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 µM), β (79.2 ± 11.4 vs. 82.8 ± 8.4 µM), and α+β (69.6 ± 5.4 vs. 68.4 ± 4.5 µM) 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 µM, 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 Con (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 (Δ111 ± 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 substantively 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 (1.03 ± 0.35 ml·kg⁻¹·min⁻¹) and α (1.00 ± 0.23 ml·kg⁻¹·min⁻¹) groups were 30% lower (P < 0.05) than that observed in the Con group (1.52 ± 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 < 0.05) during the entire stress period was performed and compared with that of net metabolic clearance rate of glucose) before ICV carbachol injection (time 0).

Table 1. Basal period (-40 to 0 min) glucose turnover data (plasma glucose, glucose production, glucose uptake, and metabolic clearance rate of glucose) before ICV carbachol injection (time 0).

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>PC (n = 7)</th>
<th>α (n = 6)</th>
<th>β (n = 5)</th>
<th>α+β (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mM</td>
<td>5.8 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>5.9 ± 0.2</td>
<td>6 ± 0.14</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>Glucose production, µmol·kg⁻¹·min⁻¹</td>
<td>147 ± 6†</td>
<td>135 ± 5†</td>
<td>128 ± 9†</td>
<td>178 ± 15</td>
<td>176 ± 17</td>
</tr>
<tr>
<td>Glucose uptake, µmol·kg⁻¹·min⁻¹</td>
<td>142 ± 7†</td>
<td>132 ± 4†</td>
<td>134 ± 9†</td>
<td>180 ± 16</td>
<td>195 ± 17</td>
</tr>
<tr>
<td>Metabolic clearance rate of glucose, ml·kg⁻¹·min⁻¹</td>
<td>133 ± 6†</td>
<td>152 ± 6†</td>
<td>128 ± 8†</td>
<td>167 ± 14</td>
<td>192 ± 18</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of dogs; ICV, intracerebroventricular; PC, pancreatic clamp; α, β, and α + β, PC + α, β-, and combined blockade, respectively. *Different from PC + β, P < 0.05. †Different from PC + α + β, P < 0.05.

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 α, 2.9-fold in β, 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 obtained 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 (88 ± 70 to 1,178 ± 65 µg/l, P < 0.05) and α (722 ± 91 to 1,064 ± 74 µg/l, P < 0.05) groups (Fig. 4). The increment in FFA was blunted in the α+β (580 ± 47 to 681 ± 63 µg/l) and PC (654 ± 44 to 746 ± 49 µg/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 µg/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 Rd, MCR, and Ra 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-
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

<table>
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<tr>
<th></th>
<th>Basal (−40 to 0)</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>120</th>
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<tr>
<td>Epinephrine, nM</td>
<td></td>
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<tr>
<td>Control†‡</td>
<td>0.7 ± 0.05</td>
<td>1.6± 0.4</td>
<td>1.8±0.3</td>
<td>2.3±0.5</td>
<td>2.1±0.3</td>
<td>1.9±0.7</td>
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<tr>
<td>PC*</td>
<td>1.3 ± 0.1</td>
<td>3.1± 0.9</td>
<td>2.3±1.0</td>
<td>2.1±0.8</td>
<td>1.6±0.5</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>α*</td>
<td>1.0 ± 0.3</td>
<td>3.5± 1.5</td>
<td>4.6±2.2</td>
<td>2.8±1.2</td>
<td>1.8±0.6</td>
<td>1.6±0.6</td>
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<tr>
<td>β*</td>
<td>0.8 ± 0.1</td>
<td>2.1± 0.5</td>
<td>4.8±1.7</td>
<td>5.6±2.4</td>
<td>3.1±1.3</td>
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<tr>
<td>α + β†‡</td>
<td>1.8 ± 0.2</td>
<td>5.9± 0.2</td>
<td>6.2±0.2</td>
<td>11±4.2</td>
<td>8.7±2.7</td>
<td>3.8±1.4</td>
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<td>Norepinephrine, nM</td>
<td></td>
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<tr>
<td>Control†‡</td>
<td>1.1 ± 0.1</td>
<td>1.7± 0.3</td>
<td>1.8±0.3</td>
<td>1.7±0.3</td>
<td>1.6±0.2</td>
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<tr>
<td>PC*</td>
<td>1.9 ± 0.2</td>
<td>3.0± 0.4</td>
<td>2.1±0.3</td>
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<td>α*</td>
<td>2.0 ± 0.7</td>
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<tr>
<td>β*</td>
<td>1.5 ± 0.2</td>
<td>2.1± 0.5</td>
<td>2.5±0.7</td>
<td>3.3±1.1</td>
<td>2.7±1.0</td>
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<tr>
<td>α + β†‡</td>
<td>2.4 ± 0.6</td>
<td>7.5± 1.4</td>
<td>7.0±2.0</td>
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<td>ACTH, pM</td>
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<td></td>
<td></td>
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<tr>
<td>Control*</td>
<td>4.2 ± 0.2</td>
<td>4.5± 0.7</td>
<td>5.2±1.1</td>
<td>10.6±0.9</td>
<td>12.7±2.4</td>
<td>8.7±1.8</td>
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<tr>
<td>PC*</td>
<td>6.1 ± 0.5</td>
<td>12.0±6.3</td>
<td>16.5±8.0</td>
<td>20.0±10.7</td>
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<td>12.5±5.2</td>
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<td>α*</td>
<td>9.5 ± 0.8</td>
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<td>30.5±15.1</td>
<td>28.9±10.6</td>
<td>42.4±18.9</td>
<td>25.1±7.6</td>
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<tr>
<td>β*</td>
<td>3.9 ± 0.2</td>
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<td>7.4±2.8</td>
<td>14.2±4.1</td>
<td>15.0±6.8</td>
<td>5.5±1.7</td>
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<tr>
<td>α + β*</td>
<td>4.5 ± 0.1</td>
<td>5.0± 0.8</td>
<td>12±5.0</td>
<td>19±9.0</td>
<td>18±11</td>
<td>19±14</td>
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<tr>
<td>Cortisol, nM</td>
<td></td>
<td></td>
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<tr>
<td>Control†‡</td>
<td>32 ± 3</td>
<td>40± 9</td>
<td>121±23</td>
<td>176±23</td>
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<tr>
<td>PC*</td>
<td>58 ± 4</td>
<td>58± 25</td>
<td>101±25</td>
<td>119±33</td>
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<td>69± 18</td>
<td>88±23</td>
<td>105±25</td>
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<tr>
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<td>20 ± 1</td>
<td>44± 17</td>
<td>63±18</td>
<td>140±31</td>
<td>182±44</td>
<td>136±41</td>
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<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).

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-

![Fig. 4. Effects of ICV carbachol injection on free fatty acid (FFA) increments in Con, PC, α, β, and α + β groups. Experimental design and symbols are as outlined in Fig. 1.](image-url)

![Fig. 5. Effects of ICV carbachol injection on plasma concentration of plasma glycerol levels in Con, PC, α, β, and α + β groups. Experimental design and symbols are as outlined in Fig. 1.](image-url)
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 $R_a$ 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 $R_a$ during the initial phase of stress. Others have shown that acute (first 15 min) infusions of glucagon (48) increase $R_a$ primarily through enhanced glycogenolysis.

Effects of adrenergic blockade on $R_a$ 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 $R_a$ at low doses but stimulates $R_a$ at higher doses (5). Also, β-adrenergic stimulation with isoproterenol increased glucose uptake in rat epi-theleares 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 $R_a$. 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 $R_a$ (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 $R_a$, 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 β- and combined α- and β-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, $R_a$, and $R_d$. The full impact of adrenergic blockade on glucose extraction is revealed when glucose uptake is measured as MCR rather than $R_a$. 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, β-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-adrenoceptors (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-adrenoceptors 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-adrenoceptor 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-adrenoceptor-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 greater 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 β_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_2-receptors and a compensatory sympathetic reflex that activates vasoconstrictive α-adrenoceptors (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 ΔR_a by 44%, but combined glucagon clamp and double adrenergic blockade suppressed ∆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 bihormonal 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 effects but may even augment them.

Conclusions. In the ICV carbachol 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 glucose uptake needs further identification. Both catecholamines and glucagon play an equal role in stimulating glucose uptake. The effect of catecholamines and glucagon on glucose uptake is mediated mainly through inhibition of lipolysis and muscle glycogenolysis.

The authors are grateful to D. Bilinski and L. Lam for excellent technical assistance. This work was supported by separate grants from the Medical Research Council of Canada (MRC) to M. Vranic and Z. Q. Shi and from the Juvenile Diabetes Foundation International and Canadian Diabetes Association (CDA) to M. Vranic. Z. Q. Shi was the recipient of a Postdoctoral Fellowship from the CDA and a Scholarship from the Juvenile Diabetes Foundation International and Canadian Medical Research Council of Canada (MRC) to M. Vranic and Z. Q. Shi and from the Juvenile Diabetes Foundation International and Canadian Medical Research Council of Canada (MRC).

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