Hepatic glucose autoregulation: responses to small, non-insulin-induced changes in arterial glucose

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Camacho, Raul C., D. Brooks Lacy, Freyja D. James, Robert H. Coker, and David H. Wasserman. Hepatic glucose autoregulation: responses to small, non-insulin-induced changes in arterial glucose. Am J Physiol Endocrinol Metab 287: E269–E274, 2004.—The purpose of this study was to determine whether the sedentary dog is able to autoregulate glucose production (Ra) in response to non-insulin-induced changes (<20 mg/dl) in arterial glucose. Dogs had catheters implanted >16 days before study. Protocols consisted of basal (−30 to 0 min) and bilateral renal arterial phloridzin infusion (0–180 min) periods. Somatostatin was infused, and glucagon and insulin were replaced to basal levels. In one protocol (Phl), glucose was allowed to fall from t = 0–90 min. This was followed by a period when glucose was infused to restore euglycemia (90–150 min) and a period when glucose was allowed to fall again (150–180 min). In a second protocol (EC), glucose was infused to compensate for the renal glucose loss due to phloridzin and maintain euglycemia from t = 0–180 min. Arterial insulin, glucagon, cortisol, and catecholamines remained at basal in both protocols. In Phl, glucose fell by ∼20 mg/dl by t = 90 min with phloridzin infusion. Ra did not change from basal in Phl despite the fall in glucose for the first 90 min. Ra was significantly suppressed with restoration of euglycemia from t = 90–150 min (P < 0.05) and returned to basal when glucose was allowed to fall from t = 150–180 min. Ra did not change from basal in EC. In conclusion, the liver autoregulates Ra in response to small changes in glucose independently of changes in pancreatic hormones at rest. However, the liver of the resting dog is more sensitive to a small increment, rather than decrement, in arterial glucose.

glucose turnover; phloridzin

THE ABILITY TO COMPENSATE for physiological increases in glucose utilization (Ra; e.g., exercise or insulin) is critical to preventing severe hypoglycemia. Compensation to frank hypoglycemia occurs via neural, hormonal, and autoregulatory responses. Individuals with type 1 diabetes mellitus (T1DM) can have an altered hypoglycemic counterregulatory hormone response (7, 10), as well as an absence of hormone-independent counterregulation (22). A rise (29) or fall (5, 29) in the level of glyceremia has been suggested as a stimulus to endogenous counterregulation (22). A rise (29) or fall (5, 29) in the level of glyceremia has been suggested as a stimulus to endogenous counterregulation (22). A rise (29) or fall (5, 29) in the level of glyceremia has been suggested as a stimulus to endogenous counterregulation (22). A rise (29) or fall (5, 29) in the level of glyceremia has been suggested as a stimulus to endogenous counterregulation (22). A rise (29) or fall (5, 29) in the level of glyceremia has been suggested as a stimulus to endogenous counterregulation (22).

MATERIALS AND METHODS

Animals and surgical procedures. Experiments were performed on a total of 12 overnight-fasted mongrel dogs (mean wt 22.7 ± 0.7 kg) of either sex that had been fed a standard diet (Pedigree beef dinner and Wayne Lab Blox, 51% carbohydrate, 31% protein, 11% fat, and 7% fiber based on dry wt). The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the Vanderbilt University Animal Care Subcommit-tee approved the protocols. At least 16 days before each experiment a laparotomy was performed under general anesthesia (0.04 mg/kg atropine and 15 mg/kg pentobarbital sodium presurgery and 1.0% isoflurane inhalation anesthetic during surgery). Catheters were inserted into the portal vein and hepatic vein for blood sampling purposes. An incision was made over the femoral artery into which a Silastic catheter (0.04 in. ID) was inserted and advanced to the level of the distal aorta (16 cm) for sampling and hemodynamic measurements during experiments. Silastic catheters (0.03 in. ID) were inserted into the vena cava for infusion purposes. A Silastic catheter (0.03 in. ID) was inserted into the splenic vein and positioned so that the catheter tip rested just beyond the point where the splenic and portal veins coalesce. This catheter was used for the intraportal infusions of glucagon and insulin. Last, Silastic catheters (0.025 mm ID) were inserted into the left and right renal arteries and advanced 0.5 cm for the infusion of phloridzin. Catheters were filled with heparinized saline and the free ends knotted. Ultrasonic transit time flow probes were fitted and secured to the portal vein and hepatic artery (Transonic Systems, Ithaca, NY). The knotted catheter ends and flow probe leads were stored in a subcutaneous pocket in the abdominal

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region (except for the carotid artery catheter, which was stored in a pocket under the skin of the neck), so that complete closure of the skin incisions was possible.

Blood samples were drawn three days before the experiment to determine the leukocyte count and the hematocrit of the animal. Only animals with a 1) leukocyte count <18,000/mm³, 2) a hematocrit >36%, 3) a good appetite (consumption of daily food ration), and 4) normal stools were used.

All studies were conducted in dogs after an 18-h fast. The free catheter ends and flow probe leads were accessed through small skin incisions made under local anesthesia (2% lidocaine) in the abdominal and neck regions the morning of the experiment. The contents of the catheters were then aspirated and flushed with saline.

Experimental procedures. Experiments consisted of a tracer equilibration period (−130 to −30 min), a basal period (−30 to 0 min), and a bilateral renal artery phloridzin infusion (8 μg·kg⁻¹·min⁻¹ per kidney) period (0−180 min). In one protocol (Phl ± Glc, n = 6), glucose was allowed to decrease (<20 mg/dl) to levels above the counterregulatory response threshold (13). This was followed by a euglycemic period (90−150 min) when glucose levels were returned to levels seen at 0 min, and a second period (150−180 min) when glucose was allowed to fall again (Fig. 1). In a second protocol (EC, n = 6), glucose was clamped at levels seen at 0 min for the duration of the study. All dogs were studied during a pancreatic clamp. A peripheral infusion of somatostatin (0.8 μg·kg⁻¹·min⁻¹) was started at −130 min to inhibit endogenous insulin and glucagon secretion in all groups. Concurrent with the infusion of somatostatin was an intraportal glucagon replacement (0.5 ng·kg⁻¹·min⁻¹). An intraportal insulin infusion was also initiated at this time. The insulin infusion rate was adjusted before the basal sampling period to maintain euglycemia and remained at this rate for the remainder of the study. A primed (33 μCi) infusion (0.33 μCi/min) of HPLC-purified [3-¹³C]glucose was initiated at −130 min. Constant-rate indocyanine green (0.1 mg·min⁻¹·kg⁻¹) was also started at −130 min and continued throughout the study. Indocyanine green was used as a backup method of blood flow measurement (17) if the Doppler probes did not provide a clear signal and as confirmation of hepatic vein catheter placement. [3-¹³C]glucose was infused in the vena cava to clamp arterial glucose when necessary and to minimize fluctuations in plasma specific activity (11). Portal vein and hepatic artery blood flows were monitored online throughout the experiments. At the end of the experiment, animals were euthanized with pentobarbital sodium, and an autopsy was performed to confirm catheter placement.

Blood sample collection and processing. Arterial, portal vein, and hepatic vein blood samples were drawn at −30, −15, 0, 30, 60, 90, 105, 120, 150, 165, and 180 min in all groups. After 90 and 150 min, arterial samples were taken every 5 min for 20 min and every 10 min throughout the rest of the experiment for the determination of plasma glucose radioactivity. Plasma glucose concentrations were determined by the glucose oxidase method using a Beckman Instruments Glucose Analyzer (Beckman Instruments, Fullerton, CA). For the determination of plasma glucose radioactivity, samples were deproteinized with barium hydroxide and zinc sulfate and centrifuged. The supernatant was then evaporated to remove ¹³H₂O and reconstituted in 1 ml of water and 10 ml of scintillation fluid [Ecolite (+), ICN Biomedicals, Irvine, CA]. Radioactivity was determined on a Packard liquid scintillation counter. Blood samples were deproteinized (1 ml of blood in 3 ml of 4% perchloric acid), and whole blood alanine, glyceral, and lactate concentrations were determined using standard enzymatic methods (18) on a Monarch 2000 Centrifugal Analyzer (Lexington, MA). Free fatty acids (FFA) were measured with the use of the Wako FFA C test kit (Wako Chemicals, Richmond, VA). Immunoreactive insulin was measured using a double-antibody procedure [interassay coefficient of variation (CV) of 16%] (25). Immunoreactive glucagon (3,500 mol wt) was measured in plasma samples by use of a double-antibody system (CV of 7%) modified from the method developed by Morgan and Lazarow (25) for insulin. Blood samples for norepinephrine and epinephrine measurement were collected into tubes containing EGTA and glutathione and centrifuged at 4°C, and plasma was stored at −70°C for subsequent HPLC analysis. Catecholamine concentrations were calculated on a linear regression basis using dihydroxybenzylamine as an internal standard. The CVs using this method were 5% and 7% for norepinephrine and epinephrine, respectively. Plasma cortisol was measured with the Clinical Assays Gamma Coat radioimmunoassay kit (Clinical Assays, Travenol-Genetech Diagnostics, Cambridge, MA) with an interassay CV of 6%.

Materials. [3-¹³C]glucose was obtained from New England Nuclear (Boston, MA). Glucagon and insulin antisera were obtained from Dr. R. L. Gingerich (Washington University School of Medicine, St. Louis, MO). ¹²⁵I-labeled glucagon, ¹²⁵I-insulin, and the standard glucagon and insulin were obtained from Linco Research (St. Louis, MO). Phloridzin, indocyanine green, and enzymes and coenzymes for metabolite analyses were obtained from Boehringer Mannheim Biochemicals and Sigma Chemical.

Calculations. Net hepatic balance of alanine, FFA, glucose, glyceral, and lactate was determined according to the formula: HAF × ([H] − [A]) + PVF × ([H] − [P]), where [A], [P], and [H] are the arterial, portal vein, and hepatic vein concentrations, and HAF and PVF are the hepatic artery and portal vein blood flows. Endogenous Ra and Rg were calculated using the two-compartment approach described by Mari (19).

Statistical analysis. SigmaStat (SPSS, Chicago, IL) software installed on a Gateway PC was used to perform statistical analyses. Statistical comparisons between groups and over time were made using ANOVA designed to account for repeated measures. Specific time points were examined for significance using contrasts solved by univariate repeated measures. Statistics are reported in Figs 3–5. Data are presented as means ± SE. Statistical significance was defined as P < 0.05.

RESULTS

Arterial hormone concentrations. Arterial plasma insulin and glucagon levels were similar between groups and did not change during the entire study (Fig. 2). Arterial plasma cortisol, epinephrine, and norepinephrine were similar between groups and did not change during the entire study (Table 1).

Arterial plasma glucose concentration, specific activity, and kinetics. Arterial plasma glucose was similar between groups during the basal period (Fig. 3). In Phl ± Glc, glucose fell from 99 ± 4 mg/dl at 0 min to 82 ± 3 mg/dl at 90 min (P < 0.05 vs. 0 min). Glucose was infused to achieve levels similar to EC within 15 min and then fell again from 101 ± 2 mg/dl at 150 min to 83 ± 2 mg/dl at 180 min (P < 0.05 vs. 150 min). The mean rate of exogenous glucose infusion required to keep
Phl ± Gc from falling below 80 mg/dl from 0 to 90 min was 0.4 ± 0.1 mg·kg⁻¹·min⁻¹ (Fig. 4). The mean rate of exogenous glucose infusion required to return glucose levels to 0-min values from 90 to 150 min was 2.9 ± 0.1 mg·kg⁻¹·min⁻¹ (P < 0.05 vs. EC). Glucose did not change in EC throughout the study. The mean rate of exogenous glucose infusion required to clamp EC at euglycemia was 1.9 ± 0.1 mg·kg⁻¹·min⁻¹. Net hepatic glucose output (NHGO) was similar between groups and did not change during the entire study (data not shown). [3-¹H]glucose specific activity varied at most by ~20% in both groups (data not shown).

Endogenous Ra was similar during the basal period between groups (Fig. 5A). With the onset of phloridzin infusion in Phl ± Gc, endogenous Ra did not change from basal despite a fall in glucose (P < 0.05 vs. EC). With the onset of exogenous glucose infusion at 90 min, endogenous Ra was significantly suppressed (2.4 ± 0.4 to 1.3 ± 0.4 mg·kg⁻¹·min⁻¹, P < 0.05) but returned to basal with the cessation of exogenous glucose infusion after 150 min. With the onset of phloridzin and exogenous glucose infusion in EC, endogenous Ra tended to be transiently suppressed (for 20 min) from 2.7 ± 0.2 to 1.6 ± 0.2 mg·kg⁻¹·min⁻¹, although this difference did not reach significance. Ra was similar during the basal period between groups (Fig. 5B). Ra initially increased in Phl ± Gc with the onset of phloridzin infusion but declined as arterial plasma glucose levels fell. The infusion of exogenous glucose to restore euglycemia in Phl ± Gc increased Ra to 4.0 ± 0.4 mg·kg⁻¹·min⁻¹.

**Table 1. Arterial plasma cortisol, epinephrine, and norepinephrine levels during baseline and bilateral renal arterial phloridzin infusion periods in the presence and absence of euglycemia**

<table>
<thead>
<tr>
<th>Phloridzin, min</th>
<th>Basal</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>105</th>
<th>120</th>
<th>150</th>
<th>165</th>
<th>180</th>
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<tbody>
<tr>
<td><strong>Arterial plasma cortisol, μg/dl</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EC</td>
<td>2.5±0.3</td>
<td>3.3±0.8</td>
<td>3.0±0.9</td>
<td>3.0±0.8</td>
<td>3.0±0.6</td>
<td>2.7±0.5</td>
<td>2.7±0.5</td>
<td>3.4±0.9</td>
<td>3.3±0.9</td>
</tr>
<tr>
<td>Phl±Gc</td>
<td>2.3±0.4</td>
<td>3.1±0.9</td>
<td>3.0±0.3</td>
<td>3.1±0.4</td>
<td>3.0±0.7</td>
<td>2.7±0.8</td>
<td>2.4±0.3</td>
<td>2.4±0.4</td>
<td>3.7±0.8</td>
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<tr>
<td><strong>Arterial plasma epinephrine, pg/ml</strong></td>
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<td></td>
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<tr>
<td>EC</td>
<td>109±13</td>
<td>118±36</td>
<td>109±21</td>
<td>119±24</td>
<td>119±27</td>
<td>122±42</td>
<td>181±63</td>
<td>169±52</td>
<td>113±31</td>
</tr>
<tr>
<td>Phl±Gc</td>
<td>112±11</td>
<td>93±13</td>
<td>138±29</td>
<td>149±27</td>
<td>152±39</td>
<td>129±24</td>
<td>127±26</td>
<td>106±12</td>
<td>156±38</td>
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<tr>
<td><strong>Arterial plasma norepinephrine, pg/ml</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>136±17</td>
<td>130±21</td>
<td>146±29</td>
<td>174±54</td>
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<td>163±39</td>
<td>159±36</td>
<td>167±49</td>
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<tr>
<td>Phl±Gc</td>
<td>123±11</td>
<td>127±23</td>
<td>158±21</td>
<td>155±26</td>
<td>140±21</td>
<td>126±25</td>
<td>161±23</td>
<td>153±19</td>
<td>196±17</td>
</tr>
</tbody>
</table>

Values are means ± SE. EC, euglycemic clamp; Phl ± Gc, phloridzin with or without glucose.
mg·kg\(^{-1}\)·min\(^{-1}\) due to a mass action effect. \(R_d\) declined again with the cessation of exogenous glucose infusion and subsequent fall in glucose from 150 to 180 min. \(R_a\) increased and remained elevated at 3.9 ± 0.1 mg·kg\(^{-1}\)·min\(^{-1}\) in EC.

Arterial concentrations and net hepatic balances of alanine, FFA, glycerol, and lactate. Arterial concentrations and net hepatic balances of alanine, FFA, glycerol, and lactate were similar between groups and did not change during the entire study (data not shown).

**DISCUSSION**

Autoregulation of \(R_a\) is impaired in non-insulin-dependent diabetes mellitus (21), contributing to hyperglycemia, as well as in T1DM (22), contributing to the pathogenesis of hypoglycemia unawareness, recurrent hypoglycemia, and further impairment of glucose counterregulation (7). Previous studies in normal rats (27), dogs (28), and humans (29) have shown that \(R_a\) is inhibited by hyperglycemia and is stimulated by hypoglycemia independently of changes in pancreatic hormones (2, 5, 6, 29). \(R_a\) has been shown to be stimulated by a mechanism independent of pancreatic hormones and hepatic adrenergic stimulation during an exercise-induced increase in \(R_d\), preventing overt hypoglycemia (4). The present study extends a role for autoregulation to rest, as exemplified by an inhibition of \(R_a\) in the presence of a small increment in glucose in the absence of hyperglycemia.

There are few models of non-insulin-induced decreases in glucose. Among them are exercise and glycogen phosphorylase inhibitors. To cause the small decrement in glucose in this study, \(R_a\) was increased by a bilateral renal arterial infusion of phloridzin to block tubular glucose reabsorption. Therefore, the metabolic environment that accompanies hyperinsulinemia-induced (3, 8, 9) or exercise-induced (23, 30, 31) increases in \(R_d\) were circumvented, whereas the ability to investigate autoregulation remained. Increasing \(R_d\) (within a physiological range) with the use of phloridzin, although different from physiological increases in \(R_d\) created by insulin or exercise, enabled us to isolate an autoregulatory mechanism. SGLT1, the transporter blocked by phloridzin, is expressed exclusively in cholangiocytes of the biliary epithelia and not in hepatocytes (16). Kolodny et al. (15) showed that phloridzin does not act directly on the dog liver to affect hepatic glucose output. Phloridzin was infused systemically at 45 \(\mu\text{g}·\text{kg}^{-1}·\text{min}^{-1}\) in the aforementioned study, as opposed to the infusion rate of 8 \(\mu\text{g}·\text{kg}^{-1}·\text{min}^{-1}\) directly into each renal artery used in the current study. Thus levels of phloridzin seen at the liver were at most about threefold higher than in the current study. Although it produces glucosuria, phloridzin fails to increase hepatic glucose output when glucose is infused to replace that lost in the urine (15). Other evidence suggests that effects of phloridzin are secondary to its effects on glucose levels and not directly on the liver. Massillon et al. (20) showed that normalization of plasma glucose in diabetic rats with phloridzin normalized hepatic glucose-6-phosphatase mRNA and protein expression. Conversely, phloridzin failed to decrease this expression when the fall in plasma glucose was prevented by glucose infusion.

With the onset of the phloridzin infusion and subsequent 17 mg/dl fall in glucose in Phl ± Glc, \(R_a\) remained unchanged from basal rates. Conversely, when glucose was increased by 21 mg/dl from 90 to 105 min in Phl ± Glc, \(R_a\) was decreased by 1.1 mg·kg\(^{-1}\)·min\(^{-1}\). This represented an autoregulatory suppression in \(R_a\). Thus the liver appears to be more sensitive
to a small increment, rather than decrement, in arterial glucose. Our results are supported by those of Nielsen et al. (26) and Ader et al. (1), who also utilized the “hot glucose infusion” method (12). Measurement of Ra was improved by minimizing the model dependence of the calculation (a variable [3-13C]glucose infusion was used to clamp specific activity) (11) and by increasing the sampling frequency at transition points. However, compared with earlier studies (1, 26) in which glucose concentrations ranged from mild (130 mg/dl) to frank (300 mg/dl) hyperglycemia to elicit progressive increases in Ra, we saw suppression of Ra with an increase of only ~20 mg/dl. Additionally, this small increase in glucose (and subsequent suppression of Ra) occurred within a normoglycemic glucose range (82–106 mg/dl). However, we cannot rule out the possibility that the prior small decrement in arterial glucose (17 mg/dl) sensitizes the liver for the autoregulatory suppression of Ra in response to a small increase in glucose. It is conceivable that, had glucose been increased from a higher concentration (e.g., from 100 to 120 mg/dl), this autoregulatory mechanism might have been absent. Because the small changes in glucose in this study occurred within a normoglycemic glucose range at rest and catecholamine levels were essentially unchanged, there was no need to perform adrenergic blockade as we had previously done during exercise (4). Arteriovenous difference measurements revealed no change in NHGO. The experimental design did not include rapid triple catheter sampling for assessment of NHGO at transition points. Measurement of NHGO is not as sensitive in detecting small, rapid, non-steady-state changes as is isotope dilution when changes in specific activity are minimized.

In this study, we have described a novel method using a renal arterial infusion of phloridzin to increase Ra independently of insulin. These results extend previous findings from our laboratory of autoregulation of Ra during exercise (4) to the resting state. In contrast to exercise when the liver is in a production mode, during rest an autoregulatory suppression of Ra is more sensitive to an increment in glycemia than stimulation of Ra is to a reduction in glycemia in the presence of phloridzin. This is consistent with work in humans (29). One may speculate that impaired suppression of Ra in the setting of a small increase in glucose may be a cause of the hyperglycemia that occurs in insulin-resistant states (14).

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GRANTS

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