Effects of glycemic control on target organ responses to epinephrine in type 1 diabetes

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Aftab Guy, Deanna, Darleen Sandoval, M. A. Richardson, Donna Tate, and Stephen N. Davis. Effects of glycemic control on target organ responses to epinephrine in type 1 diabetes. Am J Physiol Endocrinol Metab 289: E258–E265, 2005—Severe hypoglycemia occurs in intensively treated patients with type 1 diabetes mellitus (T1DM) due in part to deficient epinephrine counterregulatory responses. Previously, we have found that T1DM patients demonstrated a spectrum of altered responses to epinephrine at a variety of target organs compared with nondiabetic healthy subjects. What is not known is whether intensive glycemic control further modifies target organ responses in individuals with T1DM. Therefore, the aim of this study is to assess whether there is tissue specific (liver, muscle, adipose tissue, pancreas and cardiovascular) resistance to epinephrine in intensively controlled (IC) T1DM compared with those with conventional control (CC). Eight IC patients (age 33 ± 4 yr, BMI 24 ± 2 kg/m2, HbA1C 6.7 ± 0.1%), and 11 CC patients (age 35 ± 3 yr, BMI 25 ± 1 kg/m2, HbA1C 9.6 ± 0.1%) underwent two separate randomized, single-blind, 2-h hyperinsulinemic euglycemic clamp studies with (EPI) and without (NO EPI) epinephrine infusion. Epinephrine levels during EPI were similar in all groups (5,197 ± 344 pmol/l) and insulin levels (515 ± 44 pmol/l) were similar in all groups during the glucose clamps. Endogenous glucose production (EGP) and glucose uptake (Rg) were determined using [3-3H]glucose. Muscle biopsy was performed at the end of each study. IC had a significantly reduced EGP and Rg responses to EPI compared with CC. Glucagon responses to EPI were similarly blunted in both IC and CC. Free fatty acid and glycerol response to EPI was greater in CC compared with IC. There was a significantly greater systolic blood pressure response to EPI in CC. We conclude that, despite similar epinephrine, insulin, and glucose levels, intensively treated T1DM patients had reduced cardiovascular, skeletal muscle, hepatic, and adipose target organ responses to EPI compared with conventionally treated T1DM patients.

catecholamines; metabolic target organ; intensive glucose control; glucose clamp technique

INTENSIVE GLUCOSE CONTROL in type 1 diabetes mellitus (T1DM) is essential in the prevention of long-term microvascular complications (40). Unfortunately, intensive control is associated with a threefold increase in severe hypoglycemia (39). Defense against hypoglycemia in type 1 diabetes (T1DM) is complicated by the loss of glucagon responses to this stress with increasing duration of disease. This leaves epinephrine as the principal counterregulatory hormone in the defense against hypoglycemia in T1DM (16). Unfortunately, epinephrine responses to hypoglycemia are reduced in intensively treated T1DM patients compared with those with conventional treatment (5, 32). Thus the combination of absent glucagon and deficient epinephrine responses greatly increases the risk of severe hypoglycemia in T1DM. However, an additional component may also contribute to this scenario in T1DM. In addition to blunted plasma levels of epinephrine, the metabolic responses of this important counterregulatory hormone may also be deficient in T1DM. During low-dose insulin infusion, Amiel et al. (7) demonstrated that T1DM patients with intensive glycemic control experienced significantly deeper hypoglycemia compared with those conventionally treated, despite equivalent plasma epinephrine levels. Additionally, Fritsche et al. (21) have demonstrated relatively normal epinephrine counterregulatory responses but reduced hypoglycemia awareness in intensively controlled T1DM. These data therefore suggest that there may be reduced target organ responses to epinephrine in intensively controlled T1DM despite adequate plasma levels of the hormone. Previously, we (2) have investigated the integrated physiological effects of epinephrine independently of the confounding variable of hyperglycemia in a group of T1DM with average control and healthy nondiabetic subjects. Our data demonstrated that patients with T1DM have decreased neuroendocrine (glucagon and norepinephrine), metabolic [endogenous glucose production (EGP), glucose uptake (Rg), and glucose oxidation], cardiovascular (mean arterial pressure), and symptomatc responses but increased lipolytic responses to moderately elevated physiological levels of epinephrine compared with nondiabetic subjects (2).

Given that intensive therapy is known to blunt catecholamine responses to hypoglycemia, we wanted to test the hypothesis that intensive therapy in T1DM may also blunt epinephrine action at key target tissues. Therefore, an integrated physiological approach was used to determine glucose and glycerol flux, skeletal muscle metabolism, and neuroendocrine responses to a moderate physiological infusion of epinephrine in intensively and standard-treated T1DM patients.

MATERIALS AND METHODS

Subjects

We studied eight intensively controlled (IC) T1DM patients (5 M/3 F, age 33 ± 4 yr, BMI 24 ± 2 kg/m2, and HbA1C 6.7 ± 0.1%) and 11 conventionally controlled (CC) T1DM patients (6 M/5 F, age 35 ± 3 yr, BMI 25 ± 1 kg/m2, and HbA1C 9.6 ± 0.1%). Some of these subjects had been included in a previous study investigating the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpendo.org
effects of epinephrine in healthy individuals and patients with T1DM (2). All IC received insulin therapy via continuous subcutaneous insulin infusion or via multiple dose injections and performed blood glucose monitoring at least four times a day. CC received insulin therapy via split mixed regimen 2–3 times a day and performed blood glucose monitoring 2–4 times a day. Both groups had similar duration of disease (12 ± 4 vs. 10 ± 8 yr, IC vs. CC, respectively). None of the patients gave a history of hypoglycemia unawareness or had any clinical evidence of autonomic neuropathy or any other tissue-specific complication of diabetes as evidenced by history and bedside autonomic testing. All subjects had normal blood count, plasma electrolytes, and liver and renal function. All gave written informed consent. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board. All subjects were asked to avoid exercise and to consume their usual weight-maintaining diet for 3 days before the study. We instructed each patient to be particularly careful to avoid any hypoglycemia in the period before a study. All patients performed self-monitoring of blood glucose (SMBG) before each meal, at bedtime, and on two occasions at 3 AM for 2 wk before a study. Patients called in their SBGM results so that appropriate changes in insulin and dietary intake could be implemented. An experiment was not conducted unless all readings were 4.5 mmol/l or more. On the day preceding an experiment, intermediate-acting insulin was discontinued and replaced by injections of regular insulin before breakfast and lunch. Subjects were admitted to the Vanderbilt Clinical Research Center (GCRC) at 5 PM on the evening before each experiment. At that time, subjects received their usual evening meal, and a continuous low-dose intravenous infusion of insulin was started to normalize plasma glucose. This infusion was adjusted so that plasma glucose levels remained between 5.0 and 6.7 mmol/l overnight. All subjects were studied after a 10-h overnight fast. Subjects were blinded and randomly assigned to either protocol 1 (no epinephrine (NO EPI)) or protocol 2 (EPI) (Fig. 1). Each protocol was separated by a minimum of 2 mo.

Experimental Design

Euglycemic clamp procedure. Upon admission, all subjects had two intravenous cannulae inserted under 1% lidocaine local anesthesia. One cannula was placed in a retrograde fashion into a vein on the back of the hand. The hand was placed in a heated box (50–60°C) so that arterialized blood could be obtained (1). The other cannula was placed in the contralateral arm so that 20% glucose and potassium chloride (20 mmol/l) in normal saline could be infused via a variable-rate volumetric infusion pump (Imed, San Diego, CA). Purified tritiated glucose, as well as insulin and epinephrine, were infused via precalibrated infusion pumps on the day of the study (Harvard Apparatus, South Natick, MA). The subject remained awake in a supine position throughout each of the clamp procedures.

Protocol 1 (control study, NO EPI). On the morning of NO EPI, a primed (18 μCi) constant infusion (0.18 μCi/min) of [3-3H]glucose was started to measure glucose kinetics (Fig. 1). A period of 90 min was allowed to elapse, followed by a 30-min basal period and a 120-min hyperinsulinemic euglycemic experimental period. The low-dose intravenous insulin infusion was continued to maintain euglycemia until time 0. At that time, a primed continuous infusion of insulin was administered at a rate of 9 pmol·kg⁻¹·min⁻¹ from time 120 (min) until time 240 in all subjects (38). Plasma glucose was measured every 5 min, and a variable infusion of 20% dextrose was adjusted so that plasma glucose levels were held constant at ~5 mmol/l for the duration of the study (18). At time 240, a percutaneous muscle biopsy was performed under euglycemic conditions.

Protocol 2 (EPI). Protocol 2, or EPI, was identical to protocol 1 (NO EPI) except for the addition of a continuous infusion of epinephrine at a rate of 0.06 μg·kg⁻¹·min⁻¹ from times 120 to 240.

Muscle Biopsy

Percutaneous muscle biopsy was performed according to the Bergstrom procedure and is described elsewhere (8). The skin and fascia 12–16 cm above the patella on the lateral aspect of the thigh were anesthetized using topical anesthetic cream (EMLA) applied 1 h before the procedure followed by a local infusion of subcutaneous 1% xylocaine without epinephrine and suprafascial infusion of 2% xylocaine without epinephrine. A small incision was made using a 10-blade scalpel through the skin and underlying fascia. A 5-mm-diameter side-cutting Bergstrom percutaneous muscle biopsy needle was inserted 2–4 cm beyond the fascia into the muscle to obtain a sample of the vastus lateralis muscle. Approximately 200 mg of muscle were obtained during each protocol.

Indirect Calorimetry

Whole body carbohydrate metabolism and fat flux were assessed using indirect calorimetry. Air flow and O₂ and CO₂ concentrations in inspired and expired air were measured by a computerized open-circuit system (DeltaTrak; Sensormedics, Yorba Linda, CA). Urea nitrogen was measured by the Kjeldahl procedure (30). Rates of carbohydrate and fat oxidation were calculated from O₂ consumption and CO₂ production (corrected for protein oxidation) with the equations described by Frayn (19). Nonoxidative glucose disposal was calculated by subtracting the difference between the rates of glucose disposal (Rg) and oxidative glucose disposal.

Analyses

The collection and processing of blood samples have been described elsewhere (13). Blood samples for glucose flux were taken every 10 min throughout the control period and every 15 min during the experimental period. Blood for hormones and intermediary metabolites was drawn twice during the control period and every 15 min during the experimental period. Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Glucagon was measured according to the method of Aguilar-Parada et al. (3) with an interassay coefficient of variation (CV) of 15%. Free insulin measurements were determined as previously described, with an interassay CV of 11% (42). Catecholamines were determined by high-pressure liquid chromatography (HPLC), with an interassay CV of 12% for epinephrine and for norepinephrine (11). We made two modifications to the procedure for catecholamine determination: we used a five-point rather than a one-point standard calibration curve, and we spiked the initial and final samples of
plasma with known amounts of epinephrine and norepinephrine so that accurate identification of the relevant respective catecholamine peaks could be made. Cortisol was assayed by using the Clinical Assays Gamma Coat radioimmunoassay (RIA) kit with an interassay CV of 8%. RIA with a CV of 8% determined growth hormone (31). Pancreatic polypeptide was measured by RIA using the method of Hagopian et al. (27), with an interassay CV of 8%. Lactate, glycerol, alanine, and 3-hydroxybutyrate were measured on deproteinized whole blood with the method of Lloyd et al. (35). Nonesterified fatty acids (NEFA) were measured using the WAKO kit, adopted for use on a centrifugal analyzer (29).

Rates of glucose appearance (Ra), EGP, and glucose utilization (Rd) were calculated according to the methods of Wall et al. (41). EGP was calculated by determining the total Ra (which comprises both EGP and exogenous glucose infused to maintain the desired euglycemia) and subtracting from it the amount of glucose infused. It is now recognized that this approach is not fully quantitative, because underestimates of total Ra and Rd can be obtained. The use of highly purified tracer and the taking of measurements under steady-state conditions (i.e., constant specific activity) in the presence of a low glucose flux minimizes the major problems. To maintain constant glucose specific activity, the rate of exogenous glucose tracer was increased proportionally to increases in glucose flux.

Cardiovascular parameters (pulse, systolic, diastolic, and mean arterial pressure) were measured noninvasively using a DinaMap (Critikon, Tampa, FL) every 10 min throughout each study. Autonomic and neuroglycopenic symptoms characteristic of hypoglycemia were quantified using a previously validated semiquantitative questionnaire (15). Each subject was asked to quantify on a scale of 1 to 10 (1 being no symptoms and 10 being the most) his/her experience of the symptoms once during the control period and every 15 min during the experimental period. Symptoms measured included tiredness, confusion, hunger, dizziness, difficulty thinking, blurring vision, sweating, tremors, agitation, sensation of heat/thirst, and palpitations. The ratings of the first six symptoms were summed to get the neuroglycopenic score, and the ratings from the last five symptoms provided an autonomic symptom score.

**Materials**

HPLC-purified [3-3H]glucose (New England Nuclear, Boston, MA) was used as the glucose tracer (11.5 mCi mmol⁻¹ l⁻¹). Human regular insulin was purchased from Eli Lilly (Indianapolis, IN). The insulin infusion solution was prepared with normal saline and contained 3% (vol/vol) of the subjects' own plasma.

Epinephrine injectable solution (1:1,000, 1 mg/ml, 1-ml ampule) was prepared with normal saline for continuous infusion; 250 mg per vial was purchased from Isotech (Sigma-Aldrich).

**Muscle Enzyme Analysis**

Glycogen synthase activity was measured utilizing the technique described by Guinovart et al. (26). Glycogen phosphorylase activity was measured utilizing the technique described by Golden and Katz (25). Glycogen content of muscle was determined by enzymatic microdetermination as described by Bruss and Black (10).

**Statistical Analysis**

Data are expressed as means ± SE unless otherwise stated. Statistical comparisons between groups were performed by use of standard parametric two-way analysis of variance for repeated measurements when appropriate. This was coupled with Duncan’s post hoc test to delineate the time when statistical significance was reached. A P value of <0.05 indicated significant difference.

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**RESULTS**

**Insulin and Glucose**

Insulin and glucose levels were similar during both control (NO EPI) and EPI hyperinsulinemic euglycemic clamps (Fig. 2). Steady-state insulin levels were 522 ± 72, 546 ± 66, 558 ± 42, and 528 ± 60 pmol/l in the IC T1DM patients during NO EPI and EPI and in CC T1DM patients during NO EPI and EPI, respectively. Plasma glucose levels were maintained at 5.2 ± 0.1, 5.2 ± 0.1, 5.1 ± 0.1, and 5.3 ± 0.1 mmol/l during the clamp procedure in the IC T1DM patients during NO EPI and EPI and in CC T1DM patients during NO EPI and EPI respectively.

**Epinephrine and Norepinephrine**

Steady-state epinephrine levels during EPI were similar in both groups during the 2-h infusion, 4,980 ± 415 pmol/l in IC T1DM and 5,085 ± 344 pmol/l in CC T1DM (Fig. 3). These levels differed significantly from NO EPI in both respective groups with an average of 179 ± 44 pmol/l in IC T1DM and 185 ± 33 pmol/l in CC T1DM.

Norepinephrine responses were similar during both NO EPI and EPI studies in both groups.

**Glucagon, Cortisol, Growth Hormone, and Pancreatic Polypeptide**

Glucagon, cortisol, growth hormone (GH), and pancreatic polypeptide (PP) levels did not increase significantly during NO EPI or EPI clamps (Table 1).
Effect of epinephrine infusion on glucose specific activity during hyperinsulinemic euglycemia in IC and CC T1DM individuals

Table 2.

<table>
<thead>
<tr>
<th>Time of Euglycemic Clamp, min</th>
<th>Glucose Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>IC-NO EPI</td>
<td>364 ± 25</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>433 ± 33</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>353 ± 21</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>395 ± 56</td>
</tr>
</tbody>
</table>

Values are means ± SE in dpm/mmol.

and 7.6 ± 1.5 μmol·kg⁻¹·min⁻¹ during EPI. Final EGP in IC was 0.6 ± 1.3 during NO EPI and 4.2 ± 1.1 μmol·kg⁻¹·min⁻¹ during EPI. Glucose uptake during the final 30 min (final R) decreased significantly more during EPI in CC vs. IC (40 ± 6 during NO EPI to 16 ± 1.3 μmol·kg⁻¹·min⁻¹ during EPI in CC and 34 ± 4 during NO EPI to 16 ± 2 μmol·kg⁻¹·min⁻¹ during EPI in IC, P < 0.05).

Indirect Calorimetry and Glucose Rₚ

There was a significantly greater reduction in total glucose Rₚ with epinephrine in CC compared with IC (P < 0.05). This was accounted for by a significantly greater change in nonoxidative glucose metabolism in CC vs. IC with EPI (Δ decrease 26 ± 3 vs. −19 ± 2 μmol·kg⁻¹·min⁻¹, respectively, P < 0.05). The effect of EPI upon oxidative glucose metabolism during epinephrine infusion did not differ between the two groups (Δ increase 1.5 ± 1 μmol·kg⁻¹·min⁻¹ in CC and Δ 0.5 ± 1 μmol·kg⁻¹·min⁻¹ in IC).

Cardiovascular Parameters

Heart rate, diastolic blood pressure, and systolic blood pressure changes in response to epinephrine are summarized in Table 3. There was a significantly greater increase in systolic blood pressure in CC T1DM compared with IC (Δ 9 ± 2 vs. 2 ± 1 mmHg, P < 0.05). Heart rate increased similarly in both groups with EPI. Diastolic blood pressure and mean arterial pressure responses to EPI did not differ significantly between groups.

Intermediary Metabolism

Blood lactate and alanine increased similarly during EPI in both groups (Table 4). There was a significantly greater relative increase in glycerol response during EPI vs. NO EPI in CC T1DM compared with IC T1DM (78 ± 14 vs. 30 ± 9, P < 0.05). NEFA and β-hydroxybutyrate responses were also relatively greater in CC T1DM during EPI vs. NO EPI (P < 0.05) compared with IC T1DM.

Muscle Biopsy Assays

Skeletal muscle glycogen phosphorylase activity was greater (P < 0.05) in IC vs. CC T1DM during NO EPI (30 ± 3 vs. 9 ± 3%, respectively) as well as during EPI (19 ± 2% in IC and 13 ± 3% in CC T1DM). Skeletal muscle glycogen synthase was similar in both groups during NO EPI (19 ± 9% in IC vs. 22 ± 7% in CC) and decreased by similar significant amounts.
Adrenergic symptoms increased similarly in both groups during NO EPI (8 ± 2) and EPI (11 ± 2).

**Autonomic Symptoms**

Adrenergic symptoms increased similarly in both groups during NO EPI (8 ± 2) and EPI (11 ± 2).

**DISCUSSION**

The influence of glycemic control in T1DM on the integrated in vivo physiological responses to epinephrine has not been examined. On a background of hyperinsulinemic euglycemia in T1DM during NO EPI to 5 ± 1% in IC and 5 ± 1% in CC. There was a slightly greater decrease in total muscle glycogen content as expressed in micromoles glucose per gram of muscle in IC vs. CC. However, this was not statistically significant (19 ± 2 in IC vs. 12 ± 1 in CC T1DM during NO EPI; 13 ± 4 in IC vs. 8 ± 1 in CC T1DM during EPI).

**Table 3. Effects of epinephrine on cardiovascular parameters in euglycemia in IC and CC T1DM individuals**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>Final</th>
<th>ΔResponse Between EPI and NO EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>IC-NO EPI 116±7</td>
<td>120±7</td>
<td>2±1*</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>113±5</td>
<td>119±6</td>
<td>9±2</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>115±4</td>
<td>115±4</td>
<td>9±2</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>113±4</td>
<td>122±6</td>
<td>9±2</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>IC-NO EPI 71±4</td>
<td>73±5</td>
<td>10±2</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>69±5</td>
<td>61±5</td>
<td>10±3</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>66±2</td>
<td>68±2</td>
<td>10±3</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>70±5</td>
<td>62±5</td>
<td>10±3</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>IC-NO EPI 89±5</td>
<td>91±6</td>
<td>6±2</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>85±6</td>
<td>81±6</td>
<td>4±3</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>81±3</td>
<td>82±2</td>
<td>4±3</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>87±5</td>
<td>84±5</td>
<td>4±3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>IC-NO EPI 72±6</td>
<td>77±5</td>
<td>18±3</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>72±7</td>
<td>95±8</td>
<td>18±3</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>68±4</td>
<td>74±5</td>
<td>19±3</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>67±6</td>
<td>92±5</td>
<td>19±3</td>
</tr>
</tbody>
</table>

Data are means ± SE. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. *P < 0.05, decreased response IC vs. CC.

**Table 4. Effect of epinephrine infusion on intermediary metabolites during hyperinsulinemic euglycemia in euglycemia in IC and CC T1DM individuals**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Basal</th>
<th>Final</th>
<th>ΔResponse Between EPI and NO EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate, mmol/l</td>
<td>IC-NO EPI 0.6±0.1</td>
<td>1±0.1</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>0.6±0.1</td>
<td>2.7±0.2</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>0.5±0.1</td>
<td>1±0.1</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>0.7±0.1</td>
<td>2.7±0.2</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>Alanine, µmol/l</td>
<td>IC-NO EPI 248±40</td>
<td>252±30</td>
<td>24±8</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>243±25</td>
<td>273±30</td>
<td>16±8</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>223±18</td>
<td>226±15</td>
<td>16±8</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>259±22</td>
<td>278±23</td>
<td>16±8</td>
</tr>
<tr>
<td>Glycerol, mmol/l</td>
<td>IC-NO EPI 47±14</td>
<td>29±7</td>
<td>30±9</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>58±11</td>
<td>70±11</td>
<td>30±9</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>65±8</td>
<td>27±5</td>
<td>78±14*</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>69±17</td>
<td>109±23</td>
<td>78±14*</td>
</tr>
<tr>
<td>β-Hydroxybutyrate, mmol/l</td>
<td>IC-NO EPI 76±10</td>
<td>8±10</td>
<td>38±9</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>120±50</td>
<td>14±2</td>
<td>62±13*</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>132±40</td>
<td>13±2</td>
<td>62±13*</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>110±30</td>
<td>52±20</td>
<td>62±13*</td>
</tr>
<tr>
<td>NEFA, µmol/l</td>
<td>IC-NO EPI 356±59</td>
<td>77±16</td>
<td>42±16</td>
</tr>
<tr>
<td>IC-EPI</td>
<td>482±119</td>
<td>161±24</td>
<td>42±16</td>
</tr>
<tr>
<td>CC-NO EPI</td>
<td>445±56</td>
<td>73±7</td>
<td>252±43*</td>
</tr>
<tr>
<td>CC-EPI</td>
<td>382±33</td>
<td>256±29</td>
<td>252±43*</td>
</tr>
</tbody>
</table>

Data are means ± SE. NEFA, nonesterified fatty acids. *P < 0.05; CC responses to epinephrine are significantly greater than those of IC T1DM.
cemia, we tested the hypothesis that intensively treated T1DM patients have altered neuroendocrine, metabolic, cardiovascular, and symptom responses to epinephrine. Our data demonstrate that IC T1DM patients had decreased EGP, $R_d$, glucose oxidation, systolic blood pressure, and lipolytic responses to epinephrine compared with CC T1DM patients.

Increased duration of T1DM is associated with absent glucagon responses to hypoglycemia (24). Therefore, epinephrine’s role as a glucose-counterregulatory hormone becomes critical in T1DM. Unfortunately, intensive glycemic control results in an increased prevalence of moderate to severe hypoglycemia (40). This recurrent hypoglycemia reduces neuroendocrine- and autonomic nervous system-counterregulatory responses, which leads to further hypoglycemia (17). Thus the deficient epinephrine coupled with the absent glucagon responses are now recognized as major mechanisms responsible for the increased prevalence of hypoglycemia in intensively treated T1DM (4). Interestingly, previous studies have suggested that, despite adequate plasma levels of epinephrine, patients with intensively controlled T1DM still cannot defend their plasma glucose level in the face of hypoglycemia (6, 7). This suggests a target organ resistance or altered metabolic responsiveness to epinephrine in intensively controlled T1DM.

We used an experimental approach in this study that allowed us to measure epinephrine’s metabolic effects while controlling for the confounding variables of insulin and glucose. The physiological insulin levels used during the hyperinsulinemic euglycemic control studies resulted in complete suppression of EGP with a reciprocal increase in glucose $R_d$ as well as reduction in lipolysis. This design provided an optimal baseline from which the magnitude of epinephrine’s metabolic effects could be more easily determined.

Epinephrine’s counterregulatory hormonal effects arise from its direct actions on target tissues as well as its indirect actions on other neuroendocrine hormones. In our previous study (2), we found that similar levels of epinephrine increased glucagon secretion in nondiabetic healthy controls but had little or no effect in T1DM of moderate glycemic control ($Hb A_1C$ 8.2 ± 0.1%). In the present study, epinephrine could not stimulate glucagon secretion in either group, although there was less of a reduction in glucagon during EPI in CC compared with IC. Epinephrine increases glucagon secretion by activating β-adrenoreceptors on pancreatic α-cells (22, 23). This suggests that reduced glucagon responsiveness to epinephrine, perhaps through decreased β-adrenoreceptor sensitivity to the catecholamine at the level of the α-cell, may potentially contribute to the mechanism responsible for loss of glucagon responses to hypoglycemia with increased duration of T1DM. Furthermore, glycolytic control does not appear to play a role in epinephrine’s ability to stimulate glucagon release in T1DM.

Epinephrine’s effect on glucose kinetics was significantly reduced in IC T1DM compared with CC T1DM. EGP was suppressed similarly in both IC and CC T1DM by hyperinsulinemia during NO EPI, thereby demonstrating that the effects of glucose toxicity in the CC group could be reversed by just one evening of euglycemia. Despite similar insulin and glucose levels, epinephrine was able to combat insulin-induced suppression of EGP in both IC and CC T1DM. However, in the IC group, there was a significantly reduced EGP response compared with CC and a significantly reduced response in limiting $R_d$ during epinephrine infusion. Increases in EGP are an important defense against insulin-induced hypoglycemia. Sources of glucose production arise from both glycoligenesis and gluconeogenesis (12, 37). Recently, Chu et al. (14) have demonstrated that the majority of the increased glucose production occurring during epinephrine infusion results from hepatic gluconeogenesis. However, epinephrine can also increase renal glucose release via elevations in gluconeogenesis that can contribute to EGP in both healthy controls and T1DM patients (41, 42). Substrates for gluconeogenesis are provided to the liver by epinephrine’s ability to mobilize lactate, pyruvate, and amino acids from muscle and the gut as well as glycerol from breakdown of triglycerides in adipose tissue. There were similar increases in lactate and alanine during epinephrine infusion, yet decreased EGP in IC compared with CC T1DM. Therefore, it is likely that the reduced availability of glycerol and NEFA from blunted lipolysis contributed to the reduced gluconeogenic response to EGP in IC compared with CC.

Epinephrine infusion also demonstrated a diminished ability to limit glucose utilization in IC T1DM. Glucose utilization can be limited by various factors, including a decrease in transport and/or phosphorylation of glucose (34, 36). In the present study, total muscle glycogen content decreased by a greater extent (albeit nonsignificantly) with EPI in IC vs. CC T1DM. Detecting significant changes in skeletal muscle glycogen requires very large alterations in systemic glucose flux (or a large number of subjects). This is because alterations in glycogen metabolism will be spread across the liver and all skeletal muscle, and thus the experimental signal will be diluted at any given muscle biopsy site. Therefore, the trend of reduced glycogen content in our IC patients may have some physiological relevance. There was a reduced effect of epinephrine to inhibit insulin-mediated glucose $R_d$ in the IC compared with CC T1DM subjects. This was due to a greater reduction in whole body nonoxidative glucose metabolism in the CC T1DM subjects. Muscle biopsy data demonstrated similar effects of epinephrine on glycogen synthase and phosphorylase activity in both groups. This suggests that the impairment of epinephrine action lies at the level of glucose transport and/or phosphorylation. Our findings are supported by the study of Laurent et al. (34), who used NMR spectroscopy to investigate the effects of a similar epinephrine concentration (~800 pg/ml) on skeletal muscle metabolism in healthy volunteers. NEFA levels, consequent on lipolysis, exert profound effects on regulating the rate of glucose uptake in skeletal muscle. Increased circulating NEFA levels are now recognized to be a major mechanism for causing insulin resistance in muscle and limiting glucose uptake in the tissue (9). Therefore, the reduced availability of NEFA during EPI infusion would also have contributed to the blunted metabolic actions of the hormone on skeletal muscle in the IC group (28).

Epinephrine increased lipolysis in both groups (as evidenced by increases in both plasma glycerol and NEFA). However, there was a significantly greater lipolytic effect in CC compared with IC T1DM. Ketone bodies measured as β-hydroxybutyrate also increased significantly in CC compared with IC T1DM during EPI due to increased delivery and β-oxidation of free fatty acids at the liver. The lipolytic effect of epinephrine results from a dose-dependent response (33). At low levels, epinephrine has an inhibitory action upon lipolysis via its effect on $α_2$-adrenoreceptors. At higher levels, epinephrine exerts its
effect on β-adrenergic receptors, stimulating lipolysis. Despite similar physiological levels of epinephrine during EPI in both groups, the less pronounced lipolytic response seen in our IC T1DM patients may suggest a reduced β-adrenergic sensitivity to epinephrine at adipose tissue.

There was a relatively greater systolic blood pressure response during EPI in CC compared with IC T1DM. Diastolic blood pressure, mean arterial blood pressure, and heart rate responses were similar in both groups of T1DM during epinephrine infusion. Reduced cardiac responses to epinephrine in intensively treated T1DM have been reported previously (20, 21). Studies by Fritsche et al. (21) have demonstrated reduced cardiovascular responses to the β-adrenergic agonist isoproterenol in intensively treated T1DM patients. Thus it would appear that the reduced systolic blood pressure response is due to a specific downregulation of myocardial β-adrenergic receptor responsiveness rather than a differential effect on vascular peripheral resistance in the IC T1DM.

In summary, our results demonstrate a reduced effect of epinephrine at the level of the liver, muscle, adipose tissue, and cardiovascular system in IC T1DM. Given the increased frequency of hypoglycemia in IC T1DM, it is possible that these findings may be the result of repetitive exposure to physiological levels of epinephrine with subsequent downregulation of the β-adrenergic receptor at these target organs. Despite the lack of history of hypoglycemic unawareness or autonomic neuropathy, our patients with IC T1DM clearly demonstrated a defective response to epinephrine. This raises the concern that impaired counterregulation in T1DM may include an additional component, namely reduced tissue effectiveness of epinephrine. Additional epinephrine dose-response studies are needed to test this hypothesis, as the levels of epinephrine used in this study are physiological but higher than those typically encountered during hypoglycemia in clinical practice. In conclusion, key metabolic counterregulatory mechanisms of increased glucose production and lipolysis, together with reduced glucose uptake, are blunted during epinephrine infusion in intensively treated T1DM patients. This may imply that strategies aimed at increasing epinephrine levels during hypoglycemia may be only partially successful due to reduced action of the catecholamine at metabolic target tissues.

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