Epinephrine effects on insulin-glucose dynamics: the labeled IVGTT two-compartment minimal model approach

PAOLO VICINI,1 ANGELO AVOGARO,2 MARY E. SPILKER,1 ALESSANDRA GALLO,2 AND CLAUDIO COBELLI3

1Department of Bioengineering, University of Washington, Seattle, Washington 98195; 2Departments of Metabolic Diseases and 3Electronics and Informatics, University of Padova, 35128 Padua, Italy

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Epinephrine effects on insulin-glucose dynamics: the labeled IVGTT two-compartment minimal model approach. Am J Physiol Endocrinol Metab 283: E78–E84, 2002; 10.1152/ajpendo.00530.2001.—The hyperglycemic effects of epinephrine (Epi) are established; however, the modulation of Epi-stimulated endogenous glucose production (EGP) by glucose and insulin in vivo in humans is less clear. Our aim was to determine the effect of exogenously increased plasma Epi concentrations on insulin and glucose dynamics. In six normal control subjects, we used the labeled intravenous glucose tolerance test (IVGTT) interpreted with the two-compartment minimal model, which provides not only glucose effectiveness ($S_G^2$), insulin sensitivity ($S_I^2$), and plasma clearance rate (PCR) at basal state, but also the time course of EGP. Subjects were randomly studied during either saline or Epi infusion (1.5 μg/min). Exogenous Epi infusion increased plasma Epi concentration to a mean value of 2,034 ± 138 pmol/l. During the stable-label IVGTT, plasma glucose, tracer glucose, and insulin concentrations were significantly higher in the Epi state. The hormone caused a significant ($P < 0.05$) reduction in PCR in the Epi state when compared with the basal state. The administration of Epi has a striking effect on EGP in the Epi state when compared with the basal state. Although the hyperglycemic effects of Epi on the liver are firmly established, less clear is the modulation of Epi-stimulated endogenous glucose production (EGP) by insulin in vivo in humans. In rat studies, it was shown that, when Epi is combined with insulin infusion, there is a 50% reduction in liver glycogen content with evidence for a transient activation of hepatic glucose output by Epi in the initial 60 min of its exposure (15). In hepatocytes isolated from lean rats, the presence of insulin in the incubation medium antagonizes in a concentration-dependent manner the stimulation of gluconeogenesis by Epi (24). Although the immediate effect of Epi is the ability to prevent a compensatory increase in β-cell secretion, the ongoing hyperglycemia overcomes the Epi-mediated inhibition on insulin secretion so that compensatory hyperinsulinemia limits the excessive rise in plasma glucose. This fine modulation is absent in insulin-dependent diabetic patients and explains both the exaggerated hyperglycemic and lipolytic responses during an Epi infusion in these patients (9).

Taken together, these data suggest the existence of a fine, time-dependent interaction between Epi and insulin in determining EGP. However, this interplay has never been precisely assessed in vivo, in humans, because of the inability of the available approaches to properly describe this relationship, particularly in response to a glucose load. This is particularly important, because this situation is rather common in daily life: it is well known that even a normal subject has the propensity to develop glucose intolerance under the influence of small increments of Epi during physiological stress.

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Address for reprint requests and other correspondence: A. Avogaro, Cattedra di Malattie del Metabolismo, Via Giustiniani 2, 35128 Padua, Italy (E-mail: angelo.avogaro@unipd.it).
ion monitoring after isothermal separation at 250°C on a 30-μm J & W capillary column. Glucose pentaacetate isotopomers are monitored at mass-to-charge (m/z) 242 and 244 for [6,6-2H2]glucose and at m/z 243 for [6,6-2H2]glucose, as described previously (1). From ion intensity ratios, the value in the sample of the isotope ratio R between labeled and unlabeled species is derived. For the [6,6-2H2]glucose tracer, [6,6-2H2]glucose and [6,6-1H2]glucose are the labeled and unlabeled species with, respectively, two 2H atoms or two 1H atoms in position 6. Species are thus defined with reference to specific atoms in specific positions, and in deriving R we correct analytically for interferences in the mass spectrum from the natural isotopic composition of the other atoms of the monitored ion (7).

The ratio Z between tracer and tracee mass (or concentrations) in the sample can be evaluated from isotope ratio measurements as

\[
Z(t) = \frac{G(t)}{G_{I}(t)} = \frac{[G_{I}(t) - R_{0}] + R_{I}}{[G(t) - R(t)] + R_{I}}
\]

where \(G^*\) is the tracer glucose concentration, that is, the concentration in the sample of the exogenous administered mixture of natural and deuterated glucose; \(G_{I}\) is the tracee glucose concentration, that is, the concentration of endogenous natural glucose. Two isotope ratios, \(R\) and \(R'\), can be defined. \(R\) is the ratio of the mass of the labeled and unlabeled species, and \(R'\) is that of the incompletely labeled and unlabeled species. \(R_{I}\) (\(R_{I}'\)) and \(R_{N}\) (\(R_{N}'\)) are isotope ratios in a sample of pure tracer and tracee, respectively, and \(R(t)\) is the isotope ratio measurement at each time \(t\) (6).

The above approach based on calculating the \(Z\) variable only requires the assumption of isotopic indistinguishability. Assessment of glucose disposal by the two-compartment minimal model. The two-compartment minimal model (2CMM) was used to determine the insulin and glucose dynamics for each individual in the basal and Epi states. The model is based on fitting the following equations to glucose data to obtain best-fit parameters and metabolic indexes for the system (7, 31).

\[
Q^1(t) = -\left[ k_p + \frac{R_{I0}}{G_{I}(t)V_1} + k_{21}\right] Q^1_{I}(t) + k_{12}Q^2_{I}(t)
\]

\[
Q^2(t) = k_{21}Q^1(t) - [k_{12} + X(t)] + k_{12}Q^2_{I}(t)
\]

\[
G^*(t) = \frac{Q^2(t)}{V_1}
\]

where \(Q^1(t)\) and \(Q^2(t)\) denote tracer glucose masses in the first (accessible pool) and second (slowly equilibrating) compartments, respectively, (mg/kg for a stable-label IVGTT), \(X(t)\) is insulin action (min⁻¹), \(I(t)\) and \(I_0\) are plasma insulin and basal (end of test) insulin, respectively (μU/ml), \(G(t)\) is tracee glucose concentration in the accessible pool (mg/dl), \(G^*(t)\) is plasma tracer glucose concentration (mg/dl), \(D^*\) is the exogenous glucose dose (mg/kg), \(V_1\) is the volume of the accessible pool (dl/kg), and \(k_{21}\) (min⁻¹), \(k_{12}\) (min⁻¹), \(k_{02}\) (min⁻¹), \(p_2\) (min⁻¹), and \(sk\) (ml/μU⁻¹-min⁻¹) are parameters describing glucose kinetics and insulin action. \(G_0\) is the basal (end of test) glucose concentration. Glucose uptake by insulin-independent tissues is described as the sum of a constant and a term proportional to glucose mass in the accessible pool; the proportionality term \(k_p\) is derived from...


\[
k_p = \frac{3k_2k_0}{k_0 + k_1} + \frac{R_{40}}{G_0V_1}
\]

and the constant component is defined by the parameter \( R_{40} \), assumed known and equal to 1 mg·kg\(^{-1}\)·min\(^{-1}\). Other derived parameters include glucose effectiveness, \( S_G^{**} \), plasma glucose clearance rate at basal insulin, PCR

\[
PCR = \frac{R_{40}}{V_1G_0} + \frac{3k_2k_0}{k_0 + k_1} (\text{ml·min}^{-1}·\text{kg}^{-1})
\]

insulin sensitivity, \( S_I^{**} \)

\[
S_I^{**} = \frac{V_1}{P_3} \left( \frac{2k_1}{k_0 + k_1} \right)^2 (\text{ml·min}^{-1}·\text{kg}^{-1}/\mu\text{U}·\text{ml}^{-1})
\]

and basal endogenous glucose production, EGPb

\[
EGP_b = G_0PCR (\text{mg·min}^{-1}·\text{kg}^{-1})
\]

It is also possible to derive an estimate of the time-varying plasma glucose clearance rate during the test, PCR(t)

\[
PCR(t) = \frac{R_{40}}{V_1G(t)} + \frac{3k_2k_0}{k_0 + k_1} (\text{ml·min}^{-1}·\text{kg}^{-1})
\]

The 2CMM was run for each individual in the control study and after 90 min of epinephrine infusion. Curve fitting of the model prediction with the measured concentration values was accomplished via nonlinear weighted least squares by use of SAAM II software (SAAM Institute and University of Washington, Seattle, WA, 1998, http://www.saam.com) on a PC (4). The fractional standard deviation was calculated for each data point by use of error propagation from the isotope ratio measurements, and the weights were calculated from the measured values. This allowed us to calculate the precision of subject-specific estimates of all model parameters.

**EGP estimation by the nonparametric stochastic deconvolution method.** EGP was calculated by nonparametric stochastic deconvolution. EGP, endogenous glucose concentration \( G(t) \) calculated as total glucose minus exogenous, i.e., tracer + cold bolus glucose, and the impulse response of the system given by the 2CMM [indicated here by \( h(t,\tau) \), as this is a time-varying model] are related through the following integral equation

\[
G(t) = \int_0^t h(t,\tau)EGP(\tau)d\tau
\]

The deconvolution yields a time course of EGP for each subject in the basal and Epi states (see Table 1). The time course was calculated every 2 min, yielding an almost continuous prediction. This method of reconstructing endogenous production during an IVGTT was independently validated by using the tracer-to-tracee clamp described in Ref. 32.

The deconvolution programs were written in Matlab (The MathWorks, Natick, MA) and executed on a PC (31).

**Statistics.** To evaluate the differences between the control and Epi states, the Wilcoxon signed-ranks test for matched pairs (2-tailed) was employed, and a \( P \) value of \(<0.05 \) was considered to be significant. This statistical test was chosen on the basis of the paired nature of the data and the small sample size. Test statistics were computed with the statistical software SPSS Rel. 10.0.5 (SPSS, Chicago, IL). Values are reported as means ± SE, except where otherwise stated.

**RESULTS**

**Epinephrine, glucose, insulin, and C-peptide concentrations.** Baseline plasma Epi concentration was 520 ± 48 pmol/l. Exogenous Epi infusion increased plasma Epi concentration to a mean value of 2,034 ± 138 pmol/l \( (P < 0.001) \). The coefficient of variation (SD/mean × 100) of Epi concentrations during the IVGTT time course was 14 ± 6% during the Epi study.

Epi significantly increased baseline plasma glucose concentration \( (83 ± 10 \text{ vs. } 98 ± 12 \text{ mg/dl, } P < 0.05) \), whereas it had no effect on baseline insulin \( [9 ± 3 \text{ vs. } 11 ± 3 \mu\text{U/ml, not significant (NS)}] \) and C-peptide \( (1.4 ± 0.3 \text{ vs. } 1.5 ± 0.3 \text{ ng/ml, NS}) \) concentrations. As shown in Fig. 1, during the stable-label IVGTT, plasma glucose, tracer glucose, and insulin concentrations were significantly higher in the Epi study.

**Glucose disposal indexes.** The average trend for the derived parameters of the 2CMM showed a decrease in
magnitude for EGP_b, S_G, and S_I, with a significant (P < 0.05) reduction in PCR in the Epi compared with the basal state (Table 1). Note that the reduction of S_G, albeit substantial, was not significant; the two indexes of plasma clearance rate and glucose effectiveness were merged into one, S_I, in the one-compartment minimal model used in our previous study (2).

EGP and plasma clearance time courses. The administration of Epi has a striking effect on EGP profiles (Fig. 2): the nadir of the EGP profiles (Table 2) occurs at 21 ± 7 min in the basal state and at 55 ± 13 min in the Epi state (P < 0.05). The time-varying profile of plasma clearance rate PCR(t) was calculated from Eq. 7 and is reported in Fig. 3; elevated Epi concentrations seem to be associated with a substantial decrease in the glucose clearance rate.

DISCUSSION

The labeled IVGTT interpreted with the 2CMM allowed us to obtain accurate and precise estimates of glucose metabolism at both peripheral and liver levels; this is particularly important because Epi deeply affects both glucose uptake and its hepatic release. The present data show that exogenously increased Epi concentration not only reduced the peripheral clearance of glucose but also had a profound effect on the EGP.

In the presence of Epi levels, and with comparable baseline EGP, we observed a significant time delay in the ability of secreted insulin to exert its inhibitory action on glucose release after the glucose load. This time-dependent effect is transient, and after 2 h it has completely vanished. As is apparent from Fig. 2, the time required in the presence of Epi to have the same inhibitory action of insulin on EGP with respect to the control study is more than double. Interestingly, the maximal inhibitory effect of insulin is not reduced; this means that, in terms of insulin action on glucose release, Epi mainly reduced the ability of liver to "sense" the inhibitory action of insulin. These results would contradict previous studies showing that, in the presence of Epi, EGP is significantly less inhibited by insulin (10, 23), ten- and twofold, without and with

Table 1. 2CMM glucose production and disposal indexes

<table>
<thead>
<tr>
<th>Condition</th>
<th>Subj. No.</th>
<th>EGP_b, mg·kg⁻¹·min⁻¹</th>
<th>PCR, ml·kg⁻¹·min⁻¹</th>
<th>S_G², ml·kg⁻¹·min⁻¹</th>
<th>S_I², dl·min⁻¹·kg⁻¹ per μU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>1</td>
<td>1.31 (14)</td>
<td>1.77 (14)</td>
<td>0.42 (60)</td>
<td>0.00176 (15)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.91 (19)</td>
<td>2.49 (19)</td>
<td>1.19 (40)</td>
<td>0.00081 (40)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.36 (8)</td>
<td>3.10 (8)</td>
<td>1.79 (14)</td>
<td>0.00022 (185)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.02 (32)</td>
<td>2.49 (32)</td>
<td>1.26 (63)</td>
<td>0.00215 (5)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.98 (4)</td>
<td>2.36 (4)</td>
<td>1.17 (7)</td>
<td>0.00242 (7)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.44 (5)</td>
<td>3.37 (5)</td>
<td>2.39 (7)</td>
<td>0.00274 (16)</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
<td>2.17 ± 0.28</td>
<td>2.59 ± 0.23</td>
<td>1.37 ± 0.27</td>
<td>0.00168 ± 0.00039</td>
</tr>
</tbody>
</table>

Epinephrine

<table>
<thead>
<tr>
<th>Subj. No.</th>
<th>EGP_b, mg·kg⁻¹·min⁻¹</th>
<th>PCR, ml·kg⁻¹·min⁻¹</th>
<th>S_G², ml·kg⁻¹·min⁻¹</th>
<th>S_I², dl·min⁻¹·kg⁻¹ per μU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.36 (5)</td>
<td>1.5 (5)</td>
<td>0.39 (20)</td>
<td>0.00053 (10)</td>
</tr>
<tr>
<td>2</td>
<td>1.24 (8)</td>
<td>1.41 (8)</td>
<td>0.27 (42)</td>
<td>0.00050 (14)</td>
</tr>
<tr>
<td>3</td>
<td>1.22 (5)</td>
<td>1.4 (5)</td>
<td>0.25 (31)</td>
<td>0.00115 (10)</td>
</tr>
<tr>
<td>4</td>
<td>2.53 (5)</td>
<td>2.27 (5)</td>
<td>1.37 (8)</td>
<td>0.00059 (10)</td>
</tr>
<tr>
<td>5</td>
<td>2.10 (7)</td>
<td>1.87 (7)</td>
<td>0.98 (13)</td>
<td>0.00020 (69)</td>
</tr>
<tr>
<td>6</td>
<td>1.35 (6)</td>
<td>1.49 (6)</td>
<td>0.39 (23)</td>
<td>0.00087 (13)</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>1.63 ± 0.22</td>
<td>1.66 ± 0.14</td>
<td>0.61 ± 0.18</td>
<td>0.00064 ± 0.00013</td>
</tr>
</tbody>
</table>

P Values 0.345 0.028 0.075 0.075

Two-compartment minimal model (2CMM) metabolic indexes of basal endogenous glucose production (EGP_b), basal plasma clearance rate (PCR), glucose effectiveness (S_G²), and insulin sensitivity (S_I²). Values are parameter estimates and are shown for 6 normal subjects in the basal and epinephrine conditions. Numbers in parentheses are estimated precisions expressed as coefficients of variation in %.

Table 2. Salient temporal features of EGP estimated by the 2CMM and deconvolution

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Minimum EGP, mg·kg⁻¹·min⁻¹</th>
<th>Time, min</th>
<th>Minimum EGP, mg·kg⁻¹·min⁻¹</th>
<th>Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.256</td>
<td>10</td>
<td>0.624</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>1.190</td>
<td>30</td>
<td>0.634</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>0.140</td>
<td>18</td>
<td>0.789</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>0.236</td>
<td>50</td>
<td>1.490</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>0.264</td>
<td>12</td>
<td>0.403</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>0.356</td>
<td>18</td>
<td>0.460</td>
<td>54</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.407 ± 0.147</td>
<td>23 ± 6</td>
<td>0.733 ± 0.149</td>
<td>60 ± 12</td>
</tr>
</tbody>
</table>

P Values 0.173 0.028
EPINEPHRINE AND INSULIN RESISTANCE

Fig. 3. Average time course of plasma glucose clearance rate (PCR) calculated from model predictions in control (○) and Epi (□) states. Values are means ± SE (n = 6).

Epi, respectively. However, such data were obtained by use of different experimental protocols, such as the isoglycemic hyperinsulinemic glucose clamp and glucose infusion, which may itself alter the circulating plasma level of catecholamines (12). This altered EGP suppression during Epi infusion was observed in the presence of different insulin levels; therefore, we cannot tell whether the degree of suppression would be similar or less pronounced in the Epi group if the two treatment groups had similar insulin levels. Further studies will be needed to clarify this issue.

The following items should also be considered. Previous literature findings have shown that increased Epi levels oppose insulin action in modulating the endogenous rate of appearance of glucose (25). This hormone induces an initial rise in glucose production that is largely due to the activation of glycogenolysis; after the waning of this initial effect, Epi stimulates gluconeogenesis, which thus becomes the major factor in maintaining glucose production. Cherrington and colleagues [see Frizzell et al. (11) and Stevenson et al. (26)] have shown that the effect of Epi on glucose production lasts ~30 min.

Some hypotheses can be put forward for this waning effect of Epi on glucose production. One is related to a possible regulatory mechanism mediated by β-adrenergic receptors; however, it has been shown that, in the presence of physiological levels of hyperinsulinemia, at least in dogs, the recovery of glucose rate of appearance is not dependent on adrenergic mechanisms (29, 33). This may suggest the presence of an autoregulatory mechanism within the liver that may limit the hyperglycemic effect of Epi; this hypothesis is supported by previous studies showing that glucose autoregulates its own production or utilization by modulating the glycogen and glycolytic pathways (9, 28). Another possibility is the role of insulin in limiting the Epi response. As shown in Fig. 1, Epi infusion is paralleled by higher insulin levels determined both by the induction of insulin resistance and by a true higher secretion when the β-cell is challenged with a glucose load. This opposing effect of insulin on Epi metabolic effects is not limited to glucose but is also true for lipid metabolism (3). As far as insulin secretion is concerned, baseline and C-peptide levels were not significantly increased despite increased plasma glucose concentration; this confirms a basal inhibitory effect of Epi on insulin secretion and underscores the role of the limitation of insulin secretion in the hyperglycemic action of this hormone (5). This effect may be one of the determinants of the initially delayed suppression of EGP and of subsequent glucose intolerance during Epi infusion.

Although the labeled IVGTT provides meaningful indexes of insulin action and glucose metabolism, it does not provide figures on splanchnic glucose uptake. This parameter may play a possible role in our findings. Sacca et al. (22) found that Epi infusion decreases the uptake of glucose in the splanchnic bed, which is a pathway of glucose disposition that is relatively insensitive to insulin. They found that splanchnic glucose uptake significantly increased from baseline after 30 min from the beginning of glucose infusion, and that during Epi challenge there is a complete blunting of this process. Therefore splanchnic glucose uptake might hypothetically have a role in modulating EGP during Epi challenge.

Although we did not assess their concentrations, Epi infusion markedly increases free fatty acid levels. This action may explain our findings, because, during the IVGTT, their level drops to their nadir usually after 50–60 min from the bolus glucose administration (27).

In normal subjects, the hyperglycemic action of Epi is enhanced by the simultaneous elevations of glucagon and cortisol (25); the former increases the magnitude, but not the duration, of the rise in hepatic glucose output induced by Epi. It is likely that the different time-dependent inhibitory effects of insulin in the presence of elevated Epi levels may be partly determined by increased glucagon concentration, although we did not measure its concentration. Another potential confounder that deserves comment is the possible role of norepinephrine (NE), which can be elevated by Epi and glucose infusions (8). The simultaneous increase of NE could at least partly explain the decreased glucose clearance and the delayed suppression of EGP (17).

As was shown in our previous study, elevated Epi concentrations also have profound effects on glucose uptake (2). In Fig. 3, we provide strong, temporal evidence for this effect. Together with this marked effect on glucose clearance rate, we have found that Epi decreases, although not significantly, $S_I^2$, and $S_E^2$, the insulin action and glucose effectiveness indexes, respectively. The observed differences between this and our previous study (where insulin sensitivity was significantly decreased) may be ascribed to the different indexes provided by the 2CMM of glucose kinetics (compared with the one-compartment model used in our previous study) or to the relatively low discriminating power of this study due to the small number of subjects included. The former takes into account the fact that glucose kinetics is described by a two-compartment model (as opposed to a one-compartment model) and attempts to take into account the inhibitory
effect of glucose on its own clearance (see Eq. 4). Also, insulin sensitivity and the other indexes are more difficult to estimate with the 2CMM (which is to be expected, as the model is more complex); however, the glucose impulse response provided by the 2CMM is needed for evaluating the time course of EGP (1). It is worth noting that this approach has been used to assess an insulin sensitivity index in other pathophysiological states such as type 2 diabetes (18).

In conclusion, we have shown by using a two-compartment model of glucose kinetics that elevated plasma Epi concentrations have profound effects at both hepatic and tissue levels; these two sites of action can be dissected with the labeled IVGTT approach. In particular, at the liver site, this hormone deeply affects, in a time-dependent fashion, the inhibitory effect of insulin on glucose release. Our findings demonstrate the complexity of hepatic glucose metabolism when human subjects are exposed to catecholamine levels such as those observed during physiological stress.

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