Glucose effectiveness is the major determinant of intravenous glucose tolerance in the rat

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Glucose effectiveness is the major determinant of intravenous glucose tolerance in the rat. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E739–E746, 1999.—To determine the importance of insulin for glucose disposal during an intravenous glucose tolerance test in rats, experiments were performed in four cohorts of conscious unrestrained rats fasted overnight. In cohorts 1-3, a bolus of tracer ([3-3H]glucose, 50 μCi) was given alone, with glucose (0.3 g/kg) to induce an endogenous insulin response (−1.100 pmol/l), or with exogenous insulin to give physiological (1,700 pmol/l) or supraphysiological (12,000 pmol/l) plasma levels. Raising plasma insulin within the physiological range had no effect (P > 0.05), but supraphysiological levels induced hypoglycemia (7.3 ± 0.2 to 3.6 ± 0.2 mmol/l) and increased [3H]glucose disappearance rate (P < 0.001). In cohort 4, a primed, continuous tracer infusion was started 120 min before saline or glucose bolus injection. [3H]glucose levels fell 15–20%, and the disappearance rate rose 36% (P < 0.05) after glucose injection. These results indicate that in fasted rats a tracer bolus injection protocol is not sufficiently sensitive to measure the physiological effect of insulin released in response to a bolus of glucose because this effect of insulin is small. Glucose itself is the predominant mediator of glucose disposal after a bolus of glucose in the fasted rat.

The importance of glucose per se to mediate glucose disposal was first reported in the 1930s by Soskin et al. (30), who showed that depancreatized dogs infused with insulin to establish basal plasma levels had near-normal glucose tolerance. Vranic et al. (36) later reported that tolerance to an intravenous glucose load, quantified as the disappearance rate for glucose (Kg), was decreased by only 50% in dogs when the endogenous insulin response was prevented. In 1979, the development of the minimal-model method (4) for resolving blood glucose and insulin profiles during a frequently sampled intravenous glucose tolerance test (FSIGT) enabled quantification of both insulin sensitivity (SI) and glucose effectiveness (SG). Subsequently, there have been hundreds of studies of the effects of changes in SI to determine glucose tolerance (14). Fewer studies have addressed the role of SG, but there is a growing impetus to examine the effects of changes in SG on glucose tolerance. In healthy human beings, SI and SG appear to contribute approximately equally to glucose tolerance (18). In individuals with insulin resistance and impaired glucose tolerance, SI is decreased and SG appears responsible for ~80% of glucose tolerance, and in patients with type 2 diabetes, SI is reduced still further and SG contributes up to 99% of glucose tolerance (5, 11).

Rodents are commonly used for experimental studies of the determinants of mammalian glucose homeostasis. In our laboratory, in an effort to develop a modeling approach to investigate the physiological roles of SG and SI in rats, we made the paradoxical observation that an exogenous but physiological dose of insulin had no effect on glucose tolerance in normal rats that were fasted overnight (9). In a series of studies, a bolus injection of glucose (0.3 g/kg) was followed 9 min later by a bolus of insulin ranging from 0.01 to 0.04 U/kg (as in an insulin-modified FSIGT). In contrast to our expectation, the plasma glucose level was not affected by the insulin bolus at any dose (9). The present studies were designed to clarify this paradox. We used labeled glucose ([3-3H]glucose) to measure glucose disappearance from plasma under conditions of 1) normal IVGTT, 2) bolus injection of physiological dose of insulin, 3) bolus injection of pharmacological dose of insulin, and 4) steady-state infusion of labeled glucose with normal IVGTT.

MATERIALS AND METHODS

Animals

Male rats aged 10–14 wk (mass ~ 250–350 g) were used in these studies. The rats were housed singly in plastic cages.

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GLUCOSE EFFECTIVENESS IN RATS

under a 12:12-h light-dark photoperiod (lights on at 0600) at 22 ± 1°C and given free access to water and rat chow (Purina rodent chow). All surgical and experimental procedures adhered to the guidelines of the Canadian Council on Animal Care and were approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

Surgical Procedures

Chronic indwelling catheters were implanted in all rats, as previously described (34). Briefly, the animal was anesthetized with pentobarbital sodium (Somnotol, 35 mg/kg ip, MTC Pharmaceuticals, Cambridge, ON, Canada) and given atropine (0.04 mg/kg sc, MTC Pharmaceuticals) to minimize respiratory secretions during anesthesia. All rats received one dose of antibiotic (Baytril, 5 mg/kg sc, Bayer, Toronto, ON, Canada) and analgesic (Torbugs, 0.1 mg/kg im, Wyeth-Ayerst Laboratories, St. Laurent, QC, Canada) postoperatively. Sterile catheters (Intramedic PE-50 with 3-cm beveled Silastic tips) were placed in the left carotid artery (for sampling) and the right jugular vein (for bolus injections). For tracer infusions, a catheter was also placed into the inferior vena cava via the left femoral vein. The distal ends of all catheters were tunneled subcutaneously, exteriorized, and anchored at the nape of the neck. The catheters were back-filled with heparinized saline (80 U/ml) and sealed; every 3 days, they were flushed with normal saline and refilled with heparinized saline to maintain patency.

The animals were allowed to recover for 1 wk. Experiments were performed on conscious, unrestrained animals that had been fasted overnight by removal of Chow from the cage hoppers at 2100. At 0800 the following morning, the rat was weighed and 30-cm saline-filled PE-50 extensions were connected to the exteriorized catheters. The rat was placed into a Plexiglas experimental chamber (floor area ~600 cm²) and left to settle for 20–30 min. If two experiments were performed on an individual rat, the procedures were done in random order and were separated by 1 wk of recovery.

Experimental Protocols

The following four cohorts of rats were used: cohort 1 received a bolus of [3-3H]glucose alone or with glucose; cohort 2 received a bolus of [3-3H]glucose alone or with 0.005 U/kg insulin; cohort 3 received a bolus of [3-3H]glucose with saline or 0.10 U/kg insulin; and cohort 4 received an infusion of [3-3H]glucose plus a bolus of saline or glucose.

Bolus injection protocol. The effect of increasing plasma glucose or insulin on glucose disposal was quantified by measuring the disappearance of [3-3H]glucose (NET331C, Du Pont NEN, Boston, MA) from the plasma. Bolus injections (–1 ml) were given within 60 s via the jugular vein catheter. Rats received either [3-3H]glucose alone (50 µCi in a vehicle solution of 5.55 mmol/l d-glucose and 14 mmol/l sodium benzoate) or [3-3H]glucose in combination with glucose (0.3 mg/kg; 10% dextrose, Abbott Laboratories, Montreal, QC, Canada) or insulin (porcine insulin, Eli Lilly, Scarborough, ON, Canada) in 0.5% bovine serum albumin (RIA grade, Sigma).

Blood samples (200–300 µl) were taken from the arterial catheter at 5 and 2 min before and at 1, 2, 3, 5, 8, 13, 25, 40, 70, and 120 min after bolus injections. So that the total blood volume removed did not exceed 7 ml/kg, samples large enough (300 µl) for measurement of insulin were taken at only six time points (–5, 1, 3, 5, 13, and 120 min). Plasma volume was replaced by normal saline, but red blood cells were not reinfused after the experiment. Hematocrit was recorded before and after each experiment. Blood samples were kept on ice in microcentrifuge tubes containing heparin (10 U/tube) and sodium fluoride (0.5 mg/tube) and then were centrifuged. The plasma was stored at –20°C until it was assayed.

Infusion protocol. Rats were treated as described in Animals. Once the rat had settled, a primed, continuous infusion of [3-3H]glucose was started via the femoral vein catheter with a peristaltic pump. The priming dose (9 µCi in 0.9 ml) was given within 60 s, and the continuous dose (10 µCi/ml) was infused at 0.015 ml/min (0.15 µCi/min) throughout the experiment. After 120 min of infusion to achieve a steady-state plasma level of [3-3H]glucose, a bolus injection of saline or glucose (0.3 g/kg) was given via the jugular catheter. Arterial blood samples (200–300 µl) were taken at three time points before and at 2, 5, 10, 15, 20, 30, 60, 90, 120, and 180 min after the bolus injection. Plasma insulin was determined at no fewer than seven of these time points. Hematocrit was measured before and after the experiment. Blood samples were treated as described in Bolus injection protocol.

Assays

Unless otherwise noted, all chemicals and reagents were from Sigma (Sigma, Mississauga, ON, Canada). Plasma glucose was assayed with the glucose oxidase method (Beckman II glucose analyzer or microplate assay; Sigma Trinder). Plasma insulin was determined by RIA with a specific antibody and rat insulin standards (Linco Rat Insulin RI-13K kit, Linco Research, St. Charles, MO, or Pharmacia Insulin RIA kit, Uppsala, Sweden). To convert data obtained from the Pharmacia kit (cohorts 1 and 2) to “Linco equivalents,” 19 plasma samples were assayed simultaneously with both kits. The resulting plasma insulin values (ng/ml) were linearly related by the following equation, which was used to convert all plasma insulin data to the same range of values: Linco = (Pharmac – 0.989)/0.41 (r² = 0.96, P < 0.001).

For determination of plasma specific radioactivity, 20 µl plasma were deproteinized by addition of 180 µl of neutralized Ba(OH)₂ and ZnSO₄ solutions and centrifuged, and 100 µl of supernatant were transferred to scintillation vials. The samples were dried at 40°C to remove H₂O; 1 ml of distilled water and 10 ml of scintillation fluor were then added, and the samples were vortexed. Radioactivity of injected or infused tracer solutions was also counted. 3H was counted in a beta counter (Beckman) at ~40% efficiency, and the data were converted to disintegrations per minute (dpm).

Calculations and Statistics

Bolus injection experiments. The rate of disappearance of [3H]glucose from plasma was calculated by applying a double-exponential curve fit to the plot of [3H] counts (dpm) against time (t). The Akaike information criterion (AIC; Ref. 14) was used to determine the effect of addition of the second exponential to the model fit. Akaike number was calculated by

\[ \text{Akaike number} = N \cdot \ln \left[ \frac{1}{2} \text{SSR} \right] + \frac{N}{2} \cdot \ln(2 \pi) \]

where \( N \) is the number of estimated parameters, \( \text{SSR} \) is the sum of squared differences between observed data and model fit, and \( n \) is the number of estimated parameters (14). In experiments in which a glucose bolus was injected (cohorts 1 and 4), the rate of glucose disappearance (Kc) was calculated as the slope of the log, of plasma glucose against time between the 2-min and 13- or 15-min time points (34). As well, a monoexponential curve fit \( B \cdot e^{-S/t} \) was applied to the plasma glucose data between the 2-min and
120-min time points to estimate $S_G$ (2, 14). For this time period, the addition of a second exponential did not improve the model fit significantly in either cohort 1 (Akaike number $29.7 \pm 1.5$ vs. $28.0 \pm 1.2$; $P = 0.20$) or cohort 4 ($35.1 \pm 1.0$ vs. $26.6 \pm 3.0$; $P = 0.07$). The acute insulin response to the glucose bolus (AIRG) was quantified by calculation of the area under the curve of the rise in plasma insulin over basal insulin concentration between the 0-min and 13- and 15-min time points with the trapezoid method.

Infusion experiments. Plasma [3H]glucose disappearance rate ($R$) was calculated as follows

$$R = R_s - V \frac{dT}{dt}$$

where $R_s$ is the tracer infusion rate, $V$ is the estimated distribution volume, and $dT/dt$ is the change of plasma [3H]glucose dpm in a given time period (31). The distribution volume (ml/kg) was estimated as $0.65 \times 0.25 \times \text{mass (in kg)}$ (10, 28). The optimal segments smoothing procedure (13) was applied to the raw tracer dpm data to estimate $dT/dt$.

Statistical Analysis

All data are presented as means ± SE. The significance of changes in plasma glucose, insulin, [3-3H]glucose, and $R_s$ over time was tested by two-way repeated-measures analysis of variance with post hoc Scheffé comparisons. Hematocrit, $K_G$, AIRG, and rate constants for tracer disappearance were compared by paired or unpaired $t$-tests, as applicable. Curve fits were performed with SigmaPlot for Windows (version 6.3, SAS Institute, Cary, NC) and a significance level of $P < 0.05$. 

**RESULTS**

The basal characteristics of the four cohorts of rats are given in Table 1. Rats in cohorts 1 and 2 were somewhat smaller than those in cohorts 3 and 4, but no significant differences in basal plasma glucose or insulin concentrations were found among the groups. For all cohorts pooled, the mean hematocrit at the beginning of sampling was $39.6 \pm 0.36\%$ and that after sampling was $33.4 \pm 0.37\%$, corresponding to a mean decrease of $6.2 \pm 0.2\%$ ($P < 0.001$).

Table 1. Basal characteristics of four cohorts of rats

<table>
<thead>
<tr>
<th>Cohort</th>
<th>n</th>
<th>Body Mass, g</th>
<th>Plasma Glucose, mmol/l</th>
<th>Plasma Insulin, pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>13</td>
<td>253 ± 6</td>
<td>6.2 ± 0.1</td>
<td>176 ± 37</td>
</tr>
<tr>
<td>Glucose</td>
<td>13</td>
<td>257 ± 4</td>
<td>6.0 ± 0.1</td>
<td>153 ± 16</td>
</tr>
<tr>
<td>Saline</td>
<td>11</td>
<td>261 ± 4</td>
<td>6.6 ± 0.4</td>
<td>181 ± 40</td>
</tr>
<tr>
<td>Insulin</td>
<td>11</td>
<td>258 ± 3</td>
<td>6.2 ± 0.1</td>
<td>192 ± 24</td>
</tr>
<tr>
<td>Saline</td>
<td>5</td>
<td>344 ± 29</td>
<td>7.0 ± 0.4</td>
<td>92 ± 23</td>
</tr>
<tr>
<td>Insulin</td>
<td>5</td>
<td>343 ± 22</td>
<td>7.3 ± 0.1</td>
<td>130 ± 31</td>
</tr>
<tr>
<td>Saline</td>
<td>5</td>
<td>349 ± 8</td>
<td>6.3 ± 0.3</td>
<td>127 ± 13</td>
</tr>
<tr>
<td>Glucose</td>
<td>5</td>
<td>332 ± 23</td>
<td>7.2 ± 0.3</td>
<td>174 ± 47</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$ = no. of rats. No significant differences were found among treatment cohorts within each series tested by either paired (cohorts 1, 2, and 4) or unpaired (cohort 3) $t$-tests at $P < 0.05$.

Tracer Bolus Protocol

Figure 1 shows the changes in plasma glucose, insulin, and radioactivity ([3H]glucose) levels after bolus injection of glucose and [3-3H]glucose in cohort 1 rats. Vehicle- and glucose-injected rats had similar basal plasma levels of both glucose ($P > 0.05$) and insulin ($P > 0.05$). Within 1 min after glucose bolus injection, plasma glucose had increased to $17.8 \pm 0.7$ mmol/l ($P < 0.05$ vs. basal). The insulin response was rapid, as peak plasma insulin level ($1,084 \pm 106$ pmol/l; $P < 0.05$ vs. basal) was also reached within 1–2 min. Both plasma glucose and insulin concentrations returned to basal levels within 20 min (Fig. 1). $K_G$ was $5.38 \pm 0.22\%$/min, and AIRG was $5,027 \pm 731$ pmol·l$^{-1}$·min$^{-1}$. The estimated $S_G$ was $0.138 \pm 0.005$ min$^{-1}$.

In both vehicle- and glucose-injected rats, plasma [3H]glucose peaked at ~25,000 dpm and showed an exponential decay with time, reaching minimal levels by 120 min (Fig. 1). The tracer disappearance curve was best described by a double-exponential function, as the Akaike number was reduced significantly compared with that for a monoexponential curve for both vehicle-injected (90.1 ± 0.7 vs. 79.7 ± 1.7; $P < 0.0001$) and glucose-injected (89.8 ± 0.7 vs. 78.6 ± 1.2; $P < 0.0001$) groups. Surprisingly, no difference in [3H]glucose disappearance curves was observed between vehicle-injected and glucose-injected rats, suggesting that the acute endogenous insulin response to the glucose bolus had no effect on [3H]glucose uptake from the plasma.
The effects of exogenous insulin on plasma glucose levels and [3H]glucose disappearance are illustrated in Figs. 2 and 3. In cohort 2 rats, injection of insulin to raise plasma concentration within the physiological range gave a peak plasma insulin level of 1,765 ± 213 pmol/l (Fig. 2). The injected insulin was cleared very rapidly, as circulating insulin levels had returned to basal within 5 min. There was no significant effect of the exogenous insulin on either the plasma glucose concentration, which remained at basal levels, or the dynamics of [3H]glucose disappearance (Fig. 2).

In contrast, when exogenous insulin was given in sufficient dose (cohort 3) to raise plasma insulin to supraphysiological levels (12,000 ± 1,751 pmol/l), it evoked a significant decrease in plasma glucose and an increase in [3H]glucose disappearance (Fig. 3). In these insulin-injected rats, plasma insulin remained elevated for <13 min after injection. In insulin-injected rats, plasma glucose levels fell from the basal level of 7.3 ± 0.1 to 6.4 ± 0.1 mmol/l within 1 min of the injection (P < 0.05 vs. basal) and then further to 3.6 ± 0.2 mmol/l at 13 min postinjection (P < 0.05 vs. basal; Fig. 3). By 120 min postinjection, plasma glucose had returned to near-basal levels (6.5 ± 0.5 mmol/l; P > 0.05 vs. saline). Unlike the [3H]glucose disappearance curves shown in Figs. 1 and 2, Fig. 3 (bottom) demonstrates clearly the impact of very high insulin levels on plasma [3-3H]glucose uptake. Plasma [3H]glucose dpm decreased rapidly after insulin injection and by 5 min were significantly lower than in saline-injected rats (5,995 ± 712 in insulin-injected vs. 8,563 ± 645 in saline-injected rats; P < 0.05).

The rate constants derived from double-exponential fit to the [3H]glucose disappearance curves shown in Figs. 1–3 are summarized in Table 2. The rate of [3H]glucose disappearance from the plasma was not altered by either the endogenous insulin response to the glucose bolus (cohort 1 rats; Fig. 1) or the injection of exogenous insulin to give physiological plasma insulin levels (cohort 2 rats; Fig. 2). Only in cohort 3 rats, in which plasma insulin was raised to pharmacological levels (Fig. 3), was there a significant increase in the rate constants for [3H]glucose disappearance. In these animals, the slow rate constant was greater in insulin-injected rats than in vehicle-injected rats (Table 2).

![Fig. 2. Tracer bolus protocol in cohort 2 rats: effects of raising plasma insulin concentration within physiological range on plasma glucose and insulin concentrations and on tracer ([3H]glucose) disappearance. Values are means ± SE for 11 rats during control (tracer only, ○) or insulin-bolus (tracer + insulin, ●) treatments.](image)

![Fig. 3. Tracer bolus protocol in cohort 3 rats: effects of raising plasma insulin concentration to supraphysiological levels on plasma glucose and insulin concentrations and on tracer ([3H]glucose) disappearance. Values are means ± SE for 5 rats during control (tracer only, ○) or insulin-bolus (tracer + insulin, ●) treatments.](image)

| Table 2. Rate constants for disappearance of [3H]glucose in bolus injection experiments |
|---------------------------------------------|-----------------|-----------------|
|                                      | Fast Rate Constant | Slow Rate Constant |
| **Cohort 1**                              |                  |                  |
| Saline                                    | 0.482 ± 0.048    | 0.025 ± 0.001    |
| Glucose                                   | 0.378 ± 0.071    | 0.022 ± 0.001    |
| **Cohort 2**                              |                  |                  |
| Saline                                    | 0.539 ± 0.058    | 0.025 ± 0.001    |
| Insulin                                   | 0.633 ± 0.132    | 0.026 ± 0.001    |
| **Cohort 3**                              |                  |                  |
| Saline                                    | 0.604 ± 0.155    | 0.025 ± 0.002    |
| Insulin                                   | 1.324 ± 0.521    | 0.131 ± 0.016*   |

Values are means ± SE. Glucose bolus injection dose was 0.3 g/kg; insulin dose was 0.005 U/kg in cohort 2 rats and 0.10 U/kg in cohort 3 rats. *Significantly different from saline-injected rats at P < 0.05.
indicating a significant effect of insulin on [3H]glucose uptake.

These results suggested that, in normal fasted rats, the endogenous insulin response to an intravenous glucose load had no effect to normalize plasma glucose. It seemed possible that after bolus injection, the dynamics of mixing and equilibration of glucose or insulin were sufficiently rapid to obscure the effect of insulin on plasma glucose levels. We sought to clarify this issue by performing experiments (cohort 4) in which [3-3H]glucose was infused to steady state before the IVGTT.

Infusion Protocol

The dynamics of plasma glucose and insulin levels and [3H]glucose dpm obtained in these experiments are shown in Fig. 4. The primed, continuous infusion produced steady-state plasma [3H]glucose levels during the basal period in all rats and throughout the experiment in saline-injected rats. After glucose injection, there was a rapid increase of both plasma glucose and insulin concentrations. The changes in plasma glucose and insulin levels were similar to those we obtained in the glucose bolus-injection experiments in cohort 1 rats (Fig. 1). The peak plasma glucose concentration was 16.6 ± 1.1 mmol/l (P > 0.05 vs. cohort 1), and the peak insulin level was 1,151 ± 287 pmol/l (P > 0.05 vs. cohort 1); KG was 4.17 ± 0.08%/min, and AIRG was 7,676 ± 1,713 pmol·l⁻¹·min⁻¹ (P > 0.05 vs. cohort 1). In this group, SG was estimated to be 0.129 ± 0.021 min⁻¹ (P > 0.05 vs. cohort 1).

The infusion protocol showed clearly that there was a significant decrease in plasma [3H]glucose levels after the glucose bolus (Fig. 4). In glucose-injected rats, plasma [3H]glucose dpm had decreased by 15% at 20 min (P < 0.05 vs. basal), and it did not return to basal levels until 30–60 min postinjection. Figure 5 illustrates the time course of changes in calculated [3H]glucose disappearance rate (R*G) for cohort 4 rats. There was a rapid and significant 30% increase in R*G from 0.39 ± 0.06 to 0.51 ± 0.04 µCi·kg⁻¹·min⁻¹ (P < 0.05) after injection of the glucose bolus. R*G remained elevated for ~20 min and then returned to basal levels (Fig. 5).

The lack of correspondence between glucose disposal rate and the endogenous insulin response to intravenous glucose in rats is illustrated further by the relationship between KG and AIRG or peak plasma insulin concentration. Pooled data from all glucose-injected rats (cohorts 1 and 4; n = 18) showed no significant correlation between either KG and AIRG (slope = 0.00008; intercept = 5.70; r = 0.29; P = 0.25) or KG and peak insulin (slope = 0.00023; intercept = 3.35; r = 0.099; P = 0.70). The rate constants for tracer disappearance for cohort 1 rats also were not significantly correlated with either AIRG or peak insulin concentration (data not shown).

DISCUSSION

One of the basic tenents of glucose homeostasis is that plasma glucose is the driving force for glucose uptake and that insulin acts synergistically to increase the rate of glucose uptake (4). When plasma glucose is raised by application of an exogenous glucose load in an animal with normal pancreatic β-cell function, both plasma glucose and insulin levels are elevated, causing increased glucose uptake into tissues and decreased hepatic glucose production (1, 14). Thus glucose tolerance depends on both the actions of glucose per se on the endogenous insulin secretory response and the
sensitivity of the liver and other insulin-dependent tissues (muscle and fat) to insulin. At basal insulin, the ability of glucose per se to stimulate its own uptake and to suppress hepatic glucose output is termed glucose effectiveness (SG; Refs. 2, 4, and 5). The present studies demonstrate that in normal overnight-fasted rats, there is only a small effect of the endogenous insulin response to an intravenous glucose load to enhance glucose disposal. Tracer glucose disappearance increased on average only 30% above basal rates for ~20 min after the glucose bolus in cohort 4 rats.

The predominance of SG to mediate disposal of an intravenous glucose load in fasted rats differs from the role ascribed to SG in glucose homeostasis in humans and dogs. In healthy human beings and normal dogs, SI and SG are each responsible for ~50% of glucose tolerance (1, 2, 5, 12, 18). In conditions of insulinopenia or insulin resistance, there is an increase in the proportion of glucose disposal that is attributable to SG (5, 11). In subjects with impaired glucose tolerance, S G is only 30% above basal rates for disposal. Tracer glucose disappearance increased on average only 30% above basal rates for 20 min after the glucose bolus in cohort 4 rats.

The reason SG predominates in the fasted rat compared with the fasted human or dog is unknown but may be due to differences in basal glucose turnover. In humans and dogs, the basal rate of glucose appearance and disappearance is ~1–3 mg·kg⁻¹·min⁻¹ (11, 14), whereas in rats basal glucose turnover is ~7 mg·kg⁻¹·min⁻¹ (28, 29). This difference in turnover rate presumably influences the initial rate of glucose disappearance after a glucose bolus and may explain in part why our estimates of SG in rats (~0.130 min⁻¹) are much higher than those reported in humans and dogs (~0.02–0.04 min⁻¹). Because of the transcapillary insulin transport from plasma to tissues, the action of insulin on glucose disappearance is delayed relative to changes in plasma insulin concentration (40). If the effects of the injected glucose itself and of the elevated plasma insulin are additive to the effect of the basal glucose turnover rate, there may be little need for a large effect of insulin. In the rat, even with a small effect of insulin, plasma glucose returns rapidly to the basal level; if the action of insulin were greater, then hypoglycemia might ensue because glucose could have returned to basal by the time the insulin effect would be expected to peak (12).

In cohort 4 rats, tracer glucose disappearance rate increased immediately (within 2 min) to a maximum after the glucose bolus, which may suggest, if the tracer glucose uptake is insulin-mediated, that the delay in the action of insulin is shorter in rats than in humans and dogs. In these experiments, however, we cannot distinguish unequivocally between an effect of the injected glucose itself and that of the insulin released on tracer glucose disappearance. Additional experiments are currently under way that may help to clarify this issue (24).

Our experiments demonstrate that a tracer bolus was not sufficiently sensitive to detect the effect of insulin released in response to a glucose bolus in rats. There was also no apparent acceleration of glucose disappearance when insulin or tolbutamide was injected (9). In the present study, an insulin effect on glucose uptake was only quantifiable by a tracer bolus when insulin was injected at pharmacological doses (Fig. 3). It appears that the initial dynamics of mixing and equilibration of [³H]glucose after the bolus injection obscured the small effect of the endogenous insulin response (Fig. 1; Table 2). On the other hand, during tracer infusion where a steady state for tracer is established before injection of glucose, [³H]glucose is already well mixed in the plasma and the effect of insulin can be seen in the fall in plasma tracer concentration. The change in plasma [³H]glucose concentration provides a more sensitive measure of the effect of insulin on glucose disappearance from the plasma.

The glucose kinetics literature shows a long history of the use of tracer bolus protocols. Many investigators have used this protocol, along with various one- and two-compartment models, to assess the effects of insulin (17, 28, 36, 39). However, much of this early work was performed in dogs, where a bolus of tracer is sufficient to detect the effects of endogenous insulin (17, 36, 39). Raman and colleagues (28) used bolus injections of [³H]glucose to demonstrate increased glucose uptake in response to insulin in rats, but in their experiments insulin was infused at 12–15 mU·kg⁻¹·min⁻¹. The prolonged elevation of insulin presumably allowed for the effect to be detected. Although tracer bolus protocols were primarily used when glucose turnover was in a steady state, Wrenshall and Hetenyi (39) found the bolus sufficiently sensitive to be used in systems that are out of steady state. The successive measured injection technique was used to assess non-steady-state changes in glucose disappearance during insulin or glucose administration in dogs (17, 39). The present study, however, suggests that the bolus method is inadequate to detect the limited effect of the acute insulin response in the rat.

The difference that we found between the effects of endogenous insulin release or injection of physiological levels of insulin and those of pharmacological levels of insulin may be related to the duration of elevation of plasma insulin. In both cohorts 1 and 2, not only was plasma insulin within the physiological range, but the concentration also declined rapidly (Figs. 1 and 2). In cohort 3 rats, in contrast, plasma insulin was ~10-fold higher and it remained elevated for 14 min (Fig. 3). Plasma glucose fell significantly in these latter animals (Fig. 3). Other investigators have reported hypoglycemia after administration of pharmacological insulin doses that evoked prolonged elevation of plasma insulin concentration (28). In the fasted rat, therefore, it may be that a more prolonged effect of insulin is necessary to obtain measurable stimulation of glucose disposal. This may explain part of the paradox that an insulin effect is not quantifiable with a tracer bolus during an IVGTT, but stimulation of glucose uptake (and suppression of hepatic glucose output) is obvious during an euglycemic-hyperinsulinemic clamp in rats (20, 21, 29).
In cohorts 1 and 4 rats, the glucose disposal rate, as indicated by \( K_G \), was not significantly correlated to either \( \text{AIRG} \) (calculated over the same time period) or the peak insulin concentration measured after the glucose bolus. Buchanan et al. (8) show data for Wistar rats that also indicate no correlation between \( K_G \) and \( \text{AIRG} \), although spontaneously hypertensive rats do show a significant relationship between these variables. In contrast, in human subjects, \( K_G \) is highly correlated to both peak insulin concentration and \( \text{AIRG} \), as well as to the product of \( S_G \) and \( \text{AIRG} \) (18). The lack of a correlation between \( K_G \) and \( \text{AIRG} \) in rats supports the conclusion that insulin has little effect on glucose disposal.

The observation that \( S_G \) is the predominant mechanism for glucose disposal during an IVGTT in rats raises questions about the interpretation of IVGTTs. In rats, IVGTTs have been used to determine the effects of many physiological and environmental conditions, e.g., aging (25, 37), high-carbohydrate diets (3, 19, 25, 38), high-fat diets (6, 16, 26, 35), low-protein or calorie-restricted diets (23, 27, 32), and streptozotocin-induced diabetes (34), among others, on glucose homeostasis. Normal or increased glucose tolerance with a concomitantly lower insulin response is typically considered to reflect increased insulin sensitivity (16, 19, 22, 35), whereas normal or decreased glucose tolerance with elevated or normal insulin responses, respectively, is considered to indicate insulin resistance (6, 16, 26, 27, 32, 35, 37, 38). Our data indicate that most of glucose disposal during an IVGTT in normal rats is not due to insulin-dependent mechanisms, so that it is likely that conditions that affect intravenous glucose tolerance act mainly on the components of glucose effectiveness, rather than necessarily on insulin action or sensitivity per se. This may also explain why some investigators have not found an effect on intravenous glucose tolerance of a manipulation predicted to modify insulin action directly (7, 15, 22, 33). In light of the present results, therefore, it seems important to reassess conclusions based on simple IVGTT data alone.

The precise mechanisms by which glucose effectiveness mediates glucose disposal in the rat are not yet clear. The main effect may be one of simple mass dynamics in the normal overnight-fasted rat. The compartmental distribution of glucose, and the activity of glucose transporters and that of the enzymes involved in glucose phosphorylation, oxidation, and storage, may also be stimulated to increase glucose disposal. Under conditions in which glucose tolerance is reduced in rats (e.g., aging, obesity, streptozotocin diabetes), there are well-known, although not completely understood, changes in insulin action. Whether changes in the mechanisms that underlie glucose effectiveness also occur remains to be determined, but their importance to glucose disposal suggests that they are likely to contribute to impaired glucose tolerance.

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