Mechanisms of Epinephrine-induced Glucose Intolerance in Normal Humans

ROLE OF THE SPLANCHNIC BED

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ABSTRACT To evaluate the role of the splanchnic bed in epinephrine-induced glucose intolerance, we selectively assessed the components of net splanchnic glucose balance, i.e., splanchnic glucose uptake and hepatic glucose production, and peripheral glucose uptake by combining infusion of [3-^{3}H]glucose with hepatic vein catheterization. Normal humans received a 90-min infusion of either glucose alone (6.5 mg/kg^{-1} per min^{-1}) or epinephrine plus glucose at two dose levels: (*a*) in amounts that simulated the hyperglycemia seen with glucose alone (3.0 mg/kg^{-1} per min^{-1}); and (*b*) in amounts identical to the control study. During infusion of glucose alone, blood glucose rose twofold, insulin levels and net posthepatic insulin release increased three- to fourfold, and net splanchnic glucose output switched from a net output (1.65±0.12 mg/kg^{-1} per min^{-1}) to a net uptake (1.56±0.18). This was due to a 90–95% fall ($P < 0.001$) in hepatic glucose production and a 100% rise ($P < 0.001$) in splanchnic glucose uptake (from 0.86±0.14 to 1.71±0.12 mg/kg^{-1} per min^{-1}), which in the basal state amounted to 30–35% of total glucose uptake. Peripheral glucose uptake rose by 170–185% ($P < 0.001$). When epinephrine was combined with the lower glucose dose, blood glucose, insulin release, and hepatic blood flow were no different from values observed with glucose alone. However, hepatic glucose production fell only 40–45% ($P < 0.05$ vs. glucose alone) and, most importantly, the rise in splanchnic glucose uptake was totally blocked. As a result, splanchnic glucose clearance fell by 50% ($P < 0.05$), and net splanchnic glucose uptake did not occur. The rise in peripheral glucose uptake was also reduced by 50–60% ($P < 0.001$). When epinephrine was added to the same dose of glucose used in the control study, blood glucose rose twofold higher ($P < 0.001$). The initial rise in splanchnic glucose uptake was totally prevented; however, beyond 30 min, splanchnic glucose uptake increased, reaching levels seen in the control study when severe hyperglycemia occurred. Splanchnic glucose clearance, nevertheless, remained suppressed throughout the entire study (40%–50%, $P < 0.01$).

It is concluded that (*a*) the splanchnic bed accounts for one-third of total body glucose uptake in the basal state in normal humans; (*b*) epinephrine markedly inhibits the rise in splanchnic glucose uptake induced by infusion of glucose; and (*c*) this effect does not require a fall in insulin and is modulated by the level of hyperglycemia. Our data indicate that the splanchnic bed is an important site of glucose uptake in postabsorptive humans and that epinephrine impairs glucose tolerance by suppressing glucose uptake by both splanchnic and peripheral tissues, as well as by its well known stimulatory effect on endogenous glucose production.

INTRODUCTION

The hyperglycemic actions of epinephrine are particularly striking during exogenous glucose administration (1–4). Even small physiological increments of plasma epinephrine that cause minimal changes in fasting glucose concentration, markedly impair oral glucose tolerance in normal humans, in spite of increased insulin levels (3). The mechanisms underlying this epinephrine-induced deterioration of glucose tolerance have not, however, been fully investigated.

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Earlier studies have generally focused on the fasted state where epinephrine has been shown to produce hyperglycemia in humans by both stimulation of hepatic glucose production and inhibition of total body glucose utilization (5–8). Over 30 yr ago Somogyi postulated (1) that epinephrine interfered with the disposal of a glucose load primarily by decreasing exogenous glucose uptake rather than by increasing endogenous glucose production. Recent studies in the dog provide direct evidence supporting this view (4). However, since the methods used in these studies (radiolabeled glucose) measure only glucose uptake by the total body, it is uncertain whether epinephrine inhibits the uptake of glucose at the level of the splanchnic bed, or peripheral tissues (e.g., muscle), or both. These data are of particular interest, because the factors regulating splanchnic and peripheral glucose uptake may differ (9). For example, it has been shown that, in contrast to muscle and adipose tissues, even marked hyperinsulinemia has little effect on net glucose uptake by the splanchnic bed when glucose levels are held constant (9).

In spite of evidence that the splanchnic bed is the major site of glucose uptake after glucose ingestion (10), the factors regulating splanchnic glucose uptake in vivo remain poorly understood and the effect of epinephrine on this process has not been determined. Previous studies in vivo have been limited, because the available techniques did not permit direct quantitation of glucose uptake by the splanchnic bed. In earlier experiments, splanchnic glucose uptake was estimated indirectly from measurements of net splanchnic glucose balance using hepatic vein catheterization (9, 10). Since this technique does not separate the components of net splanchnic glucose balance, i.e., hepatic glucose production and splanchnic glucose uptake, it provides a close estimate of the rate of splanchnic glucose uptake only in circumstances when hepatic glucose production is totally suppressed, but is of little value in the basal state or during epinephrine infusion when hepatic glucose production is ongoing. We therefore developed a method for separating the components of net splanchnic glucose balance by combining hepatic vein catheterization with the tracer infusion technique (11). With this approach splanchnic glucose uptake could be measured directly in humans, making it possible to evaluate the influence of epinephrine on each of the major components of the glucoregulatory response to glucose administration, i.e., splanchnic glucose uptake, peripheral glucose uptake, and hepatic glucose production.

METHODS

Subjects. 20 male subjects aged 28–46 yr were studied. All were within 15% of their ideal body weight (Metropolitan Life Insurance Tables, 1959) and exhibited normal glucose tolerance after a 75-g oral glucose load (12). None had a history of liver or renal disease or were taking any drugs. For 3 d before the study they consumed a diet containing at least 250 g of carbohydrate.

The group (n = 15) that participated in the hepatic vein catheterization studies were asymptomatic patients, admitted to the Institute of Medical Pathology for diagnostic cardiac catheterization. They had a negative history for coronary artery disease and congestive heart failure, and had a normal exercise electrocardiogram. Only subjects with a normal cardiac output and pulmonary artery systolic blood pressure during the cardiac catheterization procedure were studied. The nature, purpose, and possible risk of the additional procedures were fully explained to each subject before his written consent to participate was obtained before cardiac catheterization. All experiments were performed in the Cardiovascular Research Laboratory of the Institute of Medical Pathology, University of Naples.

Procedures. All subjects were studied in the postabsorptive state after a 15–17-h overnight fast. Teflon catheters were inserted percutaneously in a brachial artery for blood sampling and in an antecubital vein for subsequent infusion of [3-^{3}H]glucose, glucose, indocyanine green dye, and epinephrine. A Cournard catheter (7F) was introduced percutaneously in a contralateral antecubital vein and advanced under fluoroscopic control into the right heart for diagnostic cardiac catheterization. After the diagnostic procedure was completed, the experiment was initiated by advancing the catheter into a right-sided main hepatic vein. The tip of the catheter was placed 2–3 cm from the wedge position and its location checked before each sampling by fluoroscopy. Patency of the hepatic vein catheter was maintained by a continuous infusion of saline containing no anticoagulant. At the beginning of each experiment (t = −90 min), a priming dose of [3-^{3}H]glucose (50 μCi) (Amersham Corp., Buckinghamshire, England) and indocyanine green dye (10 mg) (Cardio-Green, Hynson, Westcott, and Dunning, Baltimore, Md.) was injected rapidly followed by a continuous infusion at a rate of 0.50 μCi/min and 0.5 mg/min, respectively for the remainder of the study (180 min).

After a 90-min equilibration period, three groups of experiments were performed. In the first (n = 6), an intravenous infusion of glucose alone was administered at a rate of 6.5 mg/kg^{-1} per min^{-1} for 90 min (control group). In the second group (n = 5), epinephrine was infused at a rate of 50 ng/kg^{-1} per min^{-1} together with a smaller dose of glucose (3 mg/kg^{-1} per min^{-1}), which based on preliminary studies, raised blood glucose concentrations to comparable levels as those observed in the control study. This was done to evaluate the effect of epinephrine on glucose metabolism independent of the mass effect of glucose concentration per se (13). In the third group (n = 4), epinephrine (50 ng/kg^{-1} per min^{-1}) was infused with the same dose of glucose used in the control study (6.5 mg/kg^{-1} per min^{-1}). The epinephrine solution was prepared in sterile saline containing ascorbic acid (30 mg/100 ml) to protect against oxidation. Blood samples were withdrawn simultaneously from the arterial and hepatic vein catheters for chemical analyses and hepatic blood flow measurements in the basal state and at 15-min intervals thereafter.

To evaluate whether the epinephrine infusion caused the release of [3-^{3}H]glucose that had been sequestered in the liver, five additional experiments were performed. In these studies [2-^{3}H] glucose and [1-^{14}C]glucose were infused instead of [3-^{3}H]glucose, and after 90 min either glucose alone (6.5 mg/kg^{-1}) (n = 2), or glucose (6.5 mg/kg^{-1} per min^{-1}) plus

epinephrine (50 ng/kg^{-1} per min^{-1}) (n = 3) was added to the tracer infusion. Arterial blood samples were obtained in the basal state and at 15-min intervals thereafter for measurement of the ratio of [2-^{3}H]glucose to [1-^{14}C]glucose radioactivity in plasma. Because neither [3-^{3}H]glucose nor [1-^{14}C]glucose loses its label during passage into and out of the hepatic glycogen while [2-^{3}H]glucose for the most part does (14), a fall in the ratio of [2-^{3}H]glucose/[1-^{14}C]glucose would suggest increased release of labeled glucose from hepatic glycogen. [1-^{14}C]Glucose was used instead of [3-^{3}H]glucose to permit separation from [2-^{3}H]glucose.

Analytical methods. Blood glucose concentration was measured by the glucose oxidase method (15). The methods used for determination of plasma immunoreactive insulin and glucagon (using antibody 30K) have been previously described (16, 17). The plasma concentration of indocyanine green was measured in a Beckman spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). For the assay of [^{3}H]glucose radioactivity, blood samples were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and the Somogyi filtrate was evaporated to dryness at 70°C to remove tritiated water. The dry residue was dissolved in 1 ml of water and counted with 10 ml of Insta-Gel (Packard Instrument Co., Downers Grove, Ill.) in a liquid scintillation system. The counting error was always <1% ($\pm 2\sigma$). [1-^{14}C]Glucose radioactivity was separated from the Somogyi filtrate using an ion-exchange resin (Bio-Rad AG-2X8, Bio-Rad Laboratories, Richmond, Calif.) column method, described in detail elsewhere (4). Correction for recycling of label was made by determining the radioactivity of the sixth carbon, isolated as the dimedone derivative and multiplying by the factor 4.5 (18).

Calculations. Hepatic blood flow was estimated using Indocyanine Green dye according to the method of Leevy et al. (19). Net splanchnic glucose balance was calculated by multiplying the hepatic venous-arterial (HV-A) blood glucose difference by the estimated hepatic blood flow (EHBF). Splanchnic glucose clearance and uptake and hepatic glucose production (extrasplanchnic glucose appearance) were calculated using the following equations:

(1) Splanchnic glucose clearance = EHBF × (HV-A) blood [3-^{3}H]glucose radioactivity/arterial blood [3-^{3}H]glucose radioactivity.

(2) Splanchnic glucose uptake = splanchnic clearance × arterial blood glucose concentration.

(3) Hepatic glucose production = net splanchnic balance + splanchnic uptake. The latter calculation is based on the assumption that glucose production from splanchnic bed is entirely derived from hepatic sources. Glucose uptake by all the extrasplanchnic tissues of the body, denoted as "peripheral", was estimated according to the formula: peripheral glucose uptake = systemic (extrasplanchnic) glucose delivery − pVdg/dt where the systemic glucose delivery = net splanchnic glucose balance + exogenously infused glucose; p is the rapidly mixing compartment of the glucose space, assumed to be 0.75 (20); V is the distribution volume of glucose, assumed to be 20% of body wt (21); g is the blood glucose concentration. The values of g and dg/dt were calculated from their polynomial functions fitted by the method of least squares. Rates of peripheral glucose uptake are not presented for the combined epinephrine/high dose glucose infusion since glycosuria occurred in these studies, precluding an accurate estimate of peripheral glucose metabolism.

Rates of hepatic glucose production and splanchnic glucose uptake were also measured indirectly from the systemic tracer measurements to verify the above calculations. In the basal state the rate of glucose appearance in the systemic circulation (assumed equal to the rate of hepatic glucose production) was calculated by the isotope dilution equation: $Ra = F/SA_E$; where F = the rate of tracer infusion; and SA_E = the specific activity of plasma glucose at equilibrium. In the nonsteady state, Ra was determined by Steele's equation in its derivative form (11), using a polynomial fitting procedure previously validated (22). Splanchnic glucose uptake was calculated using the following equation: splanchnic glucose uptake = Ra − net splanchnic glucose balance.

The data presented in the text and figures are based on the direct catheter measurements, unless specifically denoted as "systemic" tracer calculations. This was done because the latter estimates are, in part, dependent on the model used to fit the time curves for glucose concentration and glucose specific activity during nonsteady-state conditions and thus may be subject to greater error. The net rate of release of insulin from the splanchnic bed or net posthepatic insulin delivery rate was calculated by multiplying the (HV-A) plasma insulin difference by the estimated hepatic plasma flow.

All calculations were performed on a Wang 2200 computer (Wang Laboratories, Inc., Lowell, Mass.). Statistical analyses were performed with the Student's t test, analysis of variance and Duncan's multiple-range test. Data are presented as the mean±SEM.

RESULTS

Infusion of glucose alone. Infusion of glucose alone (6.5 mg/kg^{-1} per min^{-1}) produced a progressive twofold increase in arterial blood glucose concentration (from 87±4 to 173±8 mg/dl) (Fig. 1). As shown in Fig. 1, net splanchnic glucose output (1.65±0.12 mg/kg^{-1} per min^{-1}, basal) fell rapidly ($P < 0.001$), switching to a net uptake of 1.5–1.6 mg/kg^{-1} per min^{-1} between 45–90 min. Estimated hepatic blood flow remained unchanged (Table I). The mechanisms responsible for this reversal of net splanchnic glucose balance are evident when one examines its components, i.e., hepatic glucose production and splanchnic glucose uptake (Fig. 1). Hepatic glucose production (2.55±0.18 mg/kg^{-1} per min^{-1}, basal) declined by 90–95% ($P < 0.001$) to levels of 0.16–0.24 mg/kg^{-1} per min^{-1}. Splanchnic glucose uptake that was one-third of total glucose uptake in the basal state (0.86±0.14 mg/kg^{-1} per min^{-1}) rose, reaching levels 100% above base line between 30–90 min ($P < 0.05$–$P < 0.001$). Since this increase in splanchnic glucose uptake was accompanied by a similar rise in plasma glucose concentration, splanchnic glucose clearance (0.99±0.15 ml/kg^{-1} per min^{-1}, basal) remained stable throughout the 90-min study period (Fig. 1). Peripheral glucose uptake (1.65±0.12 mg/kg^{-1} per min^{-1}, basal) also increased by 170–185% ($P < 0.001$) during the glucose infusion (Table II). At the end of the study, when blood glucose began to stabilize, total (splanchnic plus peripheral) glucose uptake (6.44 mg/kg^{-1} per min^{-1}) closely approximated the exogenous glucose infusion rate. At this point, glucose uptake by splanchnic and peripheral tissues was 27 and 73%, respectively.

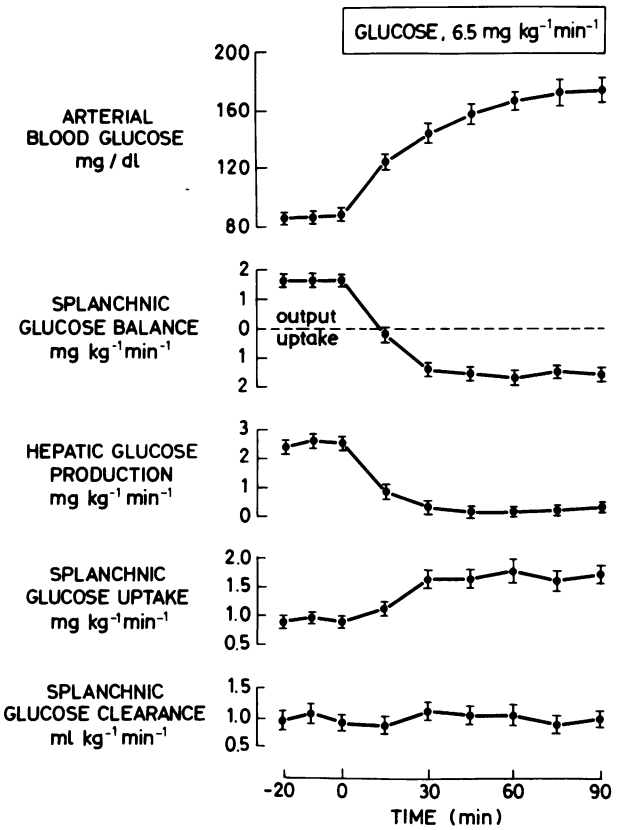

FIGURE 1 Changes in arterial blood glucose concentration, net splanchnic glucose balance, hepatic glucose production, splanchnic glucose uptake, and splanchnic glucose clearance during infusion of glucose alone.

Estimates of hepatic glucose production and splanchnic glucose uptake (2.79±0.26 and 1.00±0.19 mg/kg^{-1} per min^{-1}, respectively) based on the systemic tracer method were comparable to those obtained with the catheter in the basal state (P = NS). Furthermore, after glucose infusion a similar fall in hepatic glucose production (to 0.02±0.02 mg/kg^{-1} per min^{-1}, $P < 0.001$) and rise in splanchnic glucose uptake (to 1.68±0.12 mg/kg^{-1} per min^{-1}, $P > 0.05$) occurred between 60–90 min.

As shown in Fig. 2 (shaded area), both arterial insulin levels (10±2μU/ml, basal) and net posthepatic insulin delivery into the systemic circulation (51±13 μU/kg^{-1} per min^{-1}, basal) increased three-to fourfold after the infusion of glucose, although the latter peaked earlier. Plasma glucagon (104±17 pg/ml) decreased by 35–40% ($P < 0.05$).

Effect of epinephrine on glucose disposal during low dose glucose infusion. When epinephrine was infused along with glucose at a rate of 3 mg/kg^{-1} per min^{-1} the rise in arterial blood glucose (to 177±11 mg/dl at 90 min) was virtually identical to that observed in the control group, in spite of the lower dose of glucose used (Fig. 3 and Table II). As a result, the effect of epinephrine on splanchnic and peripheral glucose uptake could be determined under conditions of comparable hyperglycemia. Net splanchnic glucose output (1.43±0.10 mg/kg^{-1} per min^{-1}, basal) gradually decreased by 50–85% ($P < 0.01$) (Fig. 3). However, net splanchnic glucose balance differed from values in the control group ($P < 0.001$) and, most importantly, net uptake did not occur. The failure of splanchnic glucose balance to switch to a net uptake was due to incomplete suppression of hepatic glucose production as well as a lack of an increase of splanchnic glucose uptake (Fig. 3). Hepatic glucose production (2.23±0.22 mg/kg^{-1} per min^{-1}, basal) fell by only 40–50% ($P < 0.025$), remaining at levels 1.0–1.2 mg/kg^{-1} per min^{-1} above those of the control group ($P < 0.05$–$P < 0.001$). Splanchnic glucose uptake (0.79±0.15 mg/kg^{-1} per min^{-1}, basal) did not rise significantly above base line at any time during the 90-min study period, although estimated hepatic blood flow remained unchanged (Table I). Accordingly, splanchnic glucose clearance (0.94±0.18 ml/kg^{-1} per min^{-1}, basal) rapidly declined by 50% ($P < 0.05$–$P < 0.01$). Peripheral glucose uptake (1.43±0.10 mg/kg^{-1} per min^{-1}, basal) increased by 70–90% ($P < 0.001$), but markedly less than that observed in the control group (Table II).

TABLE I

*Estimated Hepatic Blood Flow during Infusion of Glucose Alone or Epinephrine plus Glucose**

Condition	0	15 min	30 min	45 min	60 min	75 min	90 min
				liters/min			
Glucose	1.24±0.14	1.43±0.26	1.47±0.25	1.34±0.17	1.20±0.08	1.23±0.12	1.35±0.17
EPI + glucose$_{LD}$‡	1.25±0.05	1.43±0.09	1.32±0.10	1.39±0.07	1.44±0.08	1.49±0.14	1.46±0.09
EPI + glucose$_{HD}$§	1.03±0.15	0.97±0.12	0.97±0.10	0.95±0.11	0.99±0.09	1.00±0.08	0.96±0.12

* Data presented as mean±SE. Base-line values (0 min) represent the mean of three observations on each subject taken at 10-min intervals preceding the infusions. None of the subsequent values are significantly different from base line.

‡ Indicates epinephrine (EPI) plus low dose (LD) glucose infusion (3 mg/kg^{-1} per min^{-1}).

§ Indicates epinephrine plus high dose (HD) glucose infusion (6.5 mg/kg^{-1} per min^{-1}).

TABLE II

*Blood Glucose and Peripheral Glucose Uptake during Infusion of Glucose Alone and Epinephrine plus Low Dose Glucose**

	Condition	0	15 min	30 min	45 min	60 min	75 min	90 min
Plasma glucose, *mg/dl*	Glucose‡	87±4	124±5	144±7	157±7	166±6	172±7	173±8
	EPI + glucose§	83±1	119±1	139±5	153±7	163±8	171±9	177±11
Peripheral glucose uptake, $mg\ kg^{-1}/min^{-1}$	Glucose	1.65±0.12	2.93±0.20	2.90±0.20	3.42±0.36	3.72±0.37	4.49±0.19	4.70±0.27
	EPI + glucose	1.43±0.10	1.22±0.30••	1.93±0.25‖	2.15±0.30‖	2.30±0.10¶	2.62±0.10••	2.56±0.16••

* Data presented as mean±SE. Base-line values (0 min) represent the mean of three observations on each subject preceding infusion. *P* values refer to significance of difference between the two groups (unpaired *t* test). Only values that differ significantly are indicated.

‡ Indicates infusion of glucose at a rate of 6.5 mg/kg^{-1} per min^{-1}.

§ Indicates infusion of epinephrine (EPI) (50 ng/kg^{-1} per min^{-1}) plus glucose (3 mg/kg^{-1} per min^{-1}).

‖ Indicates $P < 0.025$.

¶ Indicates $P < 0.005$.

•• Indicates $P < 0.001$.

Similar results were obtained when the systemic tracer method was used to assess splanchnic glucose kinetics. The decline in hepatic glucose production (from 2.36±0.17 to 1.36±0.30 mg/kg^{-1} per min^{-1}) was significantly reduced when compared to the control study ($P < 0.001$). Furthermore, splanchnic glucose uptake (0.93±0.11 mg/kg^{-1} per min^{-1}, basal) remained unchanged (0.95±0.16 mg/kg^{-1} per min^{-1} at 60–90 min) in spite of the rise in plasma glucose concentration.

As shown in Fig. 2, the increase in arterial insulin levels and net posthepatic insulin delivery was not significantly different from that observed during infusion of glucose alone. In contrast, only a small (10–15%), insignificant fall in plasma glucagon occurred during the combined epinephrine-low dose glucose infusion.

Effect of epinephrine on the glucoregulatory response to the higher dose glucose infusion. When glucose was infused at the same rate used in the control study (6.5 mg/kg^{-1} per min^{-1}) epinephrine produced a twofold greater rise in blood glucose concentration (265±18 mg/dl at 90 min, $P < 0.001$ vs. glucose alone) (Fig. 4). Net splanchnic glucose output (1.46±0.15 mg/kg^{-1} per min^{-1}, basal) progressively declined, but only switched to a net uptake in the last 30 min of the study. The latter changes in net splanchnic glucose balance were more pronounced than those observed with the smaller glucose infusion rate (where net uptake never did occur) but were less than those seen with the infusion of glucose alone ($P < 0.05$–$P < 0.001$), except at 90 min (Fig. 4). Hepatic glucose production (2.21±0.17 mg/kg^{-1} per min^{-1}, basal) gradually fell by 65–70% ($P < 0.001$), albeit to a lesser extent than in the control study ($P < 0.05$–$P < 0.001$) (Fig. 4). In addition, epinephrine totally blocked the rise in splanchnic glucose uptake (0.77±0.12 mg/kg^{-1} per min^{-1}, basal) in the first 30 min of the study. Thereafter, splanchnic glucose uptake gradually increased, reaching levels seen with glucose alone after marked hyperglycemia developed (at 75–90 min). At this time, splanchnic glucose uptake exceeded values observed with the smaller glucose dose (1.57±0.10 vs. 0.82±0.20 mg/kg^{-1} per min^{-1}, $P < 0.05$). On the other hand, epinephrine suppressed splanchnic glucose clearance (by 40–50%, $P < 0.01$) to the same extent as that seen during low dose glucose infusion (P = NS). As in previous experiments, estimated hepatic blood flow was not significantly altered from base-line values (Table I).

Changes in splanchnic glucose metabolism were quantitatively similar when the systemic tracer method was used. Hepatic glucose production (2.40±14 mg/kg^{-1} per min^{-1}, basal) fell by 85±18% between 60–90 min. Splanchnic glucose uptake (0.94±0.17 mg/kg^{-1} per min^{-1}, basal) remained constant initially (0–30 min) and then rose to 1.51±0.35 mg/kg^{-1} per min^{-1} by the completion of the study.

The effect of epinephrine on plasma insulin, net posthepatic insulin delivery, and plasma glucagon is shown in Fig. 2. The rise in plasma insulin and systemic insulin release was not significantly different from the glucose control study, despite markedly higher glucose levels. Plasma glucagon (118±19 pg/ml, basal) fell significantly within 45 min, reaching levels 20% below base line ($P < 0.005$).

Table III shows the effect of the epinephrine infusion on the ratio of [2-^{3}H]glucose to [1-^{14}C]glucose in plasma. In both the glucose control study and the combined epinephrine plus glucose infusion the [2-^{3}H] glucose/[1-^{14}C]glucose ratio remained remarkably constant throughout the entire study, suggesting that [^{14}C]glucose was not released from the liver.

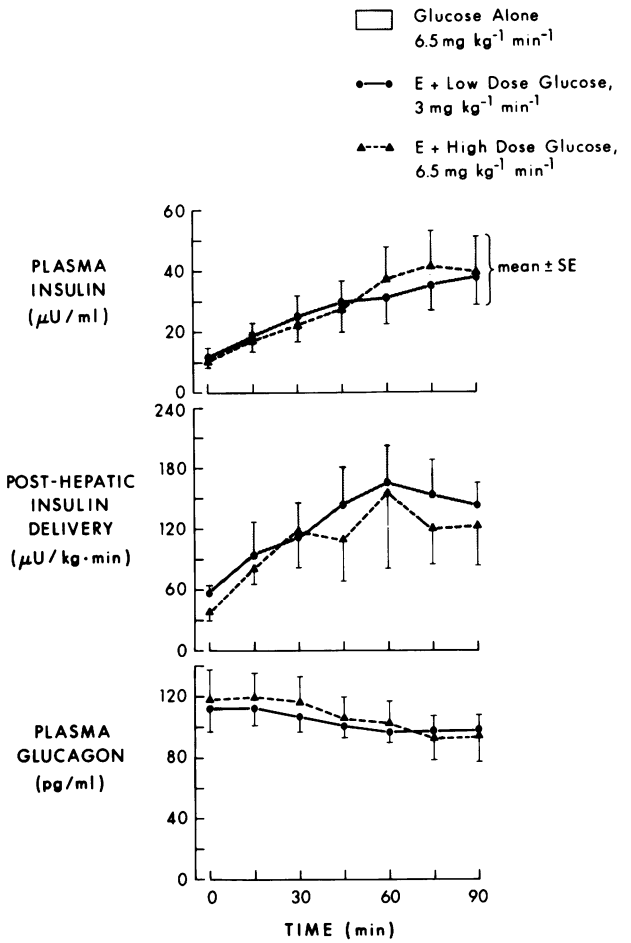

FIGURE 2 Effect of epinephrine (E) on the plasma insulin and glucagon response, and the net rate of posthepatic insulin delivery during high and low dose glucose infusion. Hormonal changes during infusion of glucose alone are denoted by the shaded area.

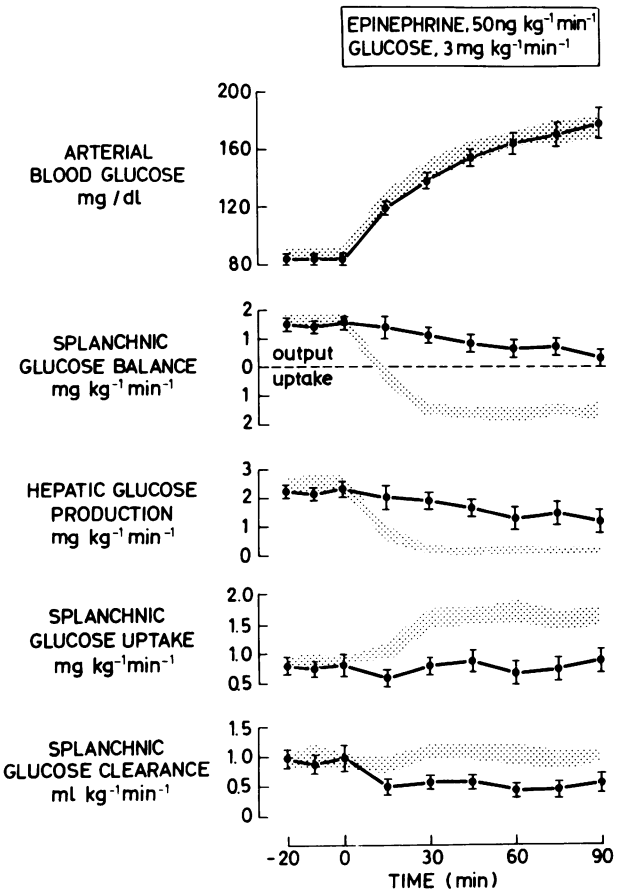

FIGURE 3 Changes in arterial blood glucose concentration, net splanchnic glucose balance, hepatic glucose production, splanchnic glucose uptake, and splanchnic glucose clearance during combined epinephrine/low dose glucose infusion. The shaded area denotes the response to infusion of glucose alone (6.5 mg/kg^{-1} per min^{-1}) that caused identical increments in blood glucose concentration.

DISCUSSION

In this study we used an experimental method that selectively assesses each of the glucoregulatory processes governing the response to an exogenous glucose load, i.e., hepatic glucose production, splanchnic glucose uptake, and peripheral glucose uptake. Similar results were obtained whether the individual components of net splanchnic glucose balance were calculated from direct measurements of tracer glucose uptake by the splanchnic bed or from the difference of net splanchnic glucose balance and indirect measurements of endogenous glucose production using systemic glucose specific activity (Methods). Although our primary objective was to evaluate the influence of epinephrine on the glucoregulatory response to intravenous glucose infusion, the data obtained in the basal state and during the control glucose infusion will be considered in detail, since neither splanchnic glucose uptake nor its contribution to total body glucose uptake has been directly measured in vivo under these conditions.

Of particular interest is the finding that the splanchnic bed is a major site of glucose uptake in overnight fasted humans. Approximately one-third of the glucose produced by the liver and exported into the systemic circulation in the basal state (~0.8 mg/kg^{-1} per min^{-1}) was taken up by splanchnic tissues. Only the brain contributes more to overall glucose uptake in the basal state (23). These observations are consistent with recent studies in the dog (using portal vein and arterial catheters) showing that extrahepatic splanchnic tissues consume significant amounts of glucose in the basal state (0.5–0.6 mg/kg^{-1} per min^{-1}) (24). Although the

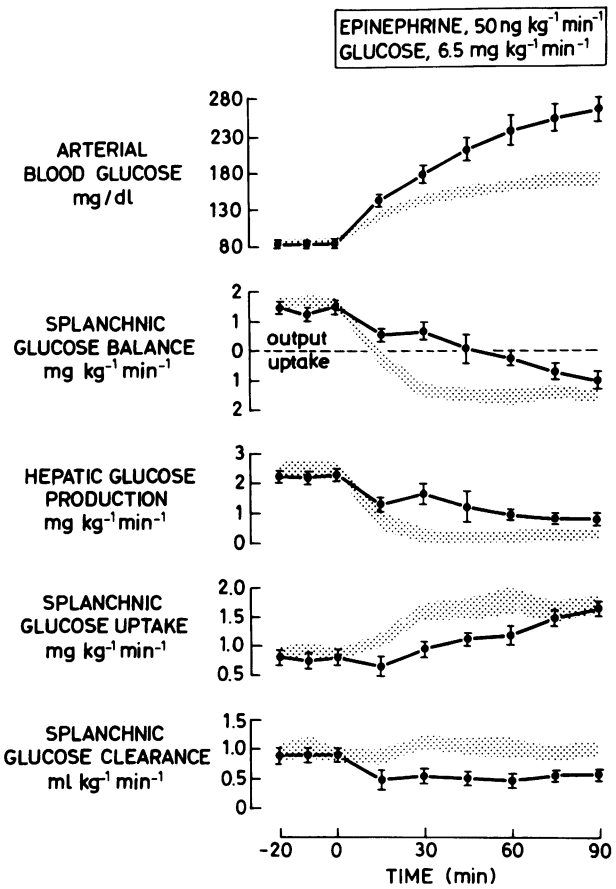

FIGURE 4 Effect of the addition of epinephrine on the response of blood glucose and splanchnic glucose metabolism to infusion of glucose. The shaded area denotes the response to glucose alone (6.5 mg/kg^{-1} per min^{-1}).

combined tracer-hepatic vein catheterization technique used in our study does not distinguish between glucose uptake by hepatic or extrahepatic splanchnic tissues, the above observations (24) suggest that the liver and gut may both contribute to basal splanchnic glucose uptake.

In keeping with the earlier studies (9, 11, 25–27), we demonstrate that hyperglycemia is minimized during exogenous glucose infusion essentially by three mechanisms: (*a*) inhibition of endogenous glucose production; (*b*) stimulation of splanchnic glucose uptake; and (*c*) stimulation of peripheral glucose uptake. The magnitude of the fall in hepatic glucose production (90–95%) is in close agreement with previous data in normal humans (9, 26, 27). With respect to the disposal of the infused glucose, approximately one-quarter of the exogenous glucose load was sequestered by the splanchnic area. This value is nearly twofold higher than that reported with the hyperglycemic clamp technique (9). However, in those studies the contribution of the splanchnic bed to total body glucose uptake may have been less because net splanchnic glucose balance rather than splanchnic glucose uptake was measured and, more importantly, plasma glucose and insulin levels were higher, which favored glucose uptake by peripheral instead of splanchnic tissues. It is of interest that the percent increase in splanchnic glucose uptake was similar to the percent increase in blood glucose concentration (or the rate of splanchnic glucose delivery since hepatic blood flow was unchanged). As a result, splanchnic glucose clearance remained relatively stable in the face of a three- to fourfold rise in net insulin release from the splanchnic bed (Figs. 1 and 2). These findings are consistent with in vitro studies by Soskin and Levine (28) and Bergman (29) showing a critical role for hyperglycemia per se in regulating hepatic glucose uptake as well as hepatic vein catheter studies by DeFronzo et al. (9) in humans, showing that the rise in net splanchnic glucose uptake induced by intravenous glucose is proportional to the rise in plasma glucose concentration.

TABLE III

The Ratio [2-^{3}H]Glucose to [1-^{14}C]Glucose Radioactivity during Infusion of Glucose with (n = 3) and without (n = 2) the Addition of Epinephrine

	Basal			Glucose±epinephrine infusion					
Condition	−20 min	−10 min	0 min	15 min	30 min	45 min	60 min	75 min	90 min
Glucose alone									
1*	0.99	0.99	0.96	1.01	1.00	1.01	1.01	1.04	1.05
2	0.84	0.87	0.84	0.82	0.83	0.80	0.81	0.84	0.84
Glucose + epinephrine									
3	0.85	0.84	0.85	0.84	0.83	0.85	0.84	0.84	0.84
4	0.86	0.85	0.86	0.84	0.86	0.86	0.83	0.82	0.83
5	0.79	0.82	0.79	0.77	0.79	0.77	0.79	0.79	0.82

* Data are presented for individual experimental subjects.

Regardless of the experimental design, our data demonstrate a considerable degree of glucose intolerance following epinephrine administration. The measurements of splanchnic and peripheral glucose kinetics during the combined epinephrine/low dose glucose infusion, in particular, provide a means of assessing the mechanisms underlying this phenomenon. Under these conditions, epinephrine entirely prevented the switch of the splanchnic bed from an area of net glucose output to one of net glucose uptake (Fig. 3). When the components of splanchnic glucose balance are individually examined, the suppression of hepatic glucose production was considerably reduced as compared with that observed with glucose alone. These findings are in keeping with earlier observations in dogs and humans showing that epinephrine blocks the rise in glucose production induced by insulin infusion (30, 31). In addition, the rise in splanchnic glucose uptake was totally suppressed in spite of a twofold increase in blood glucose and no differences in plasma insulin concentration, net posthepatic insulin release, or hepatic blood flow. Splanchnic glucose clearance, an index of the ability of splanchnic tissues to take up glucose independent of changes in glucose concentration, fell by 50% throughout the entire study. An undetected reduction in insulin secretion could not have accounted for this phenomenon, because even larger increments in plasma insulin have little or no effect on net splanchnic glucose uptake (9).

Although earlier studies have demonstrated that epinephrine inhibits total body glucose disposal (4–8), the relative contribution of reduced splanchnic vs. peripheral glucose uptake has not been previously examined for this hormone (or, for that matter, any other counterregulatory hormone). While the magnitude of epinephrine's effect on splanchnic glucose uptake could have been overestimated by virtue of the release of [3-^{3}H]glucose incorporated into hepatic glycogen during the equilibration period, this seems highly unlikely for several reasons. Previous studies have shown that negligible amounts of infused label are incorporated into hepatic glycogen in the fasting state (32), and the rise in blood glucose and insulin during these studies would be expected to offset epinephrine-stimulated hepatic glycogenolysis (32–34). This assumption is supported by experimental evidence as well. When we infused [1-^{14}C]glucose together with [2-^{3}H]glucose (a label not significantly incorporated into hepatic glycogen) the ratio of these tracers did not change in spite of the addition of epinephrine to the glucose infusion. In addition, similar results were obtained when splanchnic glucose uptake was calculated using the changes in glucose specific activity in the systemic circulation, a method that would be affected less by the release of labeled glucose from the liver. Finally, it is conceivable (but not established) that a small proportion of the [3-^{3}H]glucose taken up the liver loses its tritium within the triose-phosphate pool, only to be resynthesized without undergoing complete oxidation. If such a cycle exists, the calculated rate of splanchnic glucose uptake would slightly overestimate glucose utilization by splanchnic tissues as well as the rate of hepatic glucose production. This, however, would tend to underestimate epinephrine's inhibitory effect on splanchnic glucose utilization, since it is likely that hepatic gluconeogenesis is relatively increased by epinephrine administration (35).

Epinephrine also inhibited glucose uptake by extrasplanchnic peripheral tissues. Indeed, peripheral glucose uptake increased by only 80% in contrast to the threefold elevation observed with the infusion of glucose alone. It is of interest that the rate of peripheral and splanchnic glucose uptake reached 2.6 and 0.9 mg/kg^{-1} per min^{-1}, respectively at the end of the epinephrine/low dose glucose infusion. These values correspond to 74 and 26% of total body glucose uptake, a proportion identical to that seen in the control study (i.e., 73 and 27%). It should be noted, however, that while the proportion of total glucose uptake occurring in splanchnic and peripheral tissues did not change, splanchnic glucose uptake did not rise above base-line values, whereas peripheral glucose uptake did. Thus, one might argue that the splanchnic bed was affected to a greater extent than the periphery. Furthermore, because a greater proportion of glucose uptake occurs in the splanchnic bed when glucose is ingested (9, 10), it is possible that epinephrine would have reduced the relative contribution of splanchnic tissues to total body glucose uptake if oral rather than intravenous glucose had been given.

The experiments in which blood glucose was allowed to rise excessively (combined epinephrine/high dose glucose infusion) suggest an important influence of blood glucose concentration per se on epinephrine-mediated changes in splanchnic glucose metabolism. In this circumstance, the initial rise in splanchnic glucose uptake was delayed, but later reached levels seen in the control study after severe hyperglycemia developed. This occurred even though splanchnic glucose clearance (a measurement that compensates for hyperglycemia) remained suppressed, and plasma insulin and posthepatic insulin release did not increase excessively (Fig. 2). Furthermore, the magnitude of the suppression of hepatic glucose production (70%) was more pronounced than that observed with the infusion of epinephrine and low dose glucose (50%), although this difference did not reach statistical significance. Taken together, these observations suggest that the more pronounced hyperglycemia accompanying these experiments partially overcame epinephrine's inhibi-

tory action on splanchnic glucose metabolism. Previous studies have shown that hyperglycemia per se is capable of increasing hepatic glucose uptake (29, 33) and reducing hepatic glucose output (34) independent of changes in circulating insulin.

Finally, it should be noted that plasma glucagon levels fell to a lesser extent when epinephrine was added to the glucose infusion (Fig. 2). It is therefore conceivable that the inhibitory effects of epinephrine on splanchnic glucose metabolism are, in part, mediated by insufficient suppression of glucagon secretion. This possibility is, however, not supported by data indicating that marked hyperglucagonemia does not alter intravenous (36) or oral (37, 38) glucose tolerance in normal humans. Studies using the double tracer technique of Radziuk et al. (39) have also shown that hyperglucagonemia does not affect suppression of hepatic glucose production or the rise in total glucose uptake during glucose ingestion (4). Consequently, it is very unlikely that the small differences in glucagon levels seen in this study could exert a significant effect, particularly since both insulin and glucose concentrations were elevated during these experiments.

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