Neuroendocrine Responses to Glucose Ingestion in Man

SPECIFICITY, TEMPORAL RELATIONSHIPS, AND QUANTITATIVE ASPECTS

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ABSTRACT The mechanisms of postprandial glucose counterregulation—those that blunt late decrements in plasma glucose, prevent hypoglycemia, and restore euglycemia—have not been fully defined. To begin to clarify these mechanisms, we measured neuroendocrine and metabolic responses to the ingestion of glucose (75 g), xylose (62.5 g), mannitol (20 g), and water in ten normal human subjects to determine for each response the magnitude, temporal relationships, and specificity for glucose ingestion. Measurements were made at 10-min intervals over 5 h. By multivariate analysis of variance, the plasma glucose ($P < 0.0001$), insulin ($P < 0.0001$), glucagon ($P < 0.03$), epinephrine ($P < 0.0004$), and growth hormone ($P < 0.01$) curves, as well as the blood lactate ($P < 0.0001$), glycerol ($P < 0.001$), and β-hydroxybutyrate ($P < 0.0001$) curves following glucose ingestion differed significantly from those following water ingestion. However, the growth hormone curves did not differ after correction for differences at base line. In contrast, the plasma norepinephrine ($P < 0.31$) and cortisol ($P < 0.24$) curves were similar after ingestion of all four test solutions, although early and sustained increments in norepinephrine occurred after all four test solutions. Thus, among the potentially important glucose regulatory factors, only transient increments in insulin, transient decrements in glucagon, and late increments in epinephrine are specific for glucose ingestion. They do not follow ingestion of water, xylose, or mannitol.

Following glucose ingestion, plasma glucose rose to peak levels of 156±6 mg/dl at 46±4 min, returned to base line at 177±4 min, reached nadirs of 63±3 mg/dl at 232±12 min, and rose to levels comparable to base line at 305 min, which was the final sampling point. Plasma insulin rose to peak levels of 150±17 μU/ml ($P < 0.001$) at 67±8 min. At the time glucose returned to base line, insulin levels (49±12 μU/ml) remained fourfold higher than base line ($P < 0.01$); thereafter they declined but never fell below base line. Plasma glucagon decreased from 95±14 pg/ml to nadirs of 67±11 pg/ml ($P < 0.001$) at 84±9 min and then rose progressively to peak levels of 114±17 pg/ml ($P < 0.001$ vs. nadirs) at 265±12 min. Plasma epinephrine, which was 18±4 pg/ml at base line, did not change initially and then rose to peak levels of 119±20 pg/ml ($P < 0.001$) at 271±13 min.

These data indicate that the glucose counterregulatory process late after glucose ingestion is not solely due to the dissipation of insulin and that sympathetic neural norepinephrine, growth hormone, and cortisol do not play critical roles. They are consistent with, but do not establish, physiologic roles for the counterregulatory hormones—glucagon, epinephrine, or both—in that process.

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Received for publication 30 April 1982 and in revised form 21 March 1983.

INTRODUCTION

Following its ingestion, glucose is absorbed and the plasma glucose concentration rises. Because glucose utilization accelerates and endogenous (hepatic) glucose production is markedly suppressed, the plasma glucose concentration begins to decline long before glucose absorption is complete (1) and falls below baseline levels. As this occurs, glucose utilization diminishes and, as glucose absorption ceases, endogenous

glucose production rises, thereby returning the plasma glucose concentration to the base-line level. Clearly, endogenous glucose production must exceed glucose utilization to achieve this late rise in plasma glucose.

The regulatory mechanisms that accomplish this transition from exogenous glucose delivery to endogenous glucose production, and thus blunt the decline in plasma glucose, prevent hypoglycemia, and restore euglycemia, have not been fully defined. The well-established fact that, after glucose ingestion, plasma insulin concentrations are substantially above basal levels at the time when plasma glucose returns to base line (2–6), suggests that this transition is not solely attributable to a fall in insulin levels. Thus, factors that actively raise the plasma glucose concentration—glucose counterregulatory factors—are probably involved.

Theoretically, glucose counterregulatory factors could be hormonal, neural, autoregulatory, or a combination of these. Potentially important hormones include glucagon and epinephrine, and perhaps cortisol and growth hormone. Potentially important neural factors include norepinephrine released from sympathetic postganglionic neurons within the tissues. Lastly, evidence that hepatic glucose production is an inverse function of the plasma glucose concentration independent of hormonal and neural regulation (7–10) suggests that glucose autoregulation (11) is a potentially important glucose counterregulatory factor. Recent studies of the mechanisms of hypoglycemic counterregulation in human subjects (12–16) indicate that glucagon plays a primary role in promoting glucose recovery, that epinephrine largely compensates for deficient glucagon secretion, and that recovery from hypoglycemia fails to occur only in the absence of both glucagon and epinephrine. The extent to which these findings can be extrapolated to nonhypoglycemic glucose counterregulation is not known. However, plasma glucose decrements to levels not generally considered to represent hypoglycemia do stimulate release of glucagon, epinephrine, and other potentially important glucose counterregulatory factors (17–20).

To define the neuroendocrine responses to glucose ingestion and determine their specificity for glucose ingestion, their temporal relationships, and their quantitative aspects, we measured plasma levels of potentially important glucose counterregulatory factors at frequent intervals after ingestion of glucose, water, xylose, and mannitol by normal human subjects. The data indicate that, among the variables measured, only increments in insulin, transient decrements in glucagon, and late increments in epinephrine are specific for glucose ingestion. They are consistent with a physiologic glucose counterregulatory role for glucagon, epinephrine, or both.

METHODS

Subjects. 10 normal adults, eight men and two women, participated. Their ages ranged from 21 to 26 yr and averaged 24 yr. Their body weights ranged from 51.6 to 82.5 kg; none was more than 10% over ideal body weight (Metropolitan Life Insurance Company tables). None of the patients had a family history of diabetes. All gave their written, informed consent. These studies were approved by the Washington University Human Studies Committee.

Protocol. All studies were performed in the morning after a 12-h overnight fast with the subject in the supine position throughout each study. An intravenous needle was inserted into an antecubital vein for blood sampling 30 min prior to base-line sampling. Blood samples were drawn and blood pressures and heart rates recorded at −55, −40, −25, and −10 min before ingestion of the test solution and at 10-min intervals from 5 through 305 min after the start of ingestion.

Each subject ingested four test solutions each on four different days. The test solutions, given in varied sequence, were water, glucose (75 g), xylose (62.5 g), and mannitol (20 g), each in a volume of 300 ml. Xylose was selected because its sweet taste is not distinguishable from that of glucose and it is absorbed from the gastrointestinal tract, but does not produce changes in the hormonal variables under study. The quantity of xylose (62.5 g) was selected to produce a solution of comparable tonicity to that of the glucose solution (~1,400 mosM/kg). Mannitol was used because it is poorly absorbed from the gastrointestinal tract and would be expected to produce a shift of fluid into the gut lumen. The dose of 20 g was selected because it results in a solution that is hypertonic (~367 mosM/kg) relative to plasma but, in preliminary studies, did not generally produce gastrointestinal symptoms. Water served as a hypotonic control for the mechanical effects of ingestion.

Analytical methods. Plasma glucose was measured with a glucose oxidase method. Plasma insulin (21), glucagon (22), cortisol (23), and growth hormone (24) were measured with radioimmunoassays. Antiserum 30K was used to measure glucagon. Plasma norepinephrine and epinephrine were measured with a single isotope derivative method (25) employing 50-μl plasma samples. Microfluorometric techniques were used to measure blood lactate (26), glycerol (27), β-hydroxybutyrate (27), and alanine (28).

Statistical methods. The approach to the statistical analysis of the data is analogous to that treated in the statistical literature under the rubric of "growth curve analysis". This approach eliminates both the multiple comparisons difficulties associated with *t* tests performed at each time period and the lack of independence of the observations from one time point to the next. Each parameter of interest was subjected to a polynomial regression on time for each subject, under each condition. A sixth degree polynomial was determined to adequately describe the individual time curves. Thus it yielded seven estimated parameters for each subject, under each condition. These seven parameters were then utilized as the dependent variables for a multivariate analysis of variance (MANOVA). The MANOVA yielded overall tests of whether the four conditions taken together were different and also, three specific contrasts of primary interest: water vs. glucose ingestion, water vs. xylose ingestion, and water vs. mannitol ingestion. Each of these MANOVA effects was evaluated with the Hotelling-Lawley trace criterion and all calculations were performed using SAS79.5 (29). Logarithms of the plasma and blood concentrations were used for the polynomial regressions to control variance heterogeneity. An overall variance estimate within time point and within con-

dition was made and the pooled variance estimate was utilized to conduct t tests at each time point to further define the specific differences detected by the overall tests. The entire procedure was repeated when the original parameters were replaced with values expressed as differences from base line (the average of times −55, −40, −25, and −10 min). t Tests for differences from base line were also calculated using the pooled variance term. The data were analyzed both before and after correction for differences at base line. Base-line correction was accomplished by subtraction of the average of the base-line values from each postingestion value.

Selected variables were further analyzed by calculation of the average of individual base line, peak, and nadir values, and the average of values at the time at which plasma glucose returned to base line and that at which it reached its nadir in each individual. Comparisons were performed with a t test for paired data. Mean times for the individual peaks and nadirs were also calculated.

Data are expressed as the mean±SE throughout the manuscript.

RESULTS

Specificity for glucose ingestion. Mean (±SE) plasma glucose, insulin, glucagon, epinephrine, growth hormone, and norepinephrine concentrations before and after ingestion of water, glucose, xylose, and mannitol are shown in Fig. 1–6.

Multivariate analysis of variance (Table I) disclosed that changes in plasma glucose, insulin, glucagon, epinephrine, and growth hormone and in blood lactate, glycerol, and β-hydroxybutyrate are specific for glucose ingestion. The curves for these variables following glucose ingestion differed significantly from those following water ingestion, whereas those following xylose or mannitol ingestion did not.

After glucose ingestion, plasma glucose concentrations (Fig. 1) were significantly higher than control values (after water ingestion) and above base line from 5 through 155 min; glucose levels were lower than control values from 225 through 295 min and below base line from 225 through 305 min. Plasma insulin concentrations (Fig. 2) rose promptly after glucose ingestion and were significantly higher than control values and above base line through 235 min. Thus, on the average, hyperinsulinemia lasted 80 min longer than hyperglycemia after glucose ingestion. Notably, plasma insulin levels never fell below control or base-line values. Plasma glucagon concentrations (Fig. 3) declined transiently after glucose ingestion. Glucagon levels were lower than control values from 15 through 145 min and below base line from 5 through 155 min and then returned to levels comparable to control and base-line values. Plasma epinephrine concentrations (Fig. 4) did not change early, but rose late, after glucose ingestion. Plasma epinephrine levels were higher than control values from 175 through 305 min and above base line from 155 through 305 min. After glucose ingestion, plasma growth hormone concentrations (Fig. 5) declined initially and rose later. Plasma growth hormone levels were initially lower than control values and then higher than control values from 205 through

TABLE I
Multivariate Analysis of Variance

Measured variable	Condition	Glucose vs. water	Xylose vs. water	Mannitol vs. water
Plasma glucose	<0.0001	<0.0001	NS	NS
Plasma insulin	<0.0001	<0.0001	NS	NS
Plasma glucagon	<0.01	<0.03	NS	NS
Plasma epinephrine	<0.001*	<0.0004*	NS	NS
Plasma growth hormone	<0.005‡	<0.01§	NS	NS
Plasma cortisol	<0.02‖	NS	<0.01¶	NS
Plasma norepinephrine	NS	NS	NS	NS
Systolic blood pressure	<0.01	NS	NS	<0.03
Diastolic blood pressure	NS	NS	NS	NS
Heart rate	NS	NS	NS	NS
Blood lactate	<0.0001	<0.0001	NS	NS
Blood glycerol	<0.0001	<0.001	NS	NS
Blood β-hydroxybutyrate	<0.0001	<0.0001	NS	NS
Blood alanine	<0.01	NS	NS	NS

P values shown.
* 0.0001 after base-line correction.
‡ <0.04 after base-line correction.
§ <0.07 after base-line correction.
‖ <0.24 after base-line correction.
¶ <0.44 after base-line correction.

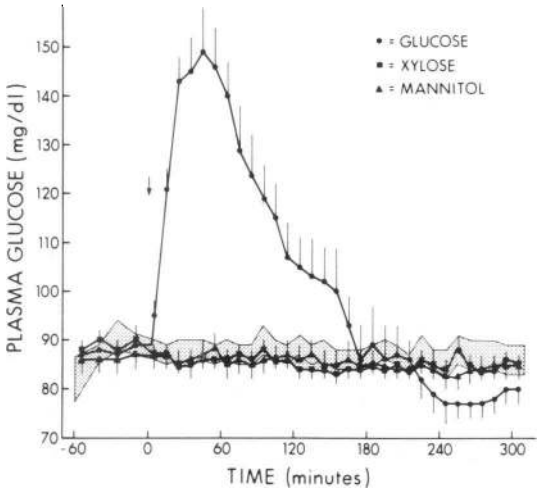

FIGURE 1 Mean (±SE) plasma glucose concentrations before and after ingestion of glucose (circles), xylose (squares), and mannitol (triangles). The stippled area is 1 SE around the mean for water ingestion.

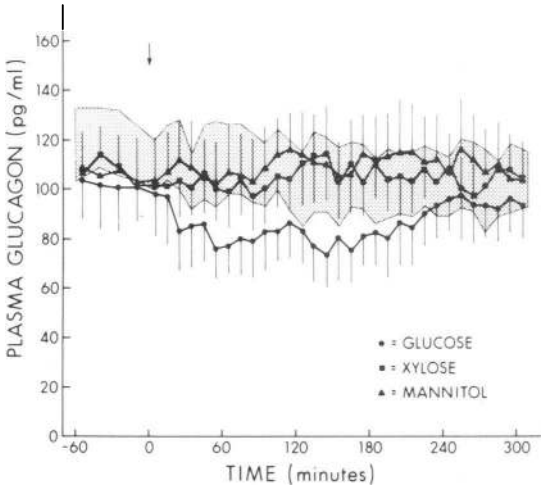

FIGURE 3 Mean (±SE) plasma glucagon concentrations before and after ingestion of glucose (circles), xylose (squares), and mannitol (triangles). The stippled area is 1 SE around the mean for water ingestion.

235 min. The latter plasma growth hormone elevations were marginally significant because of the striking variation in plasma growth hormone following water ingestion, as illustrated in Fig. 5. Indeed, with baseline correction, the plasma growth hormone curves after glucose ingestion were not significantly different by MANOVA from those after water ingestion.

In data that are not illustrated, blood lactate rose from 681±22 to a peak of 1,305±92 μmol/liter at 115 min after glucose ingestion and then declined to a final value of 867±58 μmol/liter. Lactate levels were significantly higher than control values from 35 through 275 min and above base line from 25 through 305 min. Blood glycerol declined from 68±11 to a nadir of 33±8 μmol/liter at 95 min after glucose ingestion and then rose to a final value of 103±20 μmol/liter. Glycerol levels were lower than control values from 25 through 225 min and below base line from 15 through 225 min. Blood β-hydroxybutyrate also declined from 267±55 to a nadir of 41±5 μmol/liter at 145 min after glucose

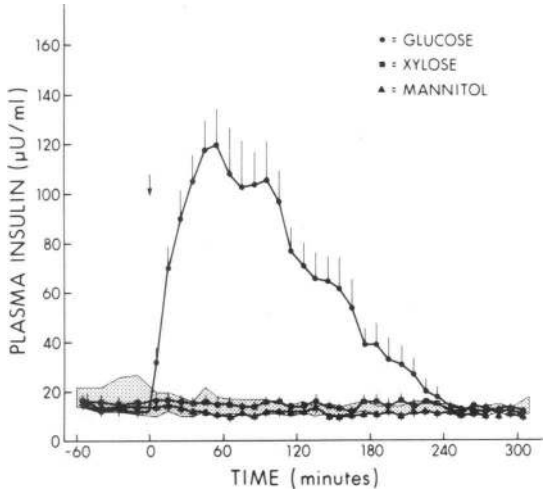

FIGURE 2 Mean (±SE) plasma insulin concentrations before and after ingestion of glucose (circles), xylose (squares), and mannitol (triangles). The stippled area is 1 SE around the mean for water ingestion.

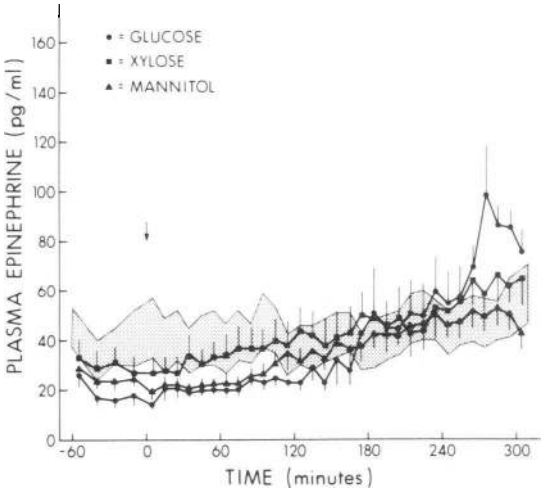

FIGURE 4 Mean (±SE) plasma epinephrine concentrations before and after ingestion of glucose (circles), xylose (squares), and mannitol (triangles). The stippled area is 1 SE around the mean for water ingestion.

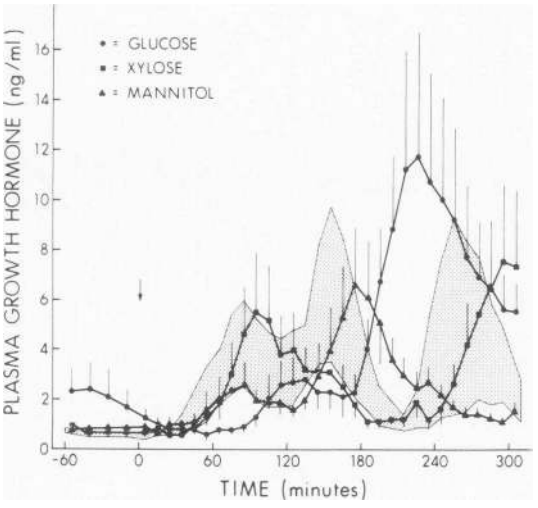

FIGURE 5 Mean (±SE) plasma growth hormone concentrations before and after ingestion of glucose (circles), xylose (squares), and mannitol (triangles). The stippled area is 1 SE around the mean for water ingestion.

ingestion and then rose to a final value of 418±86 μmol/liter. β-Hydroxybutyrate levels were lower than control values from 25 through 255 min and below base line from 25 through 265 min; they were above control values at 295 min and above base line from 285 through 305 min.

In contrast to these glucose-specific variables, changes in plasma norepinephrine (Fig. 6), cortisol (not shown), blood alanine (not shown), and blood pressure and heart rate (not shown) were not specific for glucose ingestion.

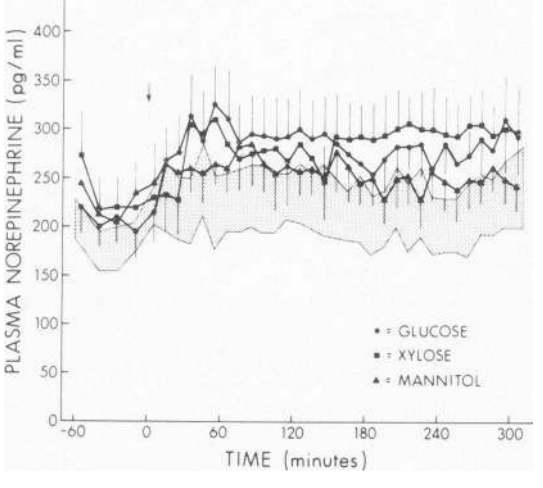

FIGURE 6 Mean (±SE) plasma norepinephrine concentrations before and after ingestion of glucose (circles), xylose (squares), and mannitol (triangles). The stippled area is 1 SE around the mean for water ingestion.

Plasma norepinephrine curves were similar after ingestion of all four test solutions. Plasma norepinephrine concentrations (Fig. 6) rose significantly above base-line values early (5–15 min) after the ingestion of water, glucose, xylose, and mannitol and remained above base line throughout the study. This pattern is quite different from that of plasma epinephrine (Fig. 4), which was unchanged early and rose late only after glucose ingestion.

The relationship between plasma glucose and plasma insulin, and glucagon and epinephrine are highlighted in Fig. 7. Plasma glucose concentrations rose from 88±2 mg/ml at base line, prior to glucose ingestion, to peaks of 156±6 mg/dl ($P < 0.001$) at 46±4 min, returned to base line (87±4 mg/dl) at 177±4 min, reached nadirs of 63±3 mg/dl ($P < 0.001$ vs. base line) at 232±12 min and rose to 80±3 mg/dl at 305 min, which was the final sampling point. Plasma insulin concentrations rose from 12±2 μU/ml at base line to peaks of 150±17 μU/ml ($P < 0.001$) at 67±8 min, which was an average of 21 min later than the glucose peak. At the time that glucose returned to base line (177±4 min), plasma insulin levels (49±12 μU/ml) remained fourfold higher than base line ($P < 0.01$); thereafter, insulin levels declined but never fell below base line. Plasma glucagon concentrations decreased from 95±14 pg/ml at base line to nadirs of 67±11 pg/ml ($P < 0.001$) at 84±9 min. Thereafter, glucagon rose progressively to 76±13 pg/ml ($P < 0.02$ vs. nadir) at the time that glucose returned to base line, to 85±15 pg/ml ($P < 0.01$ vs. nadir) at the glucose nadirs, and to peaks of 114±17 pg/ml ($P < 0.001$ vs. nadir) at 265±12 min. Plasma epinephrine concentrations were 18±4 pg/ml at base line and did not change during the first 2 h after glucose ingestion. The apparent in-

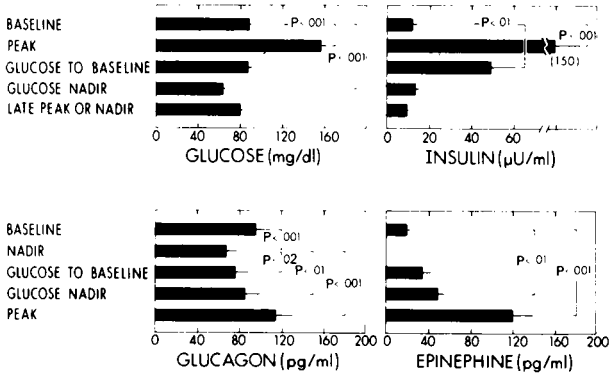

FIGURE 7 Mean (±SE) plasma glucose, insulin, glucagon, and epinephrine values before and after glucose ingestion: at baseline, before glucose ingestion; at initial peak (glucose and insulin) or nadir (glucagon); at the time that the glucose concentration had returned to baseline; at the time of the glucose concentration nadir; and at late peak (glucose, glucagon, and epinephrine) or nadir (insulin).

crements in plasma epinephrine to values of 33±9 pg/ml at the time that glucose returned to base line were not statistically significant when analyzed in this fashion, although mean plasma epinephrine levels after glucose ingestion significantly ($P < 0.001$) exceeded those after water ingestion beginning at 175 min and significantly ($P < 0.001$) exceeded base-line levels beginning at 155 min after glucose ingestion. Epinephrine levels were significantly elevated at 48±8 pg/ml ($P < 0.01$) at the glucose nadirs (232±12 min) and reached peaks of 119±20 pg/ml ($P < 0.001$) at 271 ±13 min.

DISCUSSION

These data document that increments in plasma insulin, transient decrements in plasma glucagon, and late increments in plasma epinephrine, and perhaps growth hormone follow glucose ingestion and are specific for glucose ingestion since they do not follow water, xylose, or mannitol ingestion. Similarly, transient increments in blood lactate and transient decrements in blood glycerol and β-hydroxybutyrate are specific for glucose ingestion. In contrast, early and sustained increments in plasma norepinephrine are not specific for glucose ingestion; they also follow xylose, mannitol, and water ingestion. It is reasonable to assume that these neuroendocrine responses reflect changes in release, rather than clearance, of insulin, glucagon, epinephrine, growth hormone, and norepinephrine.

Stimulation of insulin secretion after glucose ingestion is, of course, well recognized (2–6). Similarly, transient suppression of glucagon secretion (30) and late stimulation of growth hormone secretion (31) after glucose ingestion have been described. With respect to the latter, the marked variation in plasma growth hormone under all conditions in the present study warrants comment. Indeed, with correction for differences at base line, plasma growth hormone curves following glucose ingestion were not significantly different from those following water ingestion by multivariate analysis of variance.

Increments in plasma norepinephrine concentrations following glucose ingestion have been reported by at least three groups of investigators (32–35). In general, these norepinephrine increments occur within the first 15–30 min after glucose ingestion and norepinephrine levels do not return to base line through 180–240 min after glucose ingestion. Our results further confirm this pattern. Previous investigators have considered the norepinephrine response to be specific for glucose ingestion because it was not found to follow protein or fat ingestion (34); significant norepinephrine increments followed water ingestion in one study (33), but not in a second study from the same group (34), or in an earlier study (32). Perhaps because of our frequent sampling, we find that increments in plasma norepinephrine are not specific for glucose ingestion; they follow ingestion of water, xylose, and mannitol as well as glucose. The mechanism responsible for the increments in circulating norepinephrine is unclear. Although changes in plasma norepinephrine can result from changes in norepinephrine release from sympathetic postganglionic neurons, the adrenal medullae, or both (36), the temporal dissociation between the increments in plasma epinephrine (late) and those in plasma norepinephrine (early and sustained) that follow glucose ingestion is most consistent with a neural origin of the increments in plasma norepinephrine. The norepinephrine increments that follow water ingestion and the absence of an initial decrement in blood pressure after ingestion of any of the four test solutions make it clear that an osmotic mechanism is not the only stimulus to norepinephrine release. Thus, it would appear that other stimuli, such as deglutition or gut distention, trigger a sympathetic neural reflex that results in norepinephrine release.

Welle et al. observed no increments in plasma epinephrine after glucose ingestion in their initial study (33), although they took samples infrequently (hourly after the first hour) and only through 180 min. In their second study (34), these investigators observed increments in plasma epinephrine at 240 min after glucose ingestion, but judged these to be spurious in part because they did not appear to be specific for glucose ingestion. Kleinbaum and Shamoon (35) observed an increase in plasma epinephrine later after glucose ingestion but did not fully assess the specificity of that response for glucose. The present data demonstrate plasma epinephrine increments to levels significantly higher than control values (water ingestion) and significantly above base-line levels late after glucose ingestion. Furthermore, they document that the late increments in epinephrine are specific for glucose ingestion; they do not follow ingestion of xylose, mannitol, or water. These epinephrine increments are most plausibly attributed to the decreasing plasma glucose concentration (17–20) that follows initial hyperglycemia after glucose ingestion.

How might these findings relate to the mechanisms of nonhypoglycemic glucose counterregulation—those that blunt the decline in plasma glucose, prevent reactive hypoglycemia, and restore euglycemia—late after glucose ingestion? Because hyperinsulinemia persists nearly 1.5 h longer than venous (37) hyperglycemia and plasma insulin levels never fall below base line after glucose ingestion, and given also that the responsiveness to insulin does not diminish during sustained hyperinsulinemia of similar duration (38) and that the action of insulin persists after plasma insulin

falls to base line (39, 40), it is unlikely that the transition from exogenous glucose delivery to endogenous glucose production late after glucose ingestion is solely due to dissipation of insulin. Thus, counterregulatory factors, coupled with dissipation of insulin, must play a role.

The data do not support a counterregulatory role for sympathetic neural norepinephrine. Norepinephrine release is not specific for glucose ingestion; it begins early after glucose ingestion and does not increase further in temporal relation to the counterregulatory process. Also, comparable norepinephrine release after xylose ingestion does not have an impact on the plasma glucose concentration. Similarly, cortisol and growth hormone are not likely candidates. Cortisol levels were unaffected and the late increase in plasma growth hormone was of questionable specificity for glucose ingestion. Furthermore, the hyperglycemic actions of both hormones are delayed for hours and growth hormone even lowers the plasma glucose concentration initially (41).

In contrast, both glucagon and epinephrine are known to exert their hyperglycemic actions rapidly, and late increments in their plasma concentrations are specific for glucose ingestion and temporally related to the glucose counterregulatory phase. Although the observed changes in peripheral plasma glucagon are rather small, changes in portal glucagon are undoubtedly greater; the data of Fradkin et al. (42) suggest that hepatic glucose production is a function of changes in glucagon rather than the glucagon concentration per se. Yet, although the late increments in plasma epinephrine are substantial (approximately sixfold), the plasma concentrations do not commonly achieve those levels required to alter basal glucose metabolism (43). In our judgment, this makes it unlikely that epinephrine plays a counterregulatory role when other systems are intact. However, when it is infused to plasma levels lower than the peak values we observed, epinephrine has been reported to impair glucose tolerance (44) and it may interact synergistically with other hormones, including glucagon, in its hyperglycemic actions (45, 46).

The increments in blood glycerol and β-hydroxybutyrate, on the other hand, may reflect actions of epinephrine since they occur at a time when insulin levels are not below base line and epinephrine levels have achieved concentrations known to stimulate lipolysis and keytogenesis in man (43, 47).

In summary, the data indicate that the glucose counterregulatory process late after glucose ingestion is not solely attributable to dissipation of insulin and that neither sympathetic neural norepinephrine nor the hormones cortisol or growth hormone play critical roles. They are consistent with, but do not establish, physiologic roles for glucagon, epinephrine, or both in that process. Studies designed to assess the roles of glucagon and epinephrine are the subject of the report that follows.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Mr. Krishan Jethi, Ms. Eva Sorenson, Ms. Lorraine Thomas, Ms. Shirley Hill, Ms. Denise Nachowiak, and Ms. Joy Brothers; the secretarial assistance of Ms. Patricia Moore and Ms. Theresa Lautner; and the clinical assistance of the nursing and dietary staffs of the Washington University Clinical Research Center.

These studies were supported by U. S. Public Health Service grants AM 27085, AM 20579, and RR 00036.

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