The Mechanism of Glucose-Induced Catecholamine Stimulation

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Catecholamines are important hormones for maintaining homeostasis and may be secreted in response to several different stimuli. A report by Robertson and Porte in 1974 made the unexpected observation that acute administration of hypertonic glucose stimulates catecholamine secretion. Our study reassessed this observation by measuring individual catecholamines, explored its potential mechanism, and quantitated it relative to exercise and hypoglycemia-stimulated catecholamine secretion. We hypothesized that the mechanism of glucose-induced catecholamine secretion was related to an acute increase in plasma osmolality, which we tested with the nonmetabolizable hexose mannitol. In 56 studies, 14 normal adults underwent 4 partially randomized studies. The 4 study conditions consisted of the following: (1) rapid intravenous injection of 20 g of glucose; (2) rapid intravenous injection of 20 g of mannitol; (3) acute exercise (80 J/kg); and (4) insulin-induced hypoglycemia. Our results demonstrate that a significant increase in plasma catecholamine concentration occurs following each of the above stimuli, but its composition differs relative to the magnitude of epinephrine versus norepinephrine secretion. We conclude that the mechanism of glucose-induced catecholamine stimulation is the acute elevation in plasma osmolality induced by glucose, and that its stimulation is less than that which occurs following exercise for norepinephrine and less than that which occurs following hypoglycemia for epinephrine.

CIRCULATING CATECHOLAMINES have major metabolic effects on multiple metabolites in man. Both epinephrine and norepinephrine are secreted in response to various stimuli, including exercise, hypoglycemia, stress, and dehydration.[1-4] In 1974, Robertson and Porte[5] reported the unexpected observation that a bolus of intravenous glucose acutely increased total plasma catecholamine levels. This observation has not been confirmed, nor has a potential mechanism been demonstrated. Our study was designed to reassess this observation and to explore the possibility that the mechanism was related to an acute increase in plasma osmolality, such as occurs following bolus glucose administration. To determine whether or not changes in the concentration of catecholamines are attributable to the increase in osmolality that occurs after bolus glucose administration, or alternatively, to the metabolism of glucose per se, we compared the glucose-induced catecholamine response with the response observed after bolus mannitol administration. To provide semiquantitative comparative data on the relative magnitude of glucose-induced catecholamine secretion, we also compared these results with the response to 2 known catecholamine stimuli, exercise and insulin-induced hypoglycemia. Our data confirm the original observation by Robertson and Porte that bolus glucose administration acutely increases circulating catecholamines and established that this increase is secondary to increases in both epinephrine and norepinephrine. Moreover, the mechanism of these increases is related, at least in part, to the acute elevation in plasma osmolality.

MATERIALS AND METHODS

Subjects

Fourteen normal healthy adults were studied and received a series of 4 catecholamine stimulation tests during a 1-day hospital stay in a prospective, partially randomized study. All volunteers were healthy and free of major organ dysfunction.

Seven men and 7 women with a mean age of 30.4 ± 2.1 years and a mean body mass index of 24.8 ± 1.0 were studied. Mean glycosylated hemoglobin (HbA_{1c}) for all subjects was 5.5% ± 0.05% (reference range, 4.2% to 6.5%). All subjects signed informed consent for participation in the study as approved by the University of New Mexico Human Research Review Committee.

Study Protocol

All subjects participated in 4 separate studies in random sequence (except insulin-induced hypoglycemia) separated by 2 hours. All subjects were admitted to the University of New Mexico General Clinical Research Center on the evening before the studies. Forearm veins of each volunteer were catheterized for the administration of study medications in 1 arm and the withdrawal of blood samples in the contralateral arm. All subjects were served an 8.6 kcal/kg American Diabetes Association (ADA) meal at 5:00 PM, plus an additional snack of 4.3 kcal/kg at 10:00 PM the night before the study. The composition of meals given to the study subjects was identical. All subjects were normoglycemic the next morning with a mean blood glucose level of 90.5 ± 1.2 mg/dL. Subjects were allowed free access to noncaloric, noncaffeinated beverages during their stay in the General Clinical Research Center (GCRC).

No breakfast was served the morning of the study. At 8:00 AM, each subject participated in a series of 4 studies with a period of 2 hours separating each study. The 4 studies for each subject were completed within 1 day to reduce within subject variability. The studies were as follows: (1) glucose study: 20 g of 50% dextrose given intravenously within 1 minute; (2) mannitol study: 20 g of 25% mannitol given intravenously within 1 minute; (3) exercise study: aerobic exercise of 80 J/kg within 1 minute accomplished by stepping up and down a 20-cm step 40 times; and (4) hypoglycemia study: regular insulin in the dose of 0.1 U/kg given by intravenous bolus. A saline only study was not done because an *a priori* decision was made to do the statistical

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comparisons between the basal hormonal concentration and the following peak concentration poststimulation for each study. Furthermore, doing the hypoglycemic study, no significant changes in catecholamines occurred prior to the onset of hypoglycemia at 15 minutes. Blood samples were obtained for glucose, insulin, glucagon, epinephrine, and norepinephrine as follows: for the glucose, mannitol, and exercise studies, venous blood samples were obtained at -5, 0, +2, +3, +4, +5, and +10 minutes relative to the time of administration of intravenous glucose, mannitol, or the beginning of exercise, respectively. For the hypoglycemia study, venous blood samples were obtained at -5, 0, +5, +10, +15, +20, and +25 minutes relative to the time of administration of intravenous insulin. The order of the sequence of the first 3 studies was randomized. The hypoglycemia study was completed at the end of all 4 studies because of concern that the antecedent hypoglycemia might affect data obtained from subsequent studies.[6] Arterialized venous blood was used for all substrate and hormonal assays. This was obtained by using a warming device to heat the hand and forearm to approximately 60°C prior to drawing the blood sample. Hypoglycemia was defined as a blood glucose level less than 50 mg/dL with typical hypoglycemic symptoms or any blood glucose measurement less than 40 mg/dL. At the end of the hypoglycemia study, all subjects were given 50 mL of 50% dextrose for rapid correction of the insulin-induced hypoglycemia and prevention of any further reduction in the blood glucose levels. After completion of all 4 studies, the subjects were served a regular meal and observed for 2 hours before being discharged home.

Sample Analysis

For safety, bedside glucose concentrations during hypoglycemia were determined using the HemoCue Blood Glucose Analyzer (HemoCue Inc, Mission Viejo, CA).[7] Plasma glucose concentrations were later assessed using the Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Insulin concentrations were determined using the Coat-A-Count RIA kit (Diagnostic Products Corp, Los Angeles, CA). Serum glucagon concentrations were determined by radioimmunoassay by the GCRC Core Laboratory at Washington University (St Louis, MO).[8] Samples of catecholamines were placed on ice immediately after sampling and stored at -70°C until being assayed by high-performance liquid chromatography (HPLC).[9] Osmolality was measured at baseline and 2 minutes postadministration of glucose and mannitol, using the Multi-Osmette #2430 (Precision Systems, Inc, Natick, MA). Norepinephrine turnover was estimated using the model of Dvorak et al.[10]

Statistical Analysis

The primary efficacy variables assessed were the peak epinephrine, norepinephrine, and total catecholamines concentration relative to the basal value. This parameter was chosen to minimize any effect of changes in baseline values between the studies. Comparisons were made between the response of endogenous epinephrine, norepinephrine, and total catecholamines (base to peak levels) for each of the 4 studies. Secondary variables including serum insulin, plasma osmolality, plasma glucose, and plasma glucagon levels were analyzed in a similar fashion where appropriate.

All parameters were compared between the various groups using analysis of variance (ANOVA) for repeated measures with the application of Student's test for paired data where appropriate. All data are reported as the mean ± SEM.

RESULTS

Epinephrine

Figure 1A depicts comparisons between the mean basal epinephrine concentrations and mean peak epinephrine concentrations in response to each of the 4 stimuli. Mean basal epinephrine concentrations did not significantly differ among the 4 study groups and were as follows: glucose study, 12 ± 1 pg/mL; mannitol study, 32 ± 12 pg/mL; exercise study, 33 ± 16 pg/mL; and insulin-induced hypoglycemia study, 30 ± 9 pg/mL, $P > .05$. Mean peak epinephrine concentrations were as follows: glucose study, 34 ± 11 pg/mL; mannitol study, 43 ± 20 pg/mL; exercise study, 77 ± 32 pg/mL; and insulin-induced hypoglycemia study, 255 ± 75 pg/mL. As shown in Fig 1A, analysis of base to peak levels of epinephrine concentrations did not reach a statistical difference in the mannitol study, although the mean was greater postinfusion. However, there

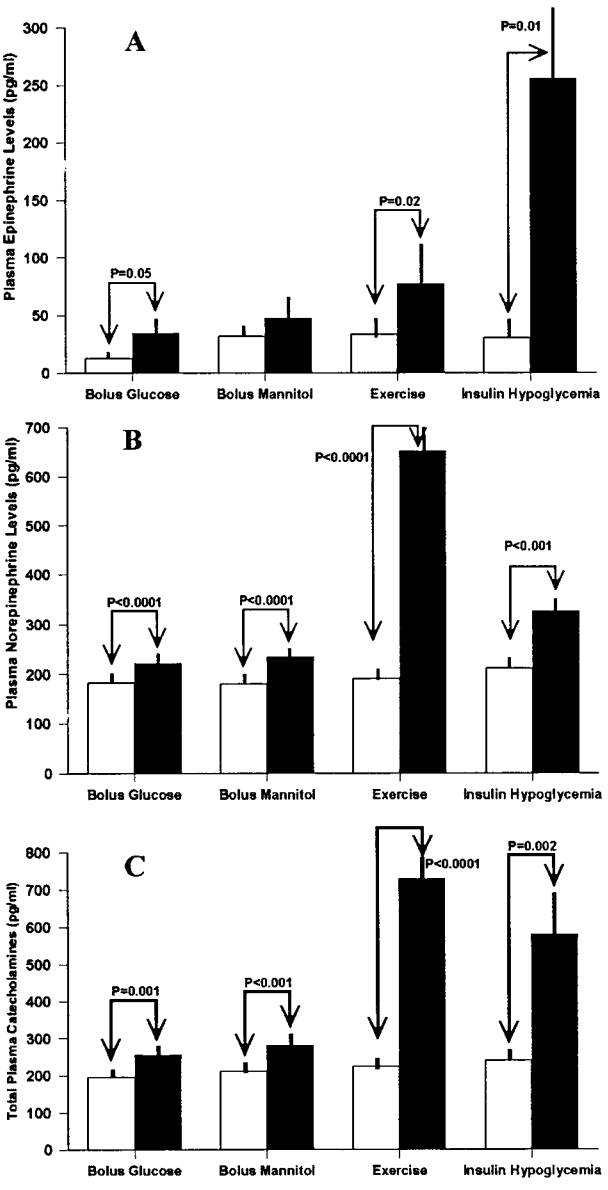

Fig 1. Changes in plasma (A) epinephrine, (B) norepinephrine, and (C) total catecholamines following 4 stimuli. Mean basal (□) and mean peak poststimulus concentrations (■) for the 14 volunteers are given for each catecholamine.

was a statistically significant difference between mean basal epinephrine concentrations and mean peak epinephrine concentrations for the glucose, exercise, and insulin-induced hypoglycemia studies ($P < .05$). This difference was more pronounced in the insulin-induced hypoglycemia study compared with the glucose and exercise studies.

Norepinephrine

Figure 1B depicts comparisons between the mean basal norepinephrine concentrations and mean peak norepinephrine concentrations in response to the same 4 stimuli. The strongest stimulus for norepinephrine secretion was exercise; with a mean peak norepinephrine level of 652 ± 58 pg/mL compared with basal norepinephrine levels of 191 ± 15 pg/mL ($P < .0001$). The difference between mean basal norepinephrine concentrations and mean peak norepinephrine concentrations for the bolus glucose study, bolus mannitol study, and insulin hypoglycemia study all reached statistical significance ($P < .001$). Norepinephrine turnover (in microgram/min) was estimated for all stimuli. There was a significant increase in turnover for all stimuli (all greater than $P < .001$) as follows: glucose from a basal of 0.403 ± 0.036 to a maximal of 0.0.486 ± 0.042, mannitol from a basal of 0.402 ± 0.030 to a maximal of 0.522 ± 0.037, exercise from a basal of 0.431 ± 0.030 to a maximal of 1.62 ± 0.19, and insulin-induced hypoglycemia from a basal of 0.466 ± 0.037 to a maximal of 0.726 ± 0.074. As anticipated from the plasma concentration, the greatest increase occurred during the exercise stimulus.

Total Plasma Catecholamine Concentrations

For the purpose of this study, we defined total plasma catecholamines as the sum of epinephrine and norepinephrine concentrations. Figure 1C depicts comparisons between the mean basal total catecholamine concentrations and mean peak total catecholamines concentrations. Mean basal total catecholamine concentrations were as follows: glucose study, 195 ± 21 pg/mL; mannitol study, 207 ± 24 pg/mL; exercise study, 222 ± 26 pg/mL; and insulin-induced hypoglycemia study, 237 ± 25 pg/mL ($P > .05$ for all comparisons). Mean peak total catecholamine concentrations were as follows: glucose study, 255 ± 28 pg/mL; mannitol study, 274 ± 32 pg/mL; exercise study, 724 ± 68 pg/mL; and insulin-induced hypoglycemia study, 580 ± 105 pg/mL. As shown in Fig 1C, analysis of base to peak levels of total plasma catecholamine concentrations demonstrated a statistically significant increase in all studies ($P < .01$). Although the sample size was half of the total study, analysis of total catecholamine responses for males (7) and females (7) as separate groups revealed the following results. Analysis of base to peak levels for total catecholamines was significant for both groups for all 4 studies: glucose study, $M = P < .02$, $F = P < .05$; mannitol study, $M = P < .01$, $F = P < .05$; exercise study, $M = P < .01$, $F = P < .0$; and insulin-induced hypoglycemia study, $M = P < .03$, $F = P < .05$.

Osmolality

Osmolality was measured at baseline (time 0) and at +2 minutes after the administration of both glucose and mannitol. This time point was chosen to approximate the maximum osmolality achieved in the circulation after hexose administration. It was not monitored during the exercise study or the insulin-induced hypoglycemia study since no osmotic substance was administered during these studies. Mean osmolality at time 0 for the glucose study was 280.2 ± 1.1 mosmol/L compared with a mean osmolality at +2 minutes of 283.7 ± 1.2 mosmol/L ($P < .01$). Mean osmolality at time 0 for the mannitol study was 280.0 ± 1.4 mosmol/L compared with mean osmolality at +2 minutes of 284.2 ± 1.3 mosmol/L ($P < .01$). There was no significant difference between the change in osmolality following glucose infusion (4.1 ± 1.2 mosmol/L) and mannitol infusion (4.9± 1.4 mosmol/L; $P > .05$).

Insulin

To monitor pancreatic endocrine activity during the 4 stimulation studies, both insulin and glucagon concentrations were measured. In the glucose study, insulin increased from a basal level of 5.3 ± 0.50 μU/mL to a stimulated value of 79.3 ± 18 μU/mL; $P < .01$. For the mannitol study, the basal value of 5.5 ± 0.5 μU/mL increased to 7.7 ± 1.3 μU/mL; $P < .05$. During the exercise study, insulin decreased from a basal value of 9.3 ± 1.6 μU/mL to 2.5 ± 0.9 μU/mL; $P < .01$. As expected, with administration of exogenous insulin, the concentration of this hormone greatly increased from a basal value of 6.5 ± 0.5 μU/mL to a 2-minute value of 431 ± 43 μU/mL; $P < .01$).

Glucagon

The pattern of change in plasma glucagon differed from that of insulin. No significant change occurred in circulating glucagon concentration when either exogenous glucose or mannitol was administered (glucose study, basal 46.1 ± 3 to 50.1 ± 7 pg/mL; $P > .05$ and mannitol study, basal 46.2 ± 2.6 to 46.4 ± 9.6 pg/mL; $P > .05$). In contrast, during the exercise study, glucagon increased from a basal value of 41.6 ± 3 pg/mL to 49.1 ± 4 pg/mL; $P < .05$ and during the insulin-induced hypoglycemia study, glucagon increased from a basal value of 43.2 ± 3 pg/mL to 54.4 ± 4 pg/mL; $P < .01$.

DISCUSSION

Catecholamines have important regulatory roles in man, including neurotransmission and the metabolic regulation of multiple substrates and hormones.[1,2] Deficiency of catecholamines can result in hypoglycemia, hypotension, and death. As a consequence, multiple secretory stimuli exist for catecholamines, preparing the individual for a "flight or fight" response. Classically, hypoglycemia is regarded to be among the most potent stimuli for epinephrine secretion, and exercise is a similarly powerful stimulus for norepinephrine secretion, although each of these hormones responds to both of these stimuli.

In 1974, Robertson and Porte[5] reported that the bolus administration of hypertonic glucose induced catecholamine secretion. Whether this response was primarily due to epinephrine or norepinephrine was not determined nor was the mechanism elucidated. Our study investigated both of these questions and also provided semiquantitative data by comparing the responses to osmotic stimuli (glucose and mannitol) to

the classical stimuli of exercise and insulin-induced hypoglycemia. Our results indicate that both epinephrine and norepinephrine concentrations are increased by bolus hypertonic glucose administration. Furthermore, the magnitude of catecholamine secretion is less than that achieved following exercise (for norepinephrine) and insulin-induced hypoglycemia (for epinephrine). As expected, the increase in norepinephrine turnover paralleled the increase in norepinephrine concentration for all 4 stimuli.

The pancreatic hormones responded to the 4 stimuli as anticipated. Glucose administration greatly increased circulating insulin concentrations, but had no observable effect on glucagon. Mannitol had minimal effects on insulin and glucagon levels. Exercise suppressed insulin, but stimulated glucagon, whereas hypoglycemia increased glucagon concentration. The reason that glucagon did not increase even further in response to hypoglycemia was probably due to the suppressive effects of exogenous insulin.[11] These observations are consistent with previous experimental observations.[12,13]

The mechanism of the glucose-induced catecholamine secretion is likely due to an acute increase in plasma osmolality. Although this may not be the only mechanism, it is likely the principal one since a similar response was observed when mannitol was administered, a nonmetabolizable hexose. The change in plasma osmolality 2 minutes after the bolus administration of mannitol was similar to that achieved with glucose. Our conclusion that the mechanism of glucose-induced catecholamine secretion is secondary to the hypertonicity of hypertonic glucose does not exclude all other possible mechanisms (such as a change in temperature induced by the administration fluids) or combination of mechanisms. Limits to the duration and quantity of blood withdrawal precluded us from testing additional possibilities, such as the temperature of the solution. A limitation of our study is that we have not excluded an osmotic effect on catecholamine extraction, which might alter the catecholamine production rate. However, we believe that the hypertonicity is the most likely mechanism because it is a known stimulus for other physiologic hormones, such as pitressin. Our data also do not indicate whether the changes observed in epinephrine and norepinephrine are due to an increase in production or removal rates from the plasma. The methodology we used to calculate catecholamine turnover requires removal rates to be constant. Additional studies will be necessary to address this issue. One interesting question that remains is why catecholamines should be secreted in response to an acute increase in osmolality. Our study does not resolve this issue. It is possible that the catecholamine response to osmolality is a continuum and that even prolonged elevations in osmolality evoke a similar response. If this were the case, then it would explain a mechanism through which hypertonic dehydration increases catecholamine secretion.[3,14] Additional studies will be required to resolve this question.

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