Sex Differences in the Adrenal Catecholamine Response to Hypoglycemia in Rats

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We studied the influence of sex on the adrenal catecholamine response to acute insulin-induced hypoglycemia in Sprague-Dawley rats. Eight male and seven female adult rats were anesthetized with pentobarbital, and a microdialysis probe was placed in the left adrenal. Dialyzed epinephrine and norepinephrine levels were measured by high-performance liquid chromatography during a control period and for 1 hour after insulin administration. The blood glucose level was measured every 15 minutes. The same protocol was applied to 23 adult females at various stages of the estrus cycle. The pattern of blood glucose changes during insulin-induced hypoglycemia was similar in both sexes, but males exhibited a significantly greater increase in epinephrine than females (261% *v* 52%, $P = .001$) in the sex-comparison experiment. A similar trend was observed for norepinephrine (73% *v* 0%, $P = .075$). The adrenal response in females for both catecholamines was not significantly affected by the estrus cycle phase ($P = .989$ for epinephrine and $P = .424$ for norepinephrine). We conclude that sex influences the magnitude of the adrenal catecholamine counterregulatory response to hypoglycemia. Males had a significantly greater increase in epinephrine release than females exposed to the same pattern of hypoglycemia. Female responses to hypoglycemia were not influenced by estrus cyclicity.

THE NOTION THAT GENDER-RELATED differences exist in the regulation of metabolism is not new,[1] but the precise nature of the interrelations of sex and metabolism is understood incompletely. Considerable information suggests there are differences in intermediary metabolism between men and women. Sex influences insulin action on glucose transport,[2] as well as insulin sensitivity at puberty[3] and in diabetic adolescents.[4] Fasting blood glucose is lower in women than in men.[5,6] Sex also affects sympathoadrenal regulation. For example, some data suggest that the sympathoadrenal system in men may be more responsive to mental stress[7,8] and to exercise[6] than in women.

Of particular interest with regard to sex effects on metabolism are homeostatic adjustments to hypoglycemia, because they involve the interaction of several factors relating to insulin and glucose regulation and to adrenal medullary function. There is, in fact, some evidence that sex governs the intensity of the catecholamine response to acute decreases in blood glucose.[1,9] We extended the knowledge of the relation between sex and the adrenal role in glucose counterregulation by studying the adrenal catecholamine response to insulin-induced hypoglycemia in male and female rats. In addition, we analyzed this response in females according to their estrus stage. We used in vivo microdialysis to sample adrenal medullary secretions directly, and observed that males responded with more catecholamine release than females exposed to equivalent hypoglycemic stress. There was no influence of cyclic estrus changes on the adrenal response.

MATERIALS AND METHODS

Two kinds of experiments were performed, both using Sprague-Dawley rats (Charles River Laboratories, Kingston, NY) weighing 300 ± 40 g (mean ± SD) in a protocol approved by the Animal Care Committee of Albert Einstein College of Medicine. Rats were sheltered in an American Association for Accreditation of Laboratory Animal Care–approved animal facility with a constant temperature and natural light-dark cycle, and were allowed Purina Rat Chow (Ralston Purina, St Louis, MO) ad libitum. Each experiment was begun between 8 and 9 AM. The animals were sedated by a 30-second exposure to carbon dioxide and then anesthetized with sodium pentobarbital (80 mg · kg^{-1}) intraperitoneally. Each rat was placed on a heating pad governed by a temperature controller (CMA/150; Carnegie Medicin, Stockholm, Sweden) connected to a rectal thermistor probe. This maintained the core body temperature at 37.5° to 38.0°C. A cervical midline incision was made to expose the trachea, which was then cannulated. The rat was ventilated through the cannula at a rate of 100 respirations · min^{-1} and a tidal volume of 1.5 mL using a volume-controlled small-animal respirator (model 683; Harvard Bioscience, South Natick, MA). The left femoral vein was cannulated with a polyvinyl catheter. The left adrenal was identified through a subcostal incision, and a CMA-11 microdialysis probe with a 2-mm membrane (Carnegie Medicin) was inserted into the gland. This allowed heat-degassed Ringer's solution to be perfused at a constant rate via a Carnegie Medicin CMA/100 microinjection pump. Consecutive 48-μL probe effluent samples were collected in glass tubes (CMA/140 microfraction collector) containing 5 μL perchloric acid, resulting in a final concentration of 0.1 mol/L in the total collection volume. Each completed fraction was injected immediately into a high-performance liquid chromatographic system described previously.[10,11]

The system (BAS 200A; Bioanalytical Systems, West Lafayette, IN) used an amperometric detector with a potential of 0.65 mV at a gain of 10 nA · V^{-1}. A BAS phase II ODS 3μ column was used, and analyte concentrations were determined in the dialysate by peak area calculations in comparison to external epinephrine and norepinephrine standards. The mobile phase consisted of monochloroacetic acid (0.075 mol/L), disodium EDTA (0.5 mmol/L), sodium octyl sulfate (1.0 mmol/L), and acetonitrile (1% to 2%) at pH 3.0, flowing at 1.0 mL · min^{-1}. The within- and between-assay coefficients of variation were less than 10%. Blood glucose levels were measured by a calibrated glucose analyzer (Glucometer M^+; Miles, Elkhart, IN).

After insertion of the adrenal probe, continuous perfusion was performed at 4 μL · min^{-1}, with samples collected every 12 minutes throughout the experiment. Catecholamine concentrations were initially high, but decreased rapidly and stabilized within 2 to 3 hours. Our previous experience with this model[10,11] showed that once stabilization occurs, levels remain constant for several hours. At least 30 minutes of stable baseline measurements were required before collection of samples for the experiment. After equilibration of the probe, two 0.1-mL control samples of blood for glucose analysis were drawn from

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the venous catheter 15 minutes apart. A bolus of insulin (0.5 U · kg^{-1}) was administered through the catheter, and the blood glucose level was then measured every 15 minutes for 1 hour. Heparinized (1:10,000) Ringer's solution was infused through the venous catheter in each sampling period in a volume equal to that of the blood drawn for analysis. Catecholamine analyte levels were determined in the dialysate collected at 12-minute intervals.

In the first experiment, eight male and seven female animals were studied and the catecholamine responses to hypoglycemia were compared. In the second design, four groups of females were studied and compared when stratified by their estrus cycle stage determined by vaginal smear. Six animals each were studied for the estrus, proestrus, and diestrus, and five for the metestrus.

Data are presented as the mean ± SEM. Differences in the response to hypoglycemia between male and female groups and among female estrus cycle groups were determined using one-way (multisample) repeated-measures ANOVA, with catecholamine concentrations as the dependent variables and time as the within-factor (Super ANOVA; Abacus Concepts, Berkeley, CA). The sequence of glucose changes during the experiments was compared among the groups in a similar manner. Statistical significance was defined as P less than .05.

RESULTS

The pattern of blood glucose changes in the two gender groups (Fig 1) was similar (F = 0.054, P = .820). The baseline glucose levels of 5.80 ± 0.22 and 6.11 ± 0.11 mmol/L in males and females, respectively, were not significantly different, and decreased to a nadir of 2.17 ± 0.11 and 2.05 ± 0.17 mmol/L at 15 minutes after insulin administration. Glucose then began to increase, but at 60 minutes it remained at about 70% of baseline levels in both groups.

During hypoglycemia, epinephrine in the dialysate increased in both groups (Fig 2), but the significant interaction of gender and time indicated that the response was considerably greater in males than in females (F = 4.093, P = .001). Males, whose baseline epinephrine levels were higher than those of females (P = .041), demonstrated a 261% mean increase in epinephrine at the peak catecholamine response, whereas the levels in females maximally increased only 52% over control levels. A similar trend was found for norepinephrine (Fig 3), in that the 73% peak increase for males was greater than for females,

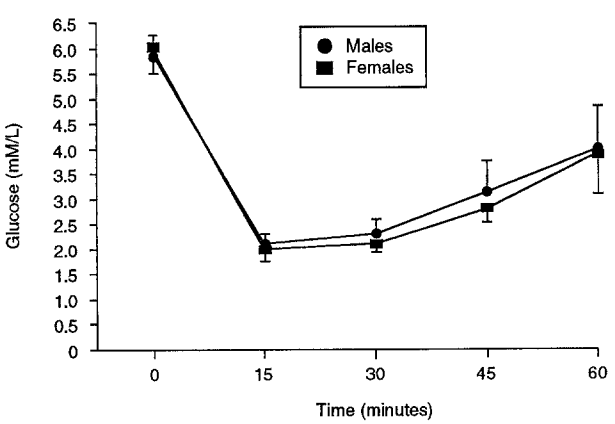

Fig 1. Pattern of insulin-induced hypoglycemia by sex. Insulin (0.5 U · kg^{-1}) was administered intravenously at time 0. Blood glucose decreased significantly from control levels in both (●) males and (■) females (P = .001), but the pattern of the glycemic response to insulin did not differ by sex (P = .820).

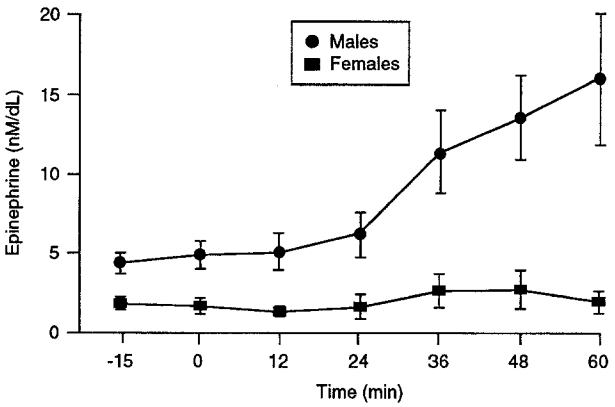

Fig 2. Epinephrine response to hypoglycemia by sex. Adrenal dialysate was collected continuously, and the epinephrine concentration was measured at indicated intervals. Two baseline samples during euglycemia preceded insulin administration at time 0. (●) Males showed a markedly greater increase in epinephrine than (■) females (P = .001) in response to equivalent patterns of hypoglycemia.

whose levels did not increase above those of the controls. This difference was not significant (F = 2.00, P = .075). The concentration of epinephrine in dialysate samples always exceeded that of norepinephrine.

Fasting and hypoglycemic glucose levels in the four estrus cycle groups were similar to those of females in the two-gender experiment (fasting, 7.30 ± 0.23 mmol/L; nadir, 1.97 ± 0.14; Fig 4). Epinephrine increased significantly overall (P = .002), but there was no significant difference among the four estrus cycle groups in the pattern of increase (F = 0.379, P = .989). Norepinephrine did not change significantly (F = 0.999, P = .422).

DISCUSSION

Our data add to the growing evidence for a sexual dimorphism in sympathoadrenal function and regulation of glucose

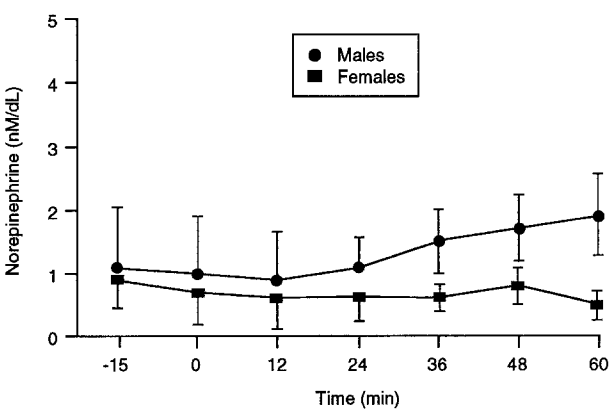

Fig 3. Norepinephrine response to hypoglycemia by sex. Adrenal dialysate was collected continuously, and the norepinephrine concentration was measured at indicated intervals. Two baseline samples during euglycemia preceded insulin administration at time 0. There was no significant difference in the pattern of norepinephrine response to hypoglycemia between (●) males and (■) females (P = .075).

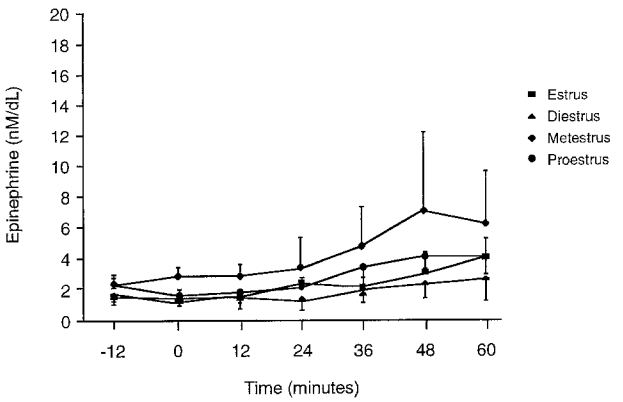

Fig 4. Epinephrine response in female rats by estrus phase: (■) estrus, (▲) diestrus, (◆) metestrus, and (●) proestrus. Adrenal dialysate was collected continuously, and the epinephrine concentration was measured at indicated intervals. Although overall epinephrine analyte levels increased during hypoglycemia ($P = .002$), there was no difference in the response pattern among estrus phases ($P = .989$).

metabolism.[1,12] We have clarified the understanding of sex differences in the adrenal response to acute insulin-induced hypoglycemia in several ways. In contrast to other experimental approaches to this problem, we used a sensitive method to assess adrenal medullary function directly and thus avoided the pitfalls of making inferences about adrenal secretion from plasma or urinary measurements. Also, we compared adrenal function in response to completely congruent patterns of hypoglycemia. Our animal model has an advantage over human investigations in that rats in the two groups were uniform in terms of genetic background, body mass, and environment. Moreover, our experimental system provides a model that could be useful to elucidate the mechanism underlying our observations.

Our data are consistent with the findings in human subjects from Diamond et al[13] and Amiel,[1] who used a clamp technique to maintain a fixed level of hypoglycemia. Diamond et al[13] suggested that the finding of higher plasma epinephrine levels in men versus women subjected to hypoglycemia could be accounted for by men having a higher glucose threshold for hormone release than women. Our findings could be explained in the same way, but our results are also consistent with a difference in the rate of epinephrine release between males and females at equivalent levels of hypoglycemia. Our finding of higher baseline dialyzable epinephrine in males versus females suggests a possible difference in resting adrenal activity as well. We chose to use an insulin bolus rather than a hypoglycemic clamp technique because the former allows assessment of the integrated ability of the animal to counterregulate acutely. Both approaches are legitimate ways to assess counterregulatory physiology, but the results may not be directly comparable.

Amiel[1] found no sex difference in human plasma epinephrine responses to 3 hours of low-dose insulin infusion. This could relate to differences in the intensity of counterregulatory responses produced by various methods of inducing hypoglycemia, or to the relative insensitivity of plasma epinephrine measurements as compared with direct assessment of adrenal secretion. It is also possible that variations in adrenal responses in different experimental approaches are the result of differential effects on the hepatic production or peripheral uptake of glucose that depend on the nature of the hypoglycemic stimulus.

Adrenal microdialysis is a useful tool for direct evaluation of medullary function in small animals.[10,11,14] Secretion of epinephrine and norepinephrine from the adrenal gland involves exocytosis into the extracellular space, from which catecholamines are readily dialyzed by a microdialysis probe inserted into the tissue. This approach is a sensitive and specific way to assess adrenal function. It has an advantage over measurement of peripheral plasma or urinary catecholamine levels in that in vivo dialysis can detect continuous and subtle variations in adrenal function. In addition, dialysate catecholamines are not admixed with those released by sympathetic nerves, and are not influenced by metabolism or by uptake into peripheral tissues.

Our studies were performed in anesthetized animals, and the potential effect of barbiturates on our results must be considered. The influence of these agents on sympathoadrenal function probably depends on a complex interaction of factors. Barbiturate induction of anesthesia was shown not to affect plasma epinephrine levels in rats[15] or humans,[16,17] but under certain conditions suppressive effects have been demonstrated.[18,19] Also, anesthesia and sympathoadrenal responses to surgery may alter glucose homeostasis.[18,20] However, pentobarbital anesthesia has been shown not to alter glycemia in rats.[21] Although we cannot exclude effects of anesthetics or surgical stress on our experiments, we think it is unlikely that these factors influenced the observed sex differences, because both groups received the same surgery and anesthetic dose. Although it is possible that sex differences in the response to barbiturates occur that could influence the adrenal, no data exist to support this notion. Insulin can suppress adrenal catecholamine release during hypoglycemia,[22] but insulin doses were the same in both groups, making direct insulin effects unlikely to explain our results.

A systematic collection error in the female animals could also explain our findings, but such a problem would be unlikely for two reasons. Both groups of animals were exposed to the same surgical and experimental techniques. Moreover, although the order of experiments was not formally randomized between the sex groups, experiments in both groups were intermixed over a period of weeks, making it unlikely that a system problem would have occurred only in females.

To determine whether the estrus phase of our female rats might have influenced our conclusion that males and females differ in the response to hypoglycemia, we studied females during all estrus phases and determined that the adrenal catecholamine responses were uniformly subdued in comparison to those of the males. There is some evidence that estrus changes may influence the medulla. The adrenal content of epinephrine varies during the estrus cycle,[23] as do the activities of catecholamine-metabolizing and -synthesizing enzymes.[24,25] Estradiol administration can increase adrenal tyrosine hydroxylase activity,[26] and affects in vitro release of catecholamines from incubated adrenal medullae.[27] Prolactin, the secretion of which varies during the estrus cycle, also can affect adrenal catecholamine levels under certain experimental conditions.[28]

However, there is no evidence that the in vivo secretory responses of the adrenal medulla are influenced by hormonal fluctuations of the estrus cycle.

Counterregulatory adjustments to hypoglycemia entail a complex comingling of hormone release involving glucagon, epinephrine, cortisol, growth hormone, and insulin-like growth factors.[29-31] The hierarchy of these responses to decreasing blood glucose probably depends on a multitude of factors, including the degree and rate of decrease in glucose and the availability of glucagon and insulin. Our results reinforce the notion that sex must also be considered a governor of adjustments to hypoglycemia. In most previously reported investigations of glucose regulation, the sex of the experimental subjects was not specified,[32] only males were studied,[33] or men and women were studied but not analyzed separately.[34,35] In future studies of glucose regulation, the sex of the subjects should be specified and, when appropriate, the data analysis should be stratified accordingly.

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