Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction

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MITRAKOU, A., C. RYAN, T. VENEMAN, M. MOKAN, T. JENSSEN, I. KISS, J. DURRANT, P. CRYER, AND J. GERICH. *Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction.* Am. J. Physiol. 260 (Endocrinol. Metab. 23): E67–E74, 1991.—To define glycemic thresholds for activation of counterregulatory hormone secretion, initiation of symptoms (autonomic and neuroglycopenic), and onset of deterioration of cognitive function, we measured indexes of these responses during glycemic plateaus of 90, 78, 66, 54, and 42 mg/dl in 10 normal volunteers, with the use of the hyperinsulinemic glucose clamp technique. Activation of glucagon, epinephrine, norepinephrine, and growth hormone secretion began at arterialized venous plasma glucose concentrations of 68 ± 1, 68 ± 1, 65 ± 1, and 67 ± 2 (SE) mg/dl, respectively. Autonomic symptoms (anxiety, palpitations, sweating, irritability, and tremor) began at 58 ± 2 mg/dl, which was significantly ($P = 0.0001$) lower. Neuroglycopenic symptoms (hunger, dizziness, tingling, blurred vision, difficulty thinking, and faintness) and deterioration in cognitive function tests began at 51 ± 3 and 49 ± 2 mg/dl, respectively, values that were both significantly ($P = 0.018$ and 0.004, respectively) lower than that for initiation of autonomic symptoms. We therefore conclude that there is a distinct hierarchy of responses to decrements in plasma glucose, such that the threshold for activation of counterregulatory hormone secretion occurs at higher plasma glucose levels than that for initiation of autonomic warning symptoms, which in turn occurs at higher plasma glucose levels than that for onset of neuroglycopenic symptoms and deterioration in cerebral function. Such a hierarchy would maximize the opportunity to avoid incapacitating hypoglycemia.

hypoglycemia; autonomic symptoms; neuroglycopenia; cognitive dysfunction

GLUCOSE IS NORMALLY the predominant fuel used by the central nervous system (31). Because brain can neither produce nor store appreciable amounts of glucose, it relies on the uptake of glucose from the circulation to supply its metabolic needs (37). As a result, severe and prolonged hypoglycemia can cause irreversible brain damage and even death. Fortunately, when the glucose counterregulatory mechanisms are intact, this is an uncommon event.

Recent studies indicate that glucose counterregulation involves a complex interaction between hormonal and nonhormonal factors (12). Glucagon and epinephrine are considered to be the first line of defense, with growth hormone, cortisol, hepatic autoregulation, and perhaps other factors becoming important when hypoglycemia is prolonged or severe (8, 15, 16, 19). In addition, it is generally thought that there are certain clues, generated as a result of a decrease in plasma glucose, which warn the individual of impending hypoglycemia, so that appropriate action may be taken before dangerous impairment in cerebral function occurs (18, 19, 39).

The exact relationship between decrements in plasma glucose concentration and the activation of counterregulation, initiation of warning symptoms, and onset of deterioration in cerebral function is not well established. For example, some studies have found glycemic thresholds for the initiation of counterregulatory hormone secretion to vary among the hormones (11, 33–35), whereas other studies have found apparently similar thresholds for all the hormones (1, 2, 10, 14, 32). With respect to warning symptoms, the plasma glucose concentration at which these symptoms initially appear in normal individuals has varied considerably among reports; in some studies they have occurred at plasma glucose concentrations as high as 65 mg/dl (11), and in other studies they have been observed at plasma glucose levels as low as 30 mg/dl (24). It is not surprising therefore that some studies suggest that warning symptoms may actually occur after detectable deterioration of cerebral function (14, 17, 22, 24), whereas other studies suggest that they precede the onset of cerebral deterioration (21), while still other studies have found warning symptoms and those indicating deterioration in cerebral function to occur concomitantly (11, 14).

To a certain extent, the differences in experimental design, subject selection, the relative sensitivity of measures used to detect changes in these parameters, and the definitions of thresholds account for some of these discrepancies. To date there has been no systematic study simultaneously examining the thresholds for activation of counterregulation, initiation of warning symptoms, and onset of deterioration of cerebral function in normal humans. Despite this, it has recently been suggested that the interrelationship among these thresholds may be altered in individuals with diabetes mellitus. On the one hand, it has been proposed that the optimization of glycemic control may predispose patients to severe hypoglycemia because of a reduced awareness of symptoms

and a delayed activation of counterregulation (1–3, 9, 24, 38). On the other hand, it has been proposed that poor glycemic control may predispose to potentially inappropriate treatment of pseudohypoglycemia because of awareness of hypoglycemic symptoms at higher plasma glucose levels (11).

However, before valid conclusions can be drawn regarding the abnormalities present in pathological states, the normal physiological condition must first be established. The present studies were therefore undertaken to quantitate simultaneously in normal volunteers the glycemic thresholds for activation of hormonal counterregulation, initiation of warning symptoms, and onset of deterioration in cerebral function to answer the following questions. *1*) Does the threshold for activation of counterregulatory hormone secretion vary among hormones? *2*) Do autonomic warning symptoms precede those attributable to neuroglycopenia? *3*) Do these symptoms precede detectable deterioration in cognitive function?

METHODS

Informed, written consent was obtained from ten (7 men and 3 women) healthy, nonobese (body mass index 24 ± 1 kg/m^2) volunteers aged 28 ± 2 (SE) yr. The protocol had been reviewed and approved by the University of Pittsburgh Institutional Review Board.

Subjects were admitted to the University of Pittsburgh Clinical Research Center the evening before experiments and were given a standard dinner between 5:30 and 6:30 P.M. [30 kcal/kg, 50% carbohydrate (CHO), 35% fat, and 15% protein] and a standard snack (~4 h later) at bedtime (10 kcal/kg, 50% CHO, 35% fat, and 15% protein). Between 7:00 and 7:30 A.M., a hand vein was cannulated retrogradely and maintained in a Plexiglas thermoregulated box (70°C) for sampling of arterialized venous blood. A deep antecubital vein of the same arm was cannulated for infusion of insulin. After a 60-min equilibration period, a continuous intravenous infusion of insulin was begun (1 $mU \cdot kg^{-1} \cdot min^{-1}$ for 270 min, followed by 2 $mU \cdot kg^{-1} \cdot min^{-1}$ for an additional 60 min). On one occasion (control study), plasma glucose concentrations were maintained at 90–100 mg/dl throughout, with the use of the glucose clamp technique (4) as previously described (8), with plasma glucose measured every 5 min. On another occasion (experimental study), plasma glucose was clamped by variable glucose infusions at sequential target glucose concentrations of 78, 66, 54, and 42 mg/dl. The plasma glucose concentration was allowed to decrease ~12 mg/dl over 45 min, and a plateau was maintained for 45 min before the next decrease. These glycemic plateaus were chosen to encompass the range of apparent thresholds for activation of counterregulatory hormone secretion, onset of symptoms, and initiation of cognitive dysfunction reported by most previous investigators; we wanted each plateau to be equidistant from the one above and below; these considerations and the need to allow 45 min to achieve the plateau and 45 min for completion of cognitive tests during each plateau led us to choose the specific plateaus used in the present study. We could have used more plateaus, but we felt that many subjects would not tolerate a longer study. Arterialized venous blood samples were drawn every 30 min from 0 to 360 min for determination of plasma insulin, growth hormone, glucagon, cortisol, epinephrine, and norepinephrine.

A semiquantitative symptom questionnaire (11, 14, 35, 39) was administered every 15 min. Subjects scored from 9 (none) to 5 (severe) on each of the following symptoms: hunger, dizziness, tingling, blurred vision, difficulty in thinking, faintness, anxiety, palpitations, sweating, irritability, or tremor. Consistent with the categorization used by previous investigators (11, 14, 23, 35), the first six symptoms were considered neuroglycopenic and the other five were considered autonomic. The sum of each of these constituted the symptom score.

In addition, during each of the plateaus and at similar times during the control euglycemic experiments, the following standard cognitive tests were administered.

Trail-making part B. Subjects were presented with a series of circled letters and numbers that were randomly arrayed across the page. Their task was to rapidly draw a line connecting the circles, alternating numbers and letters (7). The principal response measure was the time to complete the task.

Verbal fluency. Subjects were cued with a letter of the alphabet and told to name as many words as possible that begin with that letter (29). The response measure was the number of words named in 60 s.

Interference subtest from the Stroop test. Subjects were presented with a series of color names (red, green, blue) printed in different inks of different colors. Their task was to name the color of the ink in which each word was printed. The total number of correct responses in 45 s was the response measure (20).

Simple and choice visual reaction time. Visual stimuli (green or blue light) were presented on a Lafayette Instruments model 63035 reaction time (RT) module. For the simple RT subtest, subjects were told in advance which color would be illuminated. After a ready signal the light went on, and subjects pressed the button under the light. For the choice RT subtest, subjects were not told beforehand which light would be illuminated. Each subtest was comprised of 20 trials; the median RT, recorded in milliseconds, was calculated for each.

Word and color subtests from the Stroop test. Subjects were presented with a series of color names (red, green, blue) printed in black ink (word subtest) and a series of *X*'s printed in red, green, or blue ink (color subtest). Their task was to read the work or name the color of the ink. For each subtest, the response measure was the total number of correct responses in 45 s (20).

Digit vigilance test. Subjects were presented with a page of numbers and were required to search for a designated target (the numbers 6 or 9) (28). The response measure was the total number of targets found in 90 s.

Trail-making part A. Subjects were presented with a series of circled numbers, randomly arrayed across the page, and had to rapidly draw a line connecting them in order. The total time to complete the task was the principle response measure.

Verbal memory test. At the beginning of each testing session, subjects heard a series of five words, which they repeated. At the end of the session, ~30 min later, they

recalled the five words. The response measure was the number recalled correctly after the delay.

Forward and backward digit span. On the forward subtest, subjects repeated strings of digits immediately after hearing them. On the backward subtest, they repeated them in reversed order. The response measures were the longest span correctly repeated on each subtest.

On the evening before a study, the subject was provided with extensive practice on each measure. For the actual study, six alternate forms of each test were prepared. The order of the control and hypoglycemia experiments was randomized, and subjects were not informed of their plasma glucose concentrations during the experiments.

Analytical methods. Plasma glucose was measured using a Yellow Springs Instruments glucose analyzer (Yellow Springs, Ohio). Plasma insulin, glucagon, growth hormone, and cortisol were measured by previously described radioimmunoassays (9). Plasma epinephrine and norepinephrine were measured using a single isotope derivative radioenzymatic method (36).

Statistical methods. Glycemic thresholds for various parameters were determined by two methods. In the first method, as previously described (11, 35), the glycemic threshold for a given parameter in a subject was considered to be the plasma glucose concentration at which the parameter first exceeded the 95% confidence limit observed for that parameter at the corresponding time point in euglycemic control experiments, after the adjustment of experimental and control baseline data to zero. In the second method, the glycemic threshold for a parameter was considered to be the plasma glucose concentration at which the mean change in the parameter first became statistically different from the respective mean observed in control experiments, using one-tailed paired tests with Bonferroni correction for repeated comparisons. Data are given as means ± SE; the differences among thresholds were analyzed using analysis of variance followed by the least significant difference test. Data of cognitive tests were transformed to *z*-scores and analyzed using repeated-measures analysis of variance and paired *t* tests.

RESULTS

Plasma glucose and insulin concentration. In control and experimental studies, plasma insulin increased comparably to ~70 μU/ml during the 1 $mU \cdot kg^{-1} \cdot min^{-1}$ insulin infusion and to ~135 μU/ml during the 2 $mU \cdot kg^{-1} \cdot min^{-1}$ infusion (Fig. 1). Plasma glucose concentrations in experimental studies decreased from basal values of 96 ± 2 mg/dl to means of 78 ± 2, 66 ± 1, 55± 1, and 43 ± 1 mg/dl during the first (45–90 min), second (135–180 min), third (225–270 min), and fourth (315–360 min) plateaus, respectively. Corresponding values during the control euglycemic study were 101 ± 2, 98 ± 2, 101 ± 2, and 97 ± 2 mg/dl.

Plasma counterregulatory hormone concentrations. In control studies, there was no significant increase in plasma concentrations of any of the counterregulatory hormones. Indeed, plasma glucagon and cortisol concentrations decreased significantly from respective baseline values of 145 ± 16 pg/ml and 13 ± 2 μg/dl to values of 116 ± 10 pg/ml and 9 ± 2 μg/dl at the end of the study, both $P < 0.05$.

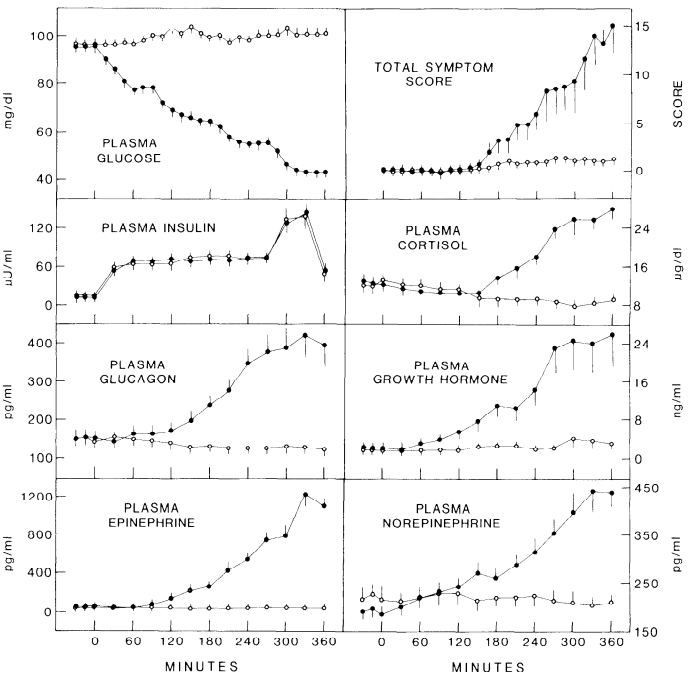

FIG. 1. Plasma glucose, insulin, glucagon, epinephrine, norepinephrine, cortisol, and growth hormone concentrations and overall symptom score. ○, euglycemia; ●, hypoglycemia.

In the experimental studies, the concentrations of all counterregulatory hormones except cortisol were significantly greater than respective values in the control studies by the end of the 66 mg/dl plateau. The increase in plasma cortisol did not reach statistical significance until initiation of the plasma glucose decrease to the subsequent (54 mg/dl) plateau.

Symptom scores. Total symptom scores (autonomic plus neuroglycopenic) are given in Fig. 1. Values in control studies tended to increase over the 6-h period, averaging 1.1 ± 0.4 at the end vs. 0 at baseline. In the experimental studies, total symptom score (which ultimately reached 15.0 ± 2.7 at 360 min) began to increase at 165 min (plasma glucose 66 ± 1 mg/dl) and first became significantly greater than values in control studies at 240 min (plasma glucose 55 ± 1 mg/dl).

Separate scores for autonomic and neuroglycemic symptom scores are given in Fig. 2. The autonomic symptom score began to increase earlier than the neuroglycopenic symptom score (165 vs. 195 min) and reached statistical significance earlier (210 min, plasma glucose 60 ± 1 mg/dl vs. 255 min, plasma glucose 54 ± 1 mg/dl).

Cognitive tests. Raw scores for each cognitive test in control and experimental studies are given in Table 1. To determine whether the performance of subjects was parallel in the two studies or whether it diverged, two-way (condition, time) repeated measures analysis of variance were calculated for each cognitive test. Statistically significant interactions were found for all but two cognitive tests (forward digit span and trail-making part A), indicating that the performance of subjects on all but two cognitive tests deteriorated during the experimental study. To facilitate the determination of the glycemic threshold for this deterioration, data for each cognitive

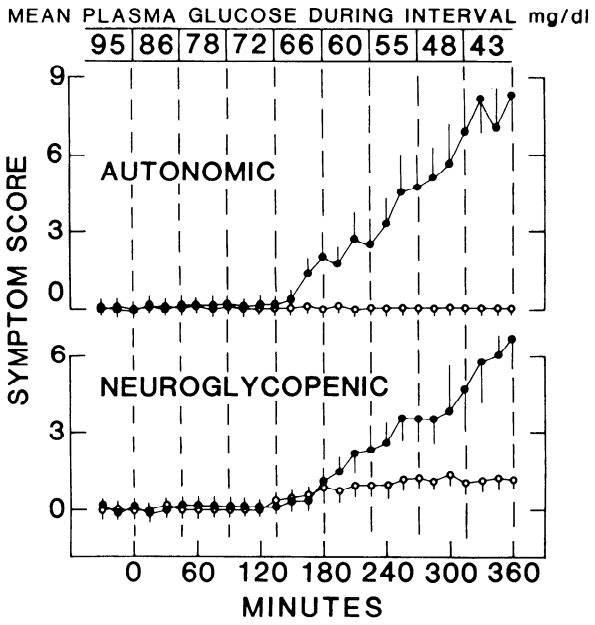

FIG. 2. Autonomic and neuroglycopenic symptom scores. ○, euglycemia; ●, hypoglycemia.

test were transformed to z-scores, and the sum of z-scores was used to compare performance at each plateau during the experimental study with performance at corresponding times during the control study. As shown in Fig. 3, performance on cognitive tests was significantly different only during the last plateau (plasma glucose 43 ± 1 mg/dl).

Assessment of glycemic thresholds for activation of counterregulation, initiation of symptoms, and onset of deterioration in cerebral function. Both methods for assessing glycemic thresholds yielded similar results (Table 2); the activation of secretion of all counterregulatory hormones except cortisol occurred at higher plasma glucose concentrations than did initiation of symptoms. Autonomic symptoms began at higher plasma glucose concentrations than did neuroglycopenic symptoms, and deterioration in cognitive function tests followed or coincided with the appearance of neuroglycopenic symptoms.

The second method for assessing glycemic thresholds, in which thresholds for individual parameters were determined in each subject, permitted statistical evaluation of differences in the threshold for each parameter (Table 2). The glycemic thresholds for increases in plasma growth hormone, glucagon, epinephrine, and norepinephrine were not significantly different from one another (~67 mg/dl) but were significantly higher than that for cortisol (55 ± 2 mg/dl, $P < 0.004$–0.0003) and for the appearance of autonomic symptoms (58 ± 2 mg/dl, $P < 0.039$–0.001). The threshold for secretion of growth hormone, glucagon, epinephrine, and norepinephrine and for autonomic symptoms all occurred at significantly higher plasma glucose concentrations than did the appearance of neuroglycopenic symptoms (51 ± 3 mg/dl, $P < 0.018$–0.00001) and the deterioration in cognitive function tests (49 ± 2 mg/dl, $P < 0.04$–0.00001). However, the thresholds for appearance of neuroglycopenic symptoms and deterioration of cognitive function tests were not significantly different from one another ($P = 0.59$).

DISCUSSION

The present studies were undertaken to quantitate simultaneously the glycemic thresholds for the major

TABLE 1. *Raw scores for each cognitive test at baseline and during each plateau period in control and experimental studies*

		Time of Test (min)					P
		0	60	150	240	330	
Trails B†	C	38.9±3.0	43.3±3.4	39.0±5.9	37.3±3.1	41.1±4.6	0.023
	E	41.6±2.9	40.9±6.6	43.2±6.5	42.6±6.2	57.4±9.6	
Fluency*	C	15.8±1.5	18.0±2.0	19.4±2.1	17.2±1.8	16.7±1.1	0.023
	E	16.4±1.6	17.7±2.4	17.5±2.2	16.0±1.9	11.7±1.3	
Interference*	C	47.0±4.1	54.3±2.9	51.8±3.1	50.4±3.5	58.2±3.4	0.0001
	E	52.6±2.9	46.4±3.6	47.0±3.8	44.3±5.9	36.4±4.9	
Simple RT‡	C	422.6±26.9	424.1±43.5	427.4±29.2	447.9±39.2	420.1±30.5	0.002
	E	390.6±14.5	395.7±10.2	394.0±13.3	440.0±31.5	496.2±41.9	
Choice RT‡	C	451.8±22.9	460.0±33.6	457.2±39.4	486.0±47.5	446.8±34.7	0.0001
	E	413.0±10.4	418.4±11.3	429.2±13.5	474.0±25.6	539.6±44.7	
Word reading*	C	109.2±6.5	106.8±7.9	105.2±6.9	103.0±6.0	106.4±8.2	0.0001
	E	104.8±6.4	108.1±6.0	109.0±6.1	96.5±7.9	82.4±9.5	
Color naming*	C	80.1±4.3	78.0±3.5	76.8±4.8	78.4±4.2	78.3±5.9	0.0001
	E	78.4±3.8	79.4±3.2	76.7±3.2	67.2±4.7	54.9±5.3	
Vigilance*	C	53.9±2.9	49.9±2.7	52.8±4.3	49.8±4.2	50.0±3.0	0.025
	E	50.0±3.7	53.7±3.2	52.1±3.4	47.4±4.4	41.1±3.7	
Trails A†	C	19.0±1.9	18.1±2.3	16.3±2.1	16.0±2.1	16.3±2.2	0.698
	E	17.9±2.2	15.4±1.1	13.9±0.9	15.5±2.5	15.1±1.2	
Word list*	C	4.4±0.4	4.3±0.4	4.3±0.4	4.1±0.4	3.2±0.6	0.019
	E	4.5±0.2	4.6±0.2	4.5±0.2	3.1±0.6	1.8±0.6	
Forward span*	C	8.6±0.3	8.1±0.4	7.9±0.3	7.9±0.4	7.8±0.2	0.616
	E	7.8±0.5	7.9±0.4	7.4±0.3	7.0±0.4	6.7±0.5	
Backward span*	C	6.9±0.5	6.3±0.4	6.7±0.5	6.3±0.5	6.4±0.4	0.034
	E	5.9±0.5	6.7±0.5	6.8±0.4	6.2±0.6	4.9±0.5	

Values are means ± SE. C, control; E, experimental; RT, reaction time. * No. of responses. † Time in s. ‡ Time in ms.

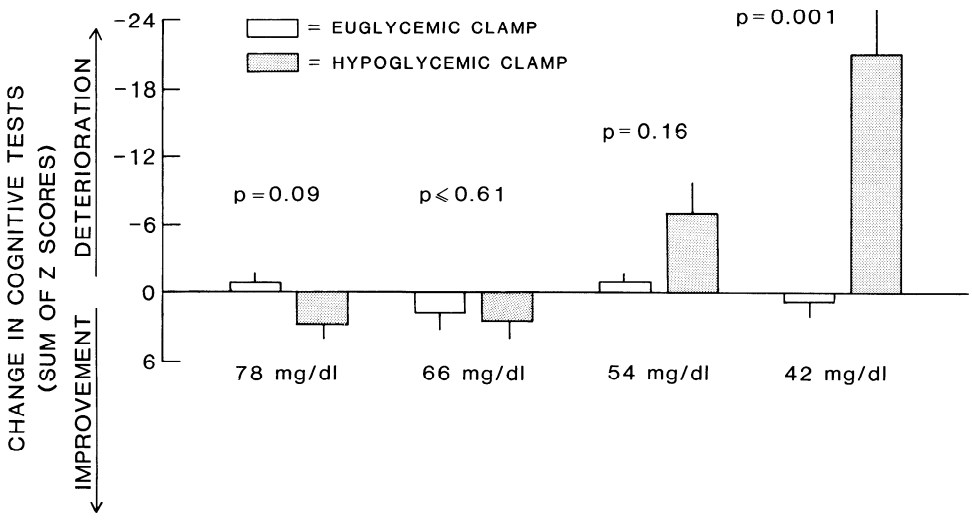

FIG. 3. Changes in sum of z-scores of cognitive function test.

TABLE 2. *Glycemic thresholds for activation for counterregulation, initiation of symptoms, and onset of deterioration in cerebral function*

	Mode of Determination		Significance Matrix							
	Method I	Method II	Growth hormone	Glucagon	Epinephrine	Norepinephrine	Cortisol	Overall symptoms	Autonomic symptoms	Neuroglycemic symptoms
Growth hormone	67±2	72±1		0.85	0.79	0.43	0.0005	0.001	0.005	0.00002
Glucagon	68±1	72±1	0.85		0.88	0.34	0.0003	0.0007	0.003	0.00002
Epinephrine	68±1	67±1	0.79	0.88		0.29	0.0003	0.0006	0.003	0.00001
Norepinephrine	65±2	67±1	0.43	0.34	0.29		0.0035	0.008	0.039	0.0001
Cortisol	55±2	55±1	0.0005	0.0003	0.0003	0.004		0.74	0.34	0.16
Overall symptoms	57±2	55±1	0.005	0.0007	0.0006	0.008	0.34		0.52	0.081
Autonomic symptoms	58±2	60±1	0.001	0.003	0.003	0.039	0.16	0.52		0.018
Neuroglycopenic symptoms	51±3	54±1	0.0002	0.00002	0.00001	0.0001	0.74	0.081	0.018	
Cognitive deterioration	49±2	43±1	0.0001	0.00001	0.00001	0.00004	0.049	0.022	0.004	0.59

Values are means ± SE in mg/dl for Method I [plasma glucose concentration at which parameter of subject exceeded 95% confidence limit observed in control (euglycemic) experiment] and for Method II [plasma glucose concentration at which mean repsonse of subjects first differed significantly from corresponding response observed in control (euglycemic) experiment]. Significance matrix was obtained using least significant difference test.

physiological responses to hypoglycemia in normal humans, i.e., activation of glucose counterregulatory hormone secretion, initiation of warning autonomic and neuroglycopenic symptoms, and onset of deterioration of cognitive function. Our results indicate that these do not occur at the same plasma glucose concentrations and that there is a clear hierarchy among the responses. Glucose counterregulatory hormone secretion was activated at plasma glucose levels (~70 mg/dl) within or just below the physiological range, levels that did not produce either autonomic or neuroglycopenic symptoms, and did not impair cognitive function. The plasma glucose concentration required to produce autonomic symptoms (~60 mg/dl) was lower than that which activated counterregulatory systems but higher than that required to produce neuroglycopenic symptoms and measurable deterioration of cognitive function (~50 mg/dl).

Two statistical approaches were used to quantitate glycemic thresholds from the experimental stepped hypoglycemic clamp and control euglycemic clamp data. In one, the glycemic threshold was defined as the plasma glucose concentration at which mean responses in the experimental study first differed significantly from the respective mean values in the control study. In the other, the glycemic threshold for a given parameter in each subject was defined as the plasma glucose concentration at which that parameter exceeded the 95% confidence limit for that parameter at the corresponding time in the control experiment (35). Both methods yielded similar thresholds. However, the latter approach permits the calculation of a threshold for each parameter in each subject.

Glycemic thresholds for the secretion of individual glucose counterregulatory factors, reported in several previous studies (1, 2, 10, 14, 33–35) and in the present study, are summarized in Table 3. The present data support the findings of Sacca et al. (32), Santiago et al. (34), Santeusanio et al. (33), and Bolli et al. (10) that the thresholds for activation of all counterregulatory systems are at plasma glucose levels greater than 55, 60, 64, and 63 mg/dl, respectively. However, the present counterregulatory thresholds, like most of those determined by Schwartz et al. (35), are at higher plasma glucose concentrations than those estimated by Amiel et al. (1, 2). The latter investigators estimated glycemic thresholds from a continuously declining plasma glucose curve, rather than the stepped hypoglycemic clamp technique (35). Because there must be an interval of time between the signal and a measurable response, thresholds estimated from a declining plasma glucose concentration

TABLE 3. *Summary of results of studies of thresholds for activation of glucose counterregulatory systems*

Reference	Glucagon	Epinephrine	Norepinephrine	Growth Hormone	Cortisol
Sacca et al. (32)*	>55, <68	>55, <68	>55, <68	>55, <68	>55, <68
Santiago et al. (34)*	~60	~60	~60	~60	<60
Santeusanio et al. (33)*	>64	>64	>64	>64	>64
Bolli et al. (10)†	>63	>63	>63	>63	>63
Schwartz et al. (35)†	68±2	69±2	51±3	66±2	58±3
Amiel et al. (2)†	55–60	63±3	55–60	55–60	55–60
Amiel et al. (1)†		56±1	55±2	57±2	53±2
DeFeo et al. (14)†	>72, <80	>72, <80	>72, <80	>72, <80	>72, <87
Present study†	68±1	68±1	65±2	67±2	55±2

Values are means ± SE of plasma glucose in mg/dl. * Venous plasma values. † Arterialized venous plasma values.

curve would be expected to be at lower plasma glucose levels. Furthermore, Amiel et al. (1, 2) defined a given threshold as the plasma glucose concentration that corresponded to an arbitrary increment in a given parameter over its baseline value; data were not compared with those from a corresponding euglycemic control study.

In general, there is remarkably good agreement between the present data and those of Schwartz et al. (35), which used a similar experimental design. The glycemic thresholds for secretion of glucagon (68 ± 1 and 68 ± 2 mg/dl, respectively), epinephrine (68 ± 1 and 69 ± 2 mg/dl, respectively), growth hormone (67 ± 2 and 66 ± 2 mg/dl, respectively), and cortisol (55 ± 2 and 58 ± 3 mg/dl, respectively) were virtually identical in the two studies. However, the thresholds for norepinephrine release were different, 65 ± 2 mg/dl in the present study and 51 ± 3 mg/dl in the data of Schwartz et al. (35). The explanation for this discrepancy is not apparent, because measurements for both studies were performed in the same laboratory. However, baseline plasma norepinephrine levels tended to be lower in the hypoglycemic experiment than in the euglycemic experiment in the report of Schwartz et al. (35); this might have contributed to the estimation of a threshold at a lower glucose level. As can be seen in Table 3, most available data (2, 14, 33–35) are consistent with the present finding of similar glycemic thresholds for the activation of epinephrine, norepinephrine, glucagon, and growth hormone release.

In the present study and that of Schwartz et al. (35), the glycemic threshold for activation of cortisol secretion occurred at a significantly lower plasma glucose concentration than those for the other counterregulatory hormones. Schwartz et al. (35) suggested that this may have resulted because a lower plasma glucose concentration was required to initiate adrenocorticotropic hormone (ACTH) secretion. However, it is conceivable that the glycemic threshold for ACTH secretion is similar to those for the other counterregulatory hormones but that initial increases ACTH were not of sufficient magnitude or duration to result in a substantial increase in cortisol secretion in the time frame of the hypoglycemic steps used. This view is supported by the fact that studies in which small decrements in plasma glucose were maintained for >60 min and those in which the decrease in plasma glucose was slow have not found the glycemic thresholds for cortisol secretion to differ from those for other counterregulatory hormones (1, 10, 14, 35).

With respect to the glycemic thresholds for symptoms, we found that the threshold for autonomic symptoms to be at a higher plasma glucose concentration (~60 mg/dl) than that for neuroglycemic symptoms (~50 mg/dl). Although it is often suggested that autonomic symptoms, presumably the result of the sympathochromaffin response, precede neuroglycopenic symptoms as hypoglycemic develops (5, 18, 19, 27, 30, 39), we are aware of only two studies in which this issue was examined (11, 14). DeFeo et al. (14) reported that both "adrenergic" and neuroglycopenic symptoms occurred simultaneously at a plasma glucose concentration of ~50 mg/dl, when plasma glucose levels were reduced rapidly in normal humans. Boyle et al. (11) did not find differences in the thresholds for autonomic and neuroglycopenic symptoms, in a study that used an experimental design similar to that used in the present study.

Thus the present study appears to be the first to document that autonomic symptoms occur at significantly higher plasma glucose concentrations than neuroglycopenic symptoms. It should be pointed out, however, that this sequence might not apply in all instances. It is quite conceivable that during rapid progression to hypoglycemia, in contrast to the slower stepped decrements in plasma glucose produced in the present study, dissociation of the thresholds for neurogenic and neuroglycopenic symptoms might not be perceptible.

To determine whether autonomic and neuroglycopenic symptoms preceded onset of deterioration in cerebral function, we chose to use a battery of cognitive function tests rather than electrophysiological measurements. We did this because changes in cognitive function tests seemed more relevant than changes in electrophysiological measurements in terms of reflecting the ability to perform daily tasks and to react to dangers in the environment, and because previous studies in normal volunteers (13, 21, 25) indicated that early changes such as the slowing of alpha waves and prolongation of the P100 visually evoked potential did not occur until the plasma glucose concentration was ~40 mg/dl. Thus, if signs of deterioration in cerebral function were to precede symptoms of hypoglycemia, changes would have to be observed at much higher plasma glucose concentrations.

In the present study, performance on 10 of the 12 cognitive tests deteriorated during the course of development of hypoglycemia. Because the responses to different tests might change at different times in different subjects and because our sample size was small relative to the number of tests performed, we transformed the

responses of each subject on each test to z-scores and summed the scores to obtain one value for cognitive performance. Using this approach, we found that the glycemic threshold for deterioration of cognitive function (49 ± 2 mg/dl) coincided with that for the appearance of neuroglycopenic symptoms (51 ± 3 mg/dl, P = not significant) but was significantly lower than that for the appearance of autonomic symptoms (58 ± 2, $P = 0.004$).

Previous studies of the effect of hypoglycemia on cognitive performance in normal volunteers have generally used fewer tests than those employed in the present study and did not examine performance at more than two hypoglycemic plateaus (21, 22, 24, 26, 39). Nevertheless, our results demonstrating a threshold for deterioration of ~50 mg/dl is in general agreement with their results. Herold et al. (24) observed significant prolongation of reaction time at plasma glucose concentrations between 40 and 50 mg/dl. Ipp and Forster (26) found significant deterioration in the trail-making test at a plasma glucose concentration of 40 but not at one of 60 mg/dl. In both of these studies, hypoglycemia was induced by a bolus intravenous injection of insulin, and thus no sustained glycemic plateau was produced, which may explain the somewhat lower plasma glucose concentrations at which impairment in cerebral function was detected for reasons discussed earlier.

Harrod et al. (21), Heller et al. (22) and Stevens et al. (39) induced hypoglycemia by intravenous infusion of insulin and studied cognitive performance at predetermined glycemic plateaus. Harrod et al. (21) found no change in the digit-recall test at a plasma glucose concentration of 36 mg/dl compared with baseline results; however, no euglycemic control experiment was performed. Heller et al. (22), who also did not have a control experiment, found prolongation of reaction time at a plasma glucose concentration of 58 mg/dl. In contrast, Stevens et al. (39), who did include a control euglycemic experiment, found no deterioration in reaction times, finger-tapping speed, and critical flicker fusion thresholds at a plasma glucose concentration of ~60 mg/dl.

In none of the above studies was deterioration in cognitive function specifically examined in relation to the onset of autonomic and neuroglycopenic symptoms. However, Ipp and Forster (26) reported that some subjects developed symptoms of sweating and palpitations at a plasma glucose concentration of ~60 mg/dl, a time at which the trail-making score of their subjects was unaltered. These observations are consistant with the findings in the present study of the appearance of autonomic symptoms before the deterioration of cerebral functions. In the study of Stevens et al. (39), overall symptoms began at a time when five of seven cognitive tests were still normal. In the study of Heller et al. (22), overall symptoms increased after the appearance of prolongation of reaction time at a plasma glucose concentration of 45 mg/dl. In the study of Herold et al. (24), the mean plasma glucose concentration at which symptoms developed was 43 mg/dl, similar to the concentration at which reaction time became prolonged. These observations may not necessarily be inconsistent with those of the present study, since we found the appearance of neuroglycopenic symptoms to coincide with the onset of deterioration in cognitive function tests, and in neither of the above studies were autonomic symptoms separately evaluated.

Our data do not support the conclusion of DeFeo et al. (14) that the glycemic threshold for cognitive impairment, based on measurements of the P300 wave latency, is 72 ± 1 mg/dl. We do not present electroneurophysiological measurements in the current study, but Blackman et al. (6) recently estimated a threshold of <50 mg/dl for the prolongation of P300 wave latency in normal humans, which coincides with the onset of neuroglycopenic symptoms and with deterioration in cognitive function tests observed in the present study.

In summary, the present study demonstrates that there is a clear hierarchy of physiological responses to decrements in plasma glucose concentration in normal humans. Small decrements in plasma glucose, to ~70 mg/dl, activate the secretion of glucose counterregulatory hormones. Under physiological conditions, this could restore euglycemia without the development of symptoms or cerebral impairment. Greater decrements in plasma glucose to ~60 mg/dl would result in a sympathochromaffin response of such intensity as to evoke symptoms that would warn of developing hypoglycemia and prompt an individual to take preventive action, e.g., eat. However, if the glucose counterregulatory system were overwhelmed (e.g., insulin overdose) or were impaired [e.g., long-standing insulin-dependent diabetes (10, 12)] and the individual did not (or could not) intervene, plasma glucose concentrations would fall further, and at ~50 mg/dl, neuroglycopenic symptoms and deterioration of cognitive function would occur. Whether such a hierarchy and the specific thresholds are preserved in pathological conditions (e.g., insulinoma or diabetes mellitus) remains to be determined.

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