Hormonal, Metabolic, and Cardiovascular Responses to Hypoglycemia in Diabetic Autonomic Neuropathy

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SUMMARY

Hormonal, metabolic, and cardiovascular responses to insulin-induced hypoglycemia were investigated in 10 juvenile-onset diabetics who showed signs of autonomic neuropathy, in 8 control patients of similar age and duration of diabetes without neuropathy, and in 6 healthy subjects. In an attempt to normalize intermediary metabolism, the diabetics were treated with soluble insulin only (given subcutaneously and intravenously) for 48 h preceding the study.

Plasma epinephrine concentrations were significantly lower in patients with autonomic neuropathy before the experiment as well as 15 min after serum glucose nadir compared with diabetics without neuropathy ($P < 0.05$), indicating impaired sympathoadrenal activity. Plasma norepinephrine responses did not differ significantly. No significant increase was found in glucagon concentrations in patients with autonomic neuropathy, whereas a small increment was found in diabetics without neuropathy. Growth hormone and cortisol responses were similar in the two patient groups, and serum free insulin concentrations were also similar. In spite of blunted responses of glucagon and of epinephrine in the patients with autonomic neuropathy, serum glucose responses were similar to those of the diabetics without autonomic neuropathy. Furthermore, rate of lipolysis, as judged from FFA and glycerol concentrations, as well as systolic blood pressure increments were significantly greater ($P < 0.05$) in patients with autonomic neuropathy than in diabetics without neuropathy.

In conclusion, during insulin-induced hypoglycemia, patients with autonomic neuropathy had impaired activation of the adrenal medulla, probably due to sympathetic neuropathy. Furthermore, they had no increase in glucagon concentrations. Compared with noneuropathic diabetics, serum glucose recovery was unaffected and lipolytic responses and blood pressure increments were exaggerated, suggesting increased sensitivity of hepatic glycogenolysis, adipose tissue lipolysis, and the cardiovascular system toward the action of catecholamines in diabetics with autonomic neuropathy. DIABETES *30*:626–633, August 1981.

In normal man, the increase of plasma concentrations of catecholamines and glucagon in response to insulin-induced hypoglycemia is considered important for glucose recovery.[1–3] Glucagon responses to hypoglycemia have been reported to be diminished in juvenile-onset diabetics.[4,5] Maher et al.[6] found that glucagon release during hypoglycemia was diminished in juvenile-onset diabetics without autonomic neuropathy and was absent in diabetics with autonomic neuropathy, whereas growth hormone and cortisol responses were identical in these patient groups.[6] Plasma catecholamines and glucose recovery were not assessed in this study. However, juvenile-onset diabetics with autonomic neuropathy display blunted responses of catecholamines to exercise[7] and standing up,[8] indicating lesions in the reflex arches controlling sympathoadrenal activity during exercise and standing up. Accordingly, in diabetics with autonomic neuropathy, blunted catecholamine responses also to hypoglycemia might be expected, and it might be speculated that blunted responses of glucagon and catecholamines would result in impaired glucose recovery and lipolysis.

In normal man, insulin-induced hypoglycemia causes a rise in systolic blood pressure, a fall in diastolic blood pressure, and tachycardia.[9] In contrast, patients with complete cervical cord transection displayed a fall in systolic as well as diastolic blood pressure during hypoglycemia.[10] Accordingly, a diminished systolic blood pressure response to hypoglycemia might be expected in diabetics with autonomic neuropathy.

Core temperature is usually lowered by hypoglycemia due to heat loss from sweating and hyperventilation.[11] Since sweating[12] may be affected in diabetics with autonomic

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Received for publication 13 October 1980 and in revised form 31 March 1981.

TABLE 1
Anthropometric and clinical data ($\bar{X}$ and range) in 18 insulin-treated juvenile-onset diabetics

	Age	Duration of diabetes (yr)	Daily insulin dose (IU)	Height (cm)	Weight (kg)	Serum creatinine (μmol/L)	Sense of vibration (V)	Beat-to-beat (min^{-1})	IgG (mU/L)	
$\bar{X}$	29	13	39	178	71	86	10	28	0.364	Diabetics without
range	(24–32)	(6–21)	(28–48)	(169–189)	(61–83)	(80–93)	(7–12)	(14–46)	(0.052–0.842)	neuropathy N = 8
$\bar{X}$	36	16	37	179	67	84	28	8	0.548	Diabetics with autonomic neuropathy
range	(26–49)	(5–31)	(24–56)	(172–190)	(57–79)	(71–115)	(11–48)	(2–20)	(0.044–1.677)	N = 10
$\bar{X}$	28			183	73		9	30		Normal subjects
range	(22–34)	—	—	(177–193)	(72–76)	—	(6–13)	(14–43)	—	N = 6
	$P < 0.025$	N.S.	N.S.	N.S.	N.S.	N.S.	$P < 0.005$	$P < 0.001$	N.S.	

neuropathy, core temperature regulation may be affected in diabetics with autonomic neuropathy. Thus, the aim of the present study was to determine whether hormonal, metabolic, cardiovascular, and core temperature responses to hypoglycemia are affected in juvenile diabetics with autonomic neuropathy.

MATERIALS AND METHODS

Patients. Eighteen insulin-treated male juvenile-onset diabetics and six normal male subjects volunteered in the study after giving written informed consent (Table 1). The patients were divided into three groups according to their beat-to-beat variation in heart rate during hyperventilation[13] (low values are considered to be due to cardiac parasympathetic neuropathy) and to blood pressure changes during change of position from supine to standing.

Diabetics without neuropathy (N = 8). Control patients with normal beat-to-beat variation in heart rate (>15 min^{-1}) and normal orthostatic blood pressure responses (decrease in systolic blood pressure ≦15 mm Hg 1 min after standing up).

Diabetics with slight autonomic neuropathy (N = 6). Patients having signs of autonomic neuropathy (beat-to-beat variation in heart rate ≦15 min^{-1}) but having normal orthostatic blood pressure responses as defined above.

Diabetics with severe autonomic neuropathy (N = 4). Patients with orthostatic hypotension (decrease in systolic blood pressure ≧30 mm Hg 1 min after standing up). Mean beat-to-beat variation was decreased (Table 1), and only one patient had a normal beat-to-beat variation (20 min^{-1}). Vibratory perception threshold was measured in the big toe with a Biothesiometer (Bio-Medical Instrument Co, Newbury, Ohio). The threshold is expressed in volts, a threshold value over 20 V being indicative of neuropathy.[14] Vibratory perception threshold was increased in the neuropathic diabetics (Table 1).

Two patients with slight neuropathy were impotent and one had retrograde ejaculation; two of these patients also lacked sweat secretion on feet and legs. One patient with slight neuropathy had loss of sweat secretion on feet and legs, but had no other clinical symptoms of neuropathy. In contrast, patients with severe neuropathy had gross clinical signs of neuropathy. All were impotent and had sweating abnormalities as described above. Three patients had atrophy of interdigital muscles and all four had paresthesias.

One patient without neuropathy and three patients with neuropathy had proteinuria. Four patients without neuropathy had background retinopathy; six patients with neuropathy had background retinopathy and three had proliferative retinopathy.

Apart from one patient with neuropathy who had minimal beta-cell function (plasma C-peptide 0.17 pmol/ml 6 min after i.v. injection of 1 mg glucagon[15]), none of the diabetics had residual beta-cell function.

No patients or normals had signs or symptoms of other endocrine, metabolic, or cardiovascular disease, nor did they take any drugs apart from insulin.

METHODS

To clear subcutaneous depots of intermediate-acting insulin, the patients only took soluble insulin (insulin Actrapid, Novo) 48 h before the start of the experiment. Soluble insulin was given subcutaneously 3–4 times daily one half-hour before meals, the individual dose being adjusted according to glucosuria as measured by Clinitest (Ames, Elkhart, Indiana). At 10 p.m., the night before the experiment, the patients arrived in the metabolic ward.

After a light meal, they had a cannula inserted into an antecubital vein in each arm, and an i.v. infusion of Actrapid dissolved in isotonic saline (1 IU/ml) was started. Infusion rate was adjusted according to frequent blood glucose measurements, aiming for blood glucose levels between 5 and 8 mmol/L throughout the night. The normal subjects were admitted to the ward at 7 a.m. after an overnight fast. At 8 a.m., hypoglycemia was induced by infusion of Actrapid 0.15 IU/kg body wt/h. The infusion was stopped when blood glucose, measured by means of a glucose reflectance meter (Eyetone, Ames), was below 2.5 mmol/L. All patients, including the neuropathic diabetics, had symptoms of hypoglycemia when the infusion was stopped: All patients had palpitations, and all patients sweated (sweating was more pronounced on the truncus than on the legs in the neuropathic diabetics). Level of consciousness seemed lower in the neuropathic diabetics than in the diabetics without neuropathy. In the normal subjects, palpitations and sweating was less pronounced and the level of consciousness seemed higher than in both patient groups. Blood samples for determination of epinephrine, norepinephrine, glucagon, growth hormone, cortisol, free insulin, C-peptide (in the normal subjects), insulin-binding antibodies, glucose, lactate,

free fatty acids, glycerol, acetoacetate, and beta-hydroxybutyrate were drawn without stasis 15 min before start of insulin infusion, at start of insulin infusion (0), 30 min after start of insulin infusion (30), at stop of insulin infusion (△), and 15, 30, 60, and 120 min later. At the same time intervals, heart rate was counted by auscultation and blood pressure was measured by the indirect auscultatory method, using a sphygmomanometer and a cuff. Rectal temperature was measured by an electrical heat conductivity meter (Ellab, Copenhagen). During the experiment, 320 ml of blood was drawn. Blood samples were cooled immediately and centrifuged within 10 min. Plasma was kept at −20°C until analysis.

Plasma epinephrine and norepinephrine were determined by a double-isotope derivative method.[16,17] Glucagon was measured with radioimmunoassy after ethanol extraction of plasma.[18] The antiserum employed, K5563, is specific for the C-terminal of glucagon. Growth hormone was measured by a commercially available solid phase radioimmunoassay kit (Phadebas). I U GH equals 0.5 mg of the first international reference preparation of human growth hormone (66/127) from the National Institute of Biological Standards and Control (Holly Hill, London). Plasma cortisol was measured by a competitive protein-binding technique.[19] Free insulin was measured after precipitation of insulin binding antibodies with polyethylene glycol.[20,21] Plasma C-peptide was measured using antibody M-1230.[15] Insulin binding IgG antibodies were measured by radioimmunoelectrophoresis.[22] Glucose (mmol/L serum) was measured by the hexokinase method,[23] and lactate, glycerol, beta-hydroxybutyrate, and acetoacetate (mmol/L blood) were determined by standard enzymatic methods.[24] Free fatty acids (FFA) (meq/L serum) were measured colorimetrically.[25]

Statistical analysis was performed using Mann-Whitney's rank sum test for unpaired comparisons and Wilcoxon's matched-pairs signed-ranks test for paired comparisons. Correlation analysis was made by means of Spearman rank correlation coefficient test. Differences and correlations were considered to be significant if P values less than 0.05 were obtained.

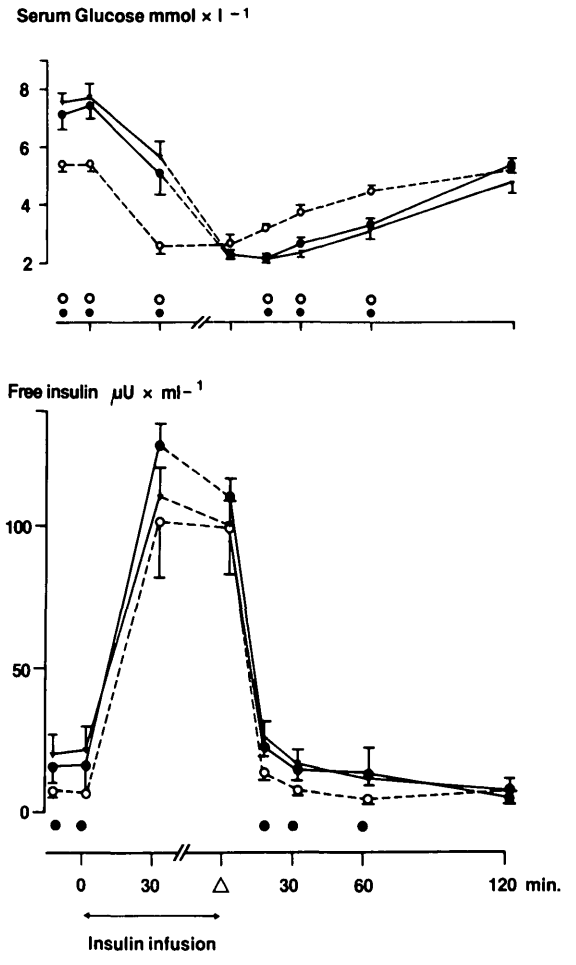

FIGURE 1. Serum concentrations of glucose (upper panel) and free insulin (lower panel) in 10 diabetics with autonomic neuropathy (▼——), 8 diabetics without neuropathy (●——), and in 6 normals (○--) before and during insulin-induced hypoglycemia. * Denotes significant differences (P < 0.05) between the patient groups, ● between normals and patients with autonomic neuropathy, and ○ between normals and patients without neuropathy. △ denotes stop of insulin infusion.

RESULTS

No significant differences in responses were found between patients with slight and severe neuropathy; these two groups are therefore regarded as one group having autonomic neuropathy (neuropathic diabetics).

Insulin was infused for 58 ± 9 min (normals), 74 ± 9 min (diabetics without neuropathy), and 90 ± 9 min (neuropathic diabetics), significantly longer in neuropathic diabetics than in normal subjects (P < 0.05).

Glucose, free insulin, and C-peptide. Serum glucose nadirs were similar in the three groups, and serum glucose concentrations (Figure 1) were similar in nonneuropathic diabetics and neuropathic diabetics throughout the experiment. Serum glucose concentrations before induction of hypoglycemia were significantly lower in the normals compared with both patient groups, whereas glucose concentrations were significantly higher in the normals 15–60 min after stop of insulin infusion.

Serum free insulin concentrations (Figure 1) were similar in diabetics without neuropathy and patients with autonomic neuropathy throughout the experiment. In the normals, serum free insulin concentrations were significantly lower than in patients with autonomic neuropathy before induction of hypoglycemia and 15–60 min after stop of insulin infusion.

In the normal subjects, plasma C-peptide was 0.33 ± 0.01 pmol/ml before induction of hypoglycemia, 0.15 ± 0.02 at stop of insulin infusion, and 0.16 ± 0.01 2 h later. Since the patients had no beta-cell function as measured by C-peptide response to 1 mg glucagon i.v., C-peptide concentrations during hypoglycemia were measured in normals only.

Counterregulatory hormones. Plasma epinephrine concentrations (Figure 2) were significantly lower in patients with autonomic neuropathy before induction of hypoglycemia (P < 0.01) and 15 min after interruption of insulin infusion (P < 0.05), compared with control patients. Plasma epinephrine concentrations in the normals were significantly lower than in control patients 15–60 min after stop of insulin infusion (P < 0.025). A significant correlation was found between plasma epinephrine concentrations 15 min after stop of insulin infusion and beat-to-beat variation in the two patient groups (r_s = 0.63, P < 0.01, N = 18).

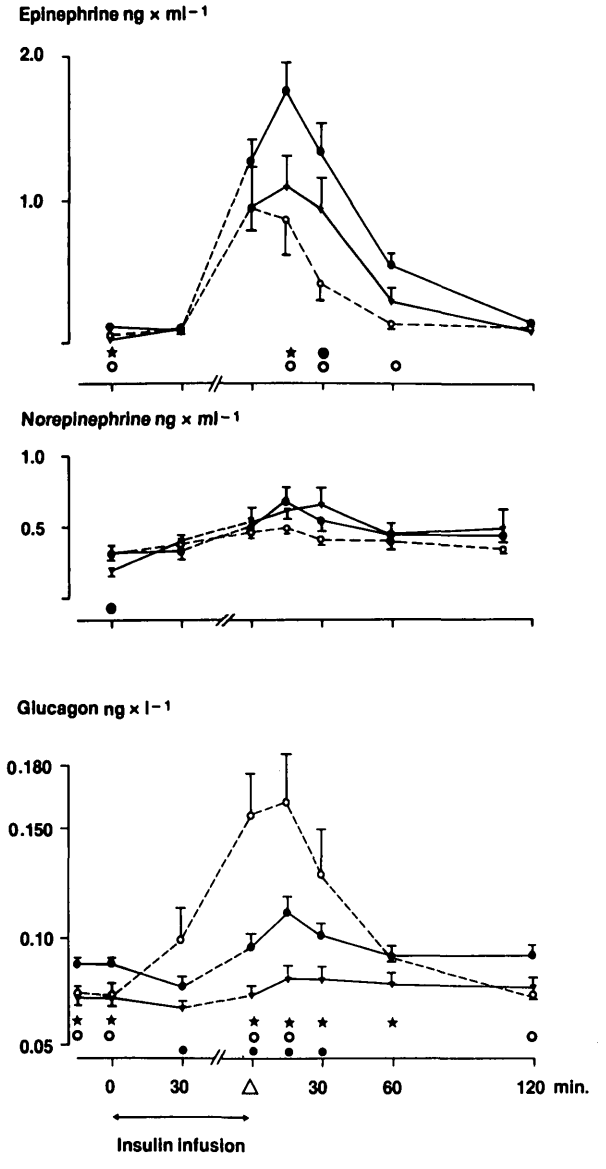

FIGURE 2. Plasma concentrations of epinephrine (upper panel), norepinephrine (middle panel), and glucagon (lower panel) during insulin-induced hypoglycemia. For details, see legend to Figure 1.

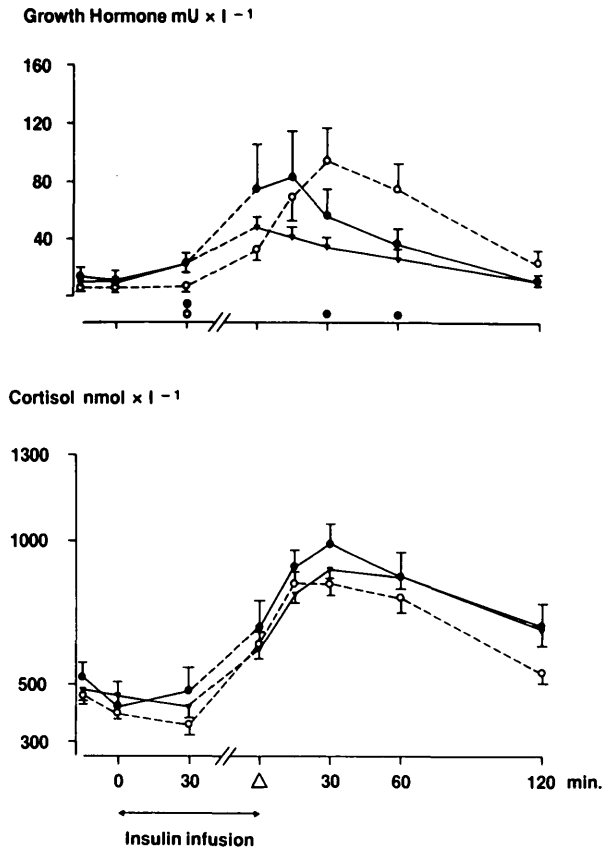

FIGURE 3. Serum growth hormone (upper panel) and plasma cortisol concentrations (lower panel) during insulin-induced hypoglycemia. For details, see legend to Figure 1.

Plasma norepinephrine responses to hypoglycemia did not differ in the three groups (Figure 2).

Plasma glucagon levels (Figure 2) were significantly higher ($P < 0.05$) before induction of hypoglycemia and 0–60 min after stop of insulin infusion in nonneuropathic diabetics compared with patients with autonomic neuropathy, in whom no significant increase above prehypoglycemic concentrations were found ($P < 0.1$). No significant correlations were found between beat-to-beat variation and plasma glucagon concentrations 0–30 min after stop of insulin infusion, whereas plasma glucagon concentrations 15 min after stop of insulin infusion correlated with threshold for sense of vibration ($r_s = -0.51$, $P < 0.05$, $N = 18$). In the normals plasma glucagon concentrations were significantly higher ($P < 0.05$) than in both patient groups 0 and 15 min after stop of insulin infusion.

No significant differences were found in growth hormone concentrations (Figure 3) in nonneuropathic diabetics compared with patients with autonomic neuropathy, whereas growth hormone concentrations in both patient groups were significantly higher than in normals ($P < 0.05$) 30 min after start of insulin infusion and higher ($P < 0.025$) in the normals than in patients with autonomic neuropathy 30 and 60 min after stop of insulin infusion.

Cortisol concentrations (Figure 3) were similar in the three groups throughout the experiment.

Metabolites. Blood lactate concentrations (Figure 4) were significantly higher in normals compared with diabetics without neuropathy following hypoglycemia but were otherwise similar in normals, control patients, and patients with autonomic neuropathy throughout the experiment. No significant differences were found between patients with and without autonomic neuropathy in plasma concentrations of free fatty acids and glycerol (Figure 4) before induction of hypoglycemia. After stop of insulin infusion, serum concentrations of free fatty acids and of glycerol were significantly higher in patients with autonomic neuropathy compared with nonneuropathic diabetics. Beat-to-beat variation and threshold for sense of vibration correlated significantly with glycerol concentrations 60 min after stop of insulin infusion ($r_s = -0.52$, $P < 0.05$, $N = 18$, and $r_s = 0.51$, $P < 0.05$, $N = 18$) but not with FFA concentrations. Glycerol was significantly lower in normals compared with both patient groups. Plasma concentrations of acetoacetate and beta-hydroxybutyrate (Figure 5) were similar in diabetics with and without autonomic neuropathy throughout the experiment, whereas plasma concentrations in the normals were significantly lower than in both patient groups.

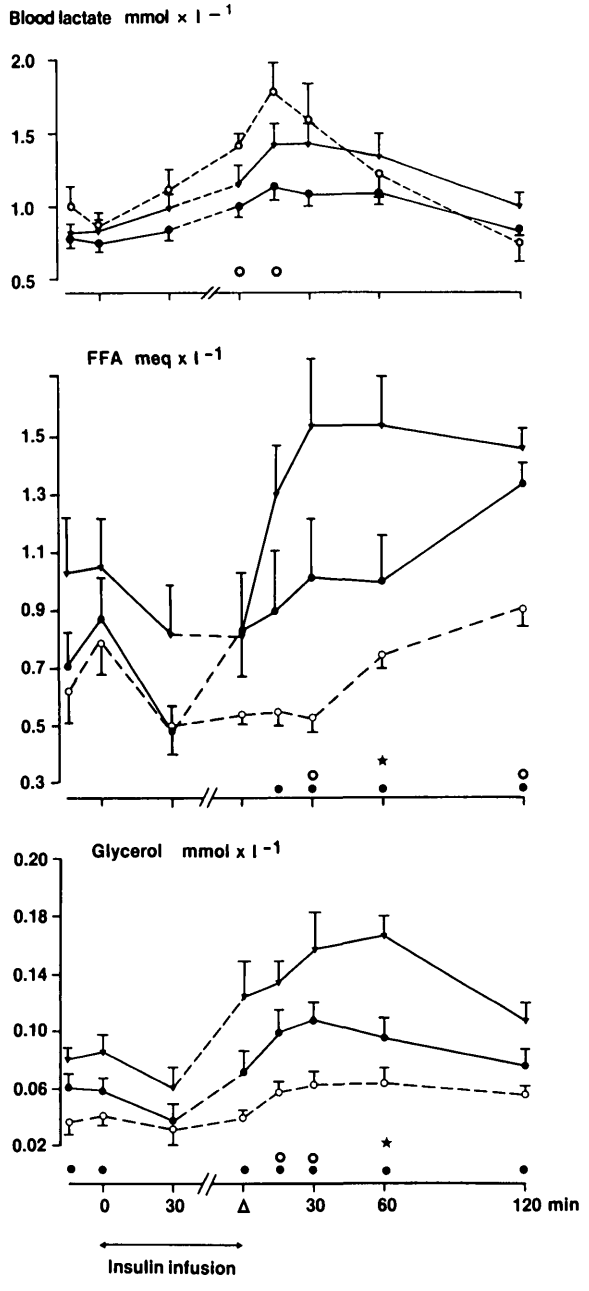

FIGURE 4. Blood concentrations of lactate (upper panel), serum free fatty acids (FFA) (middle panel), and serum glycerol (lower panel) during insulin-induced hypoglycemia. For details, see legend to Figure 1.

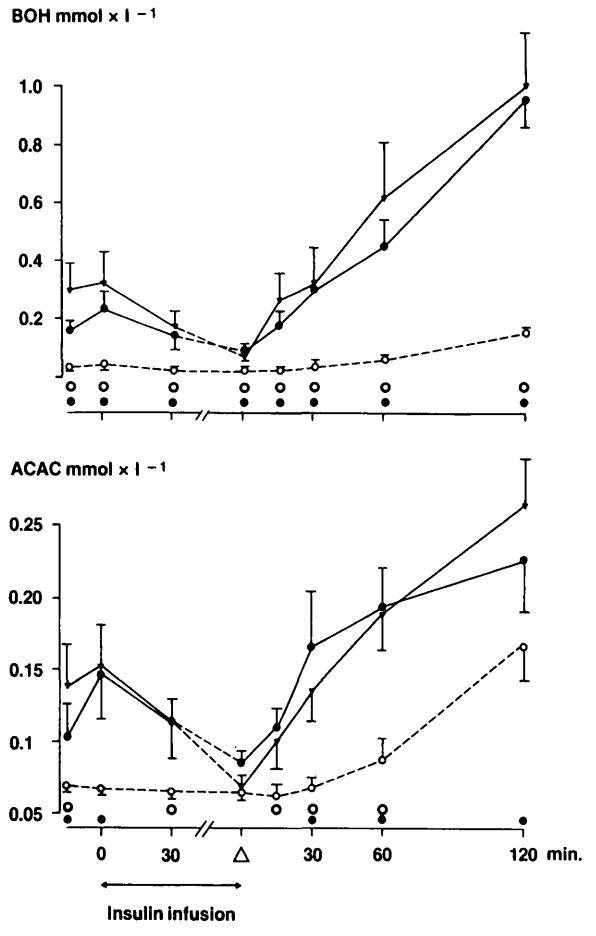

FIGURE 5. Blood concentrations of beta-hydroxybutyrate (BOH) (upper panel) and of acetoacetate (AcAc) (lower panel) during insulin-induced hypoglycemia. For details, see legend to Figure 1.

Cardiovascular variables and core temperature. Heart rate (Figure 6) was significantly higher in patients with autonomic neuropathy compared with nonneuropathic diabetics before hypoglycemia ($P < 0.05$) and also after stop of insulin infusion ($P < 0.025$). A significant difference was found in decrease in heart rate (heart rate at stop of insulin infusion minus heart rate 15 min later) between the two patient groups [10 ± 4 min^{-1} (diabetics without neuropathy) versus 1 ± 2 min^{-1} (autonomic neuropathy) ($P < 0.05$)]. Heart rate in the normals was similar to heart rate in nonneuropathic diabetics throughout the experiment.

The increase in systolic blood pressure (Figure 6) from 30 min after start of insulin infusion to stop of insulin infusion was significantly ($P < 0.05$) greater in patients with autonomic neuropathy compared with nonneuropathic diabetics and normals. A significant correlation was found between this systolic blood pressure increase and beat-to-beat variation ($r_s = -0.54$, $P < 0.05$, $N = 17$) but not between the increase in blood pressure and threshold for sense of vibration.

Rectal temperature (Figure 6) tended to be lower ($P < 0.1$) in the three groups 120 min after stop of insulin infusion compared with preinfusion temperature.

DISCUSSION

In the present study, patients with diabetic autonomic neuropathy had similar norepinephrine responses but impaired responses of epinephrine to hypoglycemia compared with patients without neuropathy who were of similar age and duration of diabetes, indicating impaired activation of the adrenal medulla. No significant increase in glucagon concentrations occurred in patients with autonomic neuropathy, while growth hormone and cortisol responses were unaffected. In spite of the differences in hormonal responses, glucose recovery was identical, and rate of lipolysis and increase in systolic blood pressure was increased in patients with autonomic neuropathy, suggesting that the sensitivity of hepatic glycogenolysis, adipose tissue lipolysis, and of the cardiovascular system toward the action of circulating

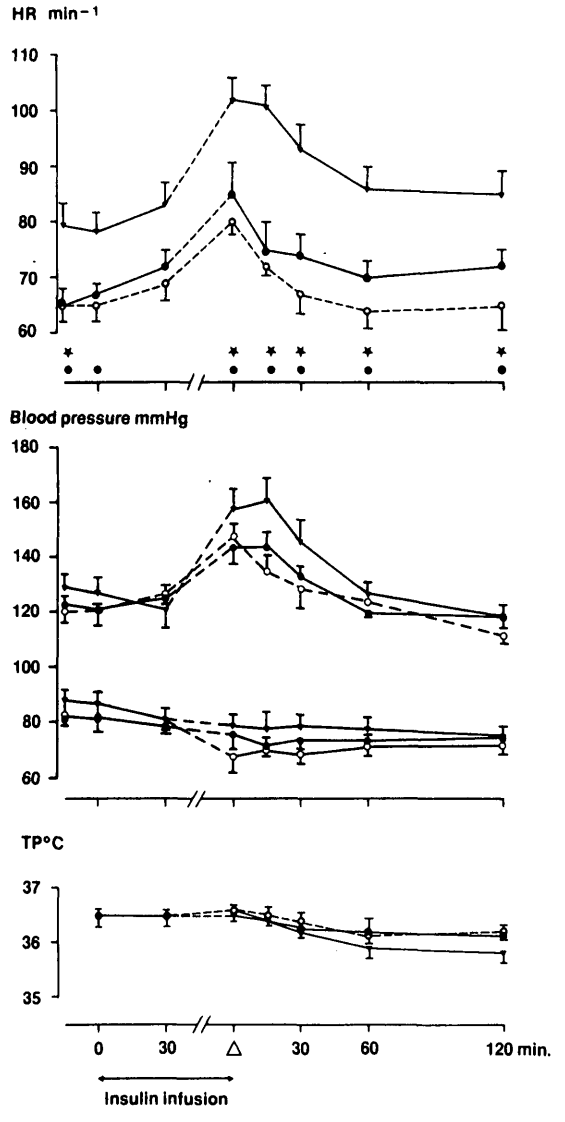

FIGURE 6. Heart rate (upper panel), systolic and diastolic blood pressure (middle panel), and rectal temperature (lower panel) responses to hypoglycemia. No significant differences in rectal temperature were found between the three groups, whereas the increase in systolic blood pressure from 30 min after start of insulin infusion to stop of insulin infusion was significantly greater ($P < 0.05$) in the neuropathic diabetics (▼——) compared with diabetics without neuropathy (●——). For details, see legend to Figure 1.

catecholamines is increased in patients with diabetic autonomic neuropathy.

The setting of the cells regulating the release of counterregulatory hormones is probably dependent on the metabolic state of the organism, insulin availability probably being of prime importance. Thus, it has been demonstrated that the hormonal response to exercise is exaggerated in poorly controlled diabetics compared with normal subjects.[26] Furthermore, blood glucose lowering from one hyperglycemic level to another lower, still hyperglycemic, level results in release of counterregulatory hormones in diabetics, whereas healthy subjects do not demonstrate release of any counterregulatory hormones despite a similar rate of decline in blood glucose concentrations.[27] Accordingly, hormonal responses to perturbations (for instance, hypoglycemia), and the influence of autonomic neuropathy on these responses, have to be evaluated in the light of the preceding metabolic state. In the present study, the two patient groups are comparable, since insulin treatment was similar during 48 h preceding the study and resulted in similar serum glucose and free insulin levels before induction of hypoglycemia. However, the metabolic state was not completely normalized. Compared with normal subjects, serum glucose and ketone body concentrations were higher in both patient groups, indicating insulin deficiency. Thus, differences in preceding metabolic state may account for the lower epinephrine response and the delayed growth hormone response in the normal subjects compared with the diabetics without neuropathy.

In patients with autonomic neuropathy, plasma epinephrine concentrations were significantly lower before the induction of hypoglycemia and 15 min after stop of insulin infusion than in diabetics without neuropathy, indicating that the sympathetic outflow to the adrenal medulla is impaired, possibly due to the degeneration of preganglionic sympathetic fibers, in patients with autonomic neuropathy. Plasma norepinephrine concentrations did not differ significantly between the two patient groups, in contrast to epinephrine findings. Since the source of circulating norepinephrine as well as its physiologic significance during hypoglycemia is unknown,[28] this dissociation cannot be explained at the present time.

After stop of insulin infusion, plasma glucagon concentrations were significantly higher in nonneuropathic diabetics compared with patients with autonomic neuropathy, in whom no significant increase was found during hypoglycemia. Thus, our results regarding the effect of autonomic neuropathy on glucagon responses to hypoglycemia are similar to those of Maher et al.[6]

The Increase in serum glucose concentration after stop of insulin infusion was similar in nonneuropathic diabetics compared with diabetics with autonomic neuropathy. This is surprising in the light of lower concentrations of the two most important counterregulatory hormones, glucagon and epinephrine, in the latter than in the former group, after stopping the insulin infusion. Recent investigations[1-3] have pointed out that in man, glucagon secretion is necessary for normal glucose counterregulation, while growth hormone is of little importance. Adrenergic mechanisms normally do not play an essential role in counterregulation, but become critical to blood glucose recovery when glucagon secretion is impaired. Since no significant increase in glucagon concentrations occurred in patients with autonomic neuropathy, it might be speculated that circulating epinephrine was responsible for blood glucose recovery in patients with autonomic neuropathy. Since the blood glucose recovery was similar to the recovery in nonneuropathic diabetics in spite of lower concentrations of circulating epinephrine in the patients with autonomic neuropathy, the sensitivity of hepatic glycogenolysis to the action of epinephrine may be increased in patients with autonomic neuropathy. Alternatively, the patients with neuropathy relied more upon gluconeogenesis, which may have been higher than in control patients due to higher concentrations of gluconeogenic precursors (glycerol and lactate). In addition, greater amounts of FFA in patients with autonomic neuropathy may have reduced peripheral glucose uptake.[29]

The rate of lipolysis, judged from serum concentrations of FFA and glycerol, was significantly greater in diabetics with autonomic neuropathy compared with nonneuropathic diabetics after stop of insulin infusion in spite of lower concentrations of epinephrine in patients with autonomic neuropathy. This is strange, but may be explained by an increase in the sensitivity of adipose tissue toward the lipolytic action of circulating catecholamines in patients with autonomic neuropathy. Since the systolic blood pressure increase during hypoglycemia is due to circulating catecholamines,[30] the negative correlation between beat-to-beat variation and systolic blood pressure increase may indicate that the sensitivity of the cardiovascular system (to the blood pressure enhancing action of circulating catecholamines) also is increased in patients with autonomic neuropathy. We suggest that the sensitivity toward circulating epinephrine is increased in neuropathic diabetics compared with nonneuropathic diabetics, whereas it recently has been shown that the sensitivity to epinephrine (and cortisol and glucagon) is increased in nonneuropathic diabetics compared with normals.[31] Compared with the normal subjects, both our diabetic groups had decreased glucagon and increased catecholamine responses to hypoglycemia; thus, it is not possible to compare the sensitivity toward epinephrine of the diabetic patients with the normal subjects in the present study. The rate of increase in plasma glucose concentration after stopping the insulin infusion was significantly greater in the normal subjects compared with both patient groups, a fact that is most likely explained by the increased amounts of circulating glucagon in the normals. Higher levels of free insulin in both groups of diabetics than in normals after stopping the insulin infusion may also have contributed to the slower glucose recovery in the diabetics and may thus even have prolonged the stimulus for epinephrine release in the diabetics. The higher blood concentrations of acetoacetate and of beta-hydroxybutyrate in the patient groups compared with the normals were probably due partly to the greater FFA load on the liver[32] and partly to greater portal concentrations of insulin in the normals, who maintained endogenous insulin secretion throughout the study as judged from C-peptide measurements. Also, the increased catecholamine levels in nonneuropathic diabetics may have been of importance, since norepinephrine infusions increase hepatic ketone body production in man.[33]

Heart rate was significantly higher before hypoglycemia in patients with autonomic neuropathy compared with nonneuropathic diabetics as well as with normals, a finding that is in accordance with previous reports.[7] The return of heart rate to preinfusion values in nonneuropathic diabetics and in the normals occurred at the time epinephrine and norepinephrine concentrations were highest, and it has been suggested that this return of heart rate is due to increased vagal activity.[34] Thus, the slower return in patients with autonomic neuropathy may be due to impaired vagal activity.

No differences between the studied groups were found in rectal temperatures during the experiment, indicating that temperature regulation during hypoglycemia is normal in patients with autonomic neuropathy.

ACKNOWLEDGMENTS

The authors thank Annette Thorburn and Lisbeth Kall for skilled technical assistance.

The study was supported in part by grants from the Danish Medical Research Council (j. no. 512-10233, j. no. 512-20044, and j. no. 512-20320) and from Erik Gardes, M.D., and Elizabeth Gardes Legat.

REFERENCES

1. Gerich, J., Davis, J., Lorenzi, M., Rizza, R., Bohannon, N., Karam, J., Lewis, S., Kaplan, R., Schultz, T., and Cryer, P.: Hormonal mechanism of recovery from insulin-induced hypoglycemia in man. Am. J. Physiol. *236*:E380–85, 1979.

2. Clarke, W. L., Santiago, J. V., Thomas, L., Ben-Galim, E., Haymond, M. W., and Cryer, P. E.: Adrenergic mechanisms in recovery from hypoglycemia in man: adrenergic blockade. Am. J. Physiol. *236*:E147–52, 1979.

3. Rizza, R. A., Cryer, P. E., and Gerich, J. E.: Role of glucagon, catecholamines, and growth hormone in human glucose counterregulation. J. Clin. Invest. *64*:62–71, 1979.

4. Gerich, J. E., Langlois, M., Noacco, C., Karam, J. H., and Forsham, P. H.: Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. Science *182*:171–73, 1973.

5. Benson, J. W., Johnson, D. G., Palmer, J. P., Werner, P. L., and Ensinck, J. W.: Glucagon and catecholamine secretion during hypoglycemia in normal and diabetic man. J. Clin. Endocrinol. Metab. *44*:459–64, 1977.

6. Maher, T. D., Tanenberg, R. J., Greenberg, B. Z., Hoffman, J. E., Doe, R. P., and Goetz, F. C.: Lack of glucagon response to hypoglycemia in diabetic autonomic neuropathy. Diabetes *26*:196–200, 1977.

7. Hilsted, J., Galbo, H., and Christensen, N. J.: Impaired responses of catecholamines, growth hormone, and cortisol to graded exercise in diabetic autonomic neuropathy. Diabetes *29*:257–62, 1980.

8. Christensen, N. J.: Plasma catecholamines in long-term diabetics with and without neuropathy and in hypophysectomized subjects. J. Clin. Invest. *51*:779–87, 1972.

9. Lloyd-Mostyn, R. H., and Oram, S.: Modification by propranolol of cardivoascular effects of insulin-induced hypoglycemia. Lancet *1*:1213–15, 1975.

10. Mathias, C. J., Frankel, H. L., Turner, R. C., and Christensen, N. J.: Physiological responses to insulin hypoglycaemia in spinal man. Paraplegia *17*:319–26, 1979–80.

11. Molnar, G. W., and Read, R. C.: Hypoglycemia and body temperature. JAMA *227*:916–21, 1974.

12. Keen, H.: Autonomic neuropathy in diabetes mellitus. The Postgraduate Medical J. *35*:272–80, 1959.

13. Hilsted, J., and Jensen, S. B.: A simple method for determination of autonomic neuropathy in juvenile diabetes. Acta Med. Scand. *205*:385–87, 1979.

14. Steiness, I.: Diabetic neuropathy. Vibration sense and abnormal tendon reflexes in diabetics. Acta Med. Scand. Suppl. *173*:394–413, 1963.

15. Faber, O. K., and Binder, C.: C-peptide response to glucagon: a test for residual beta-cell function in diabetes mellitus. Diabetes *26*:605–10, 1977.

16. Engelman, K., and Portnoy, B.: A sensitive double-isotope derivative assay for norepinephrine and epinephrine. Circ. Res, *26*:53–57, 1970.

17. Christensen, N. J.: Plasma noradrenaline and adrenaline in patients with thyrotoxicosis and myxedema. Clin. Sci. *45*:163–71, 1973.

18. Heding, L. G.: Radioimmunological determination of pancreatic and gut glucagon in plasma. Diabetologia *7*:10–15, 1971.

19. Kehlet, H., Binder, C., and Engbaek, C.: Cortisol binding capacity in plasma during anaesthesia and surgery. Acta Endocrinol. *75*:119–24, 1974.

20. Heding, L. G., and Kasperska-Czyzykowa, T.: C-peptide and proinsulin after oral glucose. Acta Med. Scand. Suppl. *639*:33–36, 1980.

21. Nakagawa, S., Nakayama, H., Sasaki, T., Yoshino, K., Yu, U. Y., Shinozaki, K., Aoki, S., and Mashimo, K.: A simple method for the determination of serum free insulin levels in insulin-treated patients. Diabetes *22*:590–600, 1973.

22. Christiansen, A. H.: Radioimmunoelectrophoresis in the determination of insulin binding to IgG. Methodological studies. Horm. Metab. Res. *5*:747–54, 1973.

23. Schmidt, F. H.: Die enzymatische Bestimmung von Glucose und Fructose nebeneinander. Klin. Wochenschr. *39*:1244–47, 1961.

24. Bergmeyer, H. U.: Methods in Enzymatic Analysis. New York and London, Academic Press, 1974.

25. Novak, M.: Colorimetric ultramicro method for the determination of free fatty acids. J. Lipid. *6*:431, 1965.

26. Berger, M., Berchtold, P., Cüppers, H. J., Drost, H., Kley, H. K., Müller, W. A., Wiegelmann, W., Zimmermann-Telschow, H., Gries, F. A., Krüskemper, H. L., and Zimmerman, H.: Metabolic and hormonal effects of muscular exercise in juvenile type diabetics. Diabetologia *13*:355–65, 1977.

27. DeFronzo, R. A., Hendler, R., and Christensen, N.: Stimulation of counterregulatory hormonal responses in diabetic man by a fall in glucose concentration. Diabetes *29*:125–31, 1980.

28. Landsberg, L. (Ed.): Catecholamines. Clin. Endocrinol. Metab. 612–17, 1977.

29. Schalch, D. S., and Kipnis, D. M.: The impairment of carbohydrate

tolerance by elevated plasma free fatty acids. J. Clin. Invest. *43*:1283–84, 1964.

[30] Christensen, N. J.: Catecholamines and diabetes mellitus. Diabetologia *16*:211–24, 1979.

[31] Shamoon, H., Hendler, R., and Sherwin, R. S.: Altered responsiveness to cortisol, epinephrine, and glucagon in insulin-infused juvenile-onset diabetics. Diabetes *29*:284–91, 1980.

[32] Sestoft, L., Trap-Jensen, J., Lyngsøe, J., Clausen, J. P., Holst, J. J., Nielsen, S. L., Rehfeld, J. F., and Schaffalitzky de Muckadell, O.: Regulation of gluconeogenesis and ketogenesis during rest and exercise in diabetic subjects and normal men. Clin. Sci. Mol. Med. *53*:411–18, 1977.

[33] Schade, D. S., and Eaton, R. P.: The regulation of plasma ketone body concentration by counter-regulatory hormones in man. Diabetes *28*:5–10, 1979.

[34] Christensen, N. J., Alberti, K. G. M. M., and Brandsborg, O.: Plasma catecholamines and blood substrate concentrations: studies in insulin-induced hypoglycaemia and after adrenaline infusions. Eur. J. Clin. Invest. *5*:415–23, 1975.