Studies on Plasma Glucagon Concentration in Maturity-Onset Diabetics with Autonomic Neuropathy

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SUMMARY

The mean fasting plasma immunoreactive glucagon (IRG) concentration in 25 non-insulin-dependent, maturity-onset diabetics (MODs) was 15.4 ± 3.1 pmol/L, which was not significantly different from the 16.8 ± 2.4 pmol/L in 17 normal controls of similar age. However, in seven MODs with autonomic neuropathy (AN) the mean fasting IRG concentration of 44.7 ± 4.4 was significantly greater than the 17.4 ± 1.3 pmol/L in 18 MODs without neuropathy and compared with controls. We, therefore, investigated IRG concentrations in five age-matched, non-insulin-dependent MODs with AN and five without it. Plasma IRG concentrations were measured in the basal state on three separate occasions and in response to insulin and to a mixed meal. Fasting or basal IRG concentrations were measured in the morning with and without a preceding 12-h insulin infusion to determine the effects of reduction in fasting plasma glucose concentrations and after 12 h of nasogastric aspiration to exclude continued stimulation caused by delayed gastric emptying. In the select group of subjects the mean basal plasma IRG concentration was raised above the normal in diabetics with AN (49.4 ± 5.1) and was 16.2 ± 2.1 in diabetics without AN, which was within normal limits. Neither 12 h of insulin infusion (0.5—0.8 U/h) nor 12 h of nasogastric aspiration altered basal IRG concentrations in either group. The maximum incremental IRG response to acute insulin administration (0.2 U/kg) in diabetics with AN was 6.8 ± 3.9, which was significantly impaired compared with 24.9 ± 4.8 in nine MODs without AN and with 26.3 ± 6.7 in five normal controls. The maximum increment in IRG response to acute insulin administration in diabetics with AN rose significantly to 32.8 ± 12.9 after 12 h of insulin infusion. Although the maximum IRG responses to a mixed meal were higher in both diabetic groups than in the normal controls, there was a delayed initial rise and persistent elevation in diabetics with AN. We suggest that (1) basal IRG concentrations may be dependent on the integrity of the autonomic nervous system in MODs; (2) MODs without AN seem to have a normal response to insulin-induced hypoglycemia, whereas in MODs with AN the response is impaired and can be corrected by an adequate degree of both insulinization and reduction in fasting blood glucose concentration; and (3) the maximum IRG response to a mixed meal is exaggerated in all MODs and is not dependent on the integrity of the autonomic nervous system, although the pattern of response may be altered in the presence of AN. DIABETES 28:1015–1021, November 1979.

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There is controversy as to the role of glucagon in the pathogenesis of the hyperglycemia of diabetes.[1–6] Fasting hyperglucagonemia,[7–14] both absolute and relative to the blood glucose concentration, has been found in all forms of diabetes mellitus in man. Unger et al.[15] originally suggested that the alpha cell dysfunction in diabetes may be caused, among other possibilities, by a genetic defect, which resulted in a loss of the glucose-sensing ability of the pancreatic alpha cell. We have shown, however, that patients with acquired diabetes secondary to pancreatitis also have inappropriately elevated fasting plasma levels of immunoreactive glucagon (IRG) for the prevailing glucose levels and, thus, that the defect can be acquired.[13,14] Weir et al.[16] suggested that the alpha cell dysfunction in juvenile-onset diabetics (JODs) may be caused by a paracrine defect with failure of intraislet insulin to gain access to alpha cells. Unger et al.[17] have shown that injections of supraphysiologic amounts of insulin restored the IRG responses to arginine, a protein meal, and a glucose meal to normal, which suggests that the unrestrained alpha cell secretion in JODs may be a result of insulin lack within pancreatic islets.

In contrast, in mild maturity-onset diabetics (MODs), despite often raised levels of endogenous insulin, IRG con-

TABLE 1
Clinical data of the patients studied

Name	Age (yr)	Weight (kg)	Height (cm)	% Standard weight	Duration of treatment (yr)	Agent used	Postural hypo-tension	R–R vari-ation	Sus-tained hand-grip	Val-salva's maneu-ver	Heart rate on standing	Testicular sensation	Sweating	Acids
	Diabetics with AN													
WR	45	91	179	110	6	Phenformin + Chlorpropamide	N	A	N	N	A	A	A	A
RB	52	76	164	110	2	Diet	N	A	N	A	A	N	A	N
CT	45	56	161	85	6	Metformin	N	A	A	A	A	N	N	N
SH	51	75	167	105	5	Chlorpropamide + Phenformin	N	A	N	A	A	N	N	A
CL	63	84	180	104	4	Tolbutamide	N	N	N	N	A	A	N	A
Mean	51.2	76.4	170.2	102.8	5.5									
SEM	3.3	5.9	3.9	4.6	1.09									
	Diabetics without AN													
JL	46	63	172	85	5	Chlorpropamide	N	N	N	N	N	N	N	N
DG	35	65	165	96	5	Chlorpropamide	N	N	N	N	N	N	N	N
PR	58	63	164	91	2	Glibenclamide	N	N	N	N	N	N	N	N
WN	54	70	171	95	10	Metformin + Glibenclamide	N	N	N	N	N	N	N	N
WG	49	69	167	99	3	Diet	N	N	N	N	N	N	N	N
Mean	48.4	66	167.8	93.2	5									
SEM	3.9	1.5	1.6	2.4	1.38									

N = normal; A = abnormal.
R–R variation = variation in heart rate during deep breathing.
Acids = gastric acid secretory response to insulin-hypoglycemia.
Standard weight = average weight of same-sexed individuals of the same age and height is derived from the Society of Actuaries, Build and Blood Pressure Study, Chicago, 1959.

centrations are inappropriately raised for the prevailing glucose level,[15] suggesting that other factors may be important in the regulation of IRG secretion.

In man, the autonomic nervous system has been implicated in the regulation of glucagon secretion.[18–21] We, therefore, investigated the IRG status in MODs with and without autonomic neuropathy (AN). Our findings suggest that fasting IRG concentrations are above normal in the presence of AN, although, in all forms of non-insulin-dependent MODs, IRG levels may be within normal limits, but they are raised relative to the fasting glucose concentration.

PATIENTS AND METHODS

Non-insulin-dependent MODs, attending Groote Schuur Hospital's Diabetes Clinic, were questioned for symptoms and examined for signs of autonomic dysfunction. AN was clinically diagnosed if three or more of the following acknowledged tests of autonomic function were abnormal: (1) a fall in systolic blood pressure of greater than 30 mm Hg immediately on assuming the erect from the supine position; (2) a variation in heart rate of less than 10 beats per minute between deep inspiration and expiration;[22] (3) a rise in diastolic blood pressure of less than 10 mm Hg in response to sustained handgrip at 30% maximum voluntary contraction using a handgrip dynamomanometer;[23] (4) a ratio of less than 1:1 between the heart rate during and 15 s after Valsalva's maneuver—again, the heart rate was measured by a continuous ECG recording;[24] (5) impaired testicular sensation associated with other evidence of AN but in the absence of other causes, e.g., carcinoma;[25] (6) an abnormal gastric acid response to insulin-induced hypoglycemia (Hollander's criteria)[26] in the presence of a gastric acid secretory response to pentagastrin stimulation;[27] (7) absent or reduced sweat on the lower limbs during hypoglycemia (sweat was detected using quinizarin powder[28]); (8) a ratio of 1 or less of the ECG-recorded R-R interval of the 15th and 30th heartbeat on assuming the standing from lying position.[24]

Twenty-five MODs and 17 normal controls of similar age (35 to 65 yr) were examined. Five age-matched male non-insulin-dependent MODs with AN (mean age, 51.2 ± 3.3 yr) and five non-insulin-dependent MODs without AN (mean age, 50.7 ± 2.9 yr) were selected for detailed studies. The studies were approved by the Hospital Ethics Committee. Informed consent was obtained from all subjects. The clinical data of the patients with and without AN who were selected for detailed studies are enumerated in Table 1.

1. In the pilot study, basal plasma IRG concentrations were estimated in 25 MODs, 7 with and 18 without AN, and in 17 normal healthy people.

2. In 14 MODs, 9 without and 5 with AN, and in 5 normal subjects after a 12-h overnight fast, an indwelling cannula was placed in an antecubital vein. After half an hour at rest, an intravenous bolus of 0.2 U/kg (0.1 U/kg in normal subjects) Actrapid monocomponent insulin (Novo Laboratories Industries, Ltd.) was given. Blood was sampled immediately before and at 20, 40, 60, and 90 min after the injection.

3. In an attempt to attain normalization of fasting plasma glucose levels, the 10 selected MODs (Table 1) were admitted to a medical ward; an indwelling cannula was inserted into an antecubital vein, and a 12-h continuous insulin infusion by means of a Harvard infusion pump was begun. Patients were also fasted overnight. An indwelling cannula for blood sampling was inserted into a contralateral antecubital vein. Blood glucose estimations were done throughout the night using Dextrostix and the Eyetone reflectance meter,*

* Kindly loaned by Ames Company.

and the insulin infusion rate was altered accordingly. The insulin infusion rate was 0.5–0.8 U/h. In the morning, three basal blood samples were taken at intervals for 30 min during the insulin infusion, then the infusion was stopped, and 0.2 U/kg insulin was given as an intravenous bolus. Blood samples were again taken 20, 40, 60, and 90 min after the insulin injection.

4. Because there existed the possibility of continuous stimulation of glucagon release by delayed gastric emptying, each of the 10 selected patients had a nasogastric tube passed 5 h postprandially on the second night of their stay in the hospital. The correct position was confirmed by radiologic screening. Two hourly aspirations were done during the night to ensure complete gastric emptying. In the morning the nasogastric tube was removed and basal samples were taken. Then a mixed breakfast, consisting of two boiled eggs, two slices of bread, 20 g butter, 20 g honey, 250 ml milk, and 30 g skim milk and containing 36.4 g protein, 43 g fat, and 72 g carbohydrate, was eaten in 10 min. Blood was collected once during the meal and at regular intervals for 240 min thereafter.

Laboratory methods. In all studies the 5-ml blood samples for plasma glucose and glucagon estimation were immediately placed in heparinized tubes also containing 0.5 ml (5000 KIU) Aprotinin (Trasylol, Bayer), centrifuged, and the plasma was aliquoted. The blood for insulin estimation was allowed to clot and the serum was separated by centrifugation. All aliquots were stored at −20°C until the relevant estimations were done. Plasma glucose was estimated by the ferricyanide method[29] using the Technicon AutoAnalyzer. Plasma levels of immunoreactive glucagon using Unger's 30K antiserum was assayed by the method previously described.[13] Serum insulin was measured using the Amersham kit.

Statistics. The results are expressed as mean ± SEM. Significant differences in plasma glucose, glucagon, and serum insulin concentrations were analyzed using the Mann-Whitney *U* test for unpaired samples and the Wilcoxon test for paired samples. A value of $P < 0.05$ was regarded as statistically significant.

RESULTS

The mean fasting plasma IRG concentration was 15.4 ± 3.1 pmol/L in the 25 MODs and 16.8 ± 2.4 pmol/L in 17 healthy subjects. The differences are not significant. In the seven MODs with AN, the fasting plasma IRG concentration was 44.7 ± 4.4 pmol/L, which was significantly higher than the level of 17.4 ± 1.3 pmol/L in the 18 diabetics without AN. The mean maximum increment in IRG concentration in response to insulin-induced hypoglycemia in 14 MODs was 29.1 ± 4.2 pmol/L, which was not significantly different from the 26.3 ± 6.7 pmol/L in normal controls. However, when divided into MODs with and without AN, the mean maximum increment in MODs with AN was 6.8 ± 3.9, which was significantly less than the 24.9 ± 4.8 pmol/L in MODs without AN and the 26.3 ± 6.7 pmol/L in normal controls.

In the 10 selected subjects the fasting or basal glucose concentrations were raised (>6.6 pmol/L) in both groups of MODs, and the levels before the initial acute administration of insulin and after nasogastric aspiration did not differ significantly from each other. A 12-h insulin infusion lowered the mean glucose concentration in both groups, but the fall was not significant (Table 2). Twelve-hour gastric aspiration had no significant effects on plasma glucose concentrations in diabetics with AN (7.9 ± 1.5–10.1 ± 1.7) or in diabetics without AN (8.1 ± 1.9–7.9 ± 0.5).

The fasting or basal IRG concentration was significantly greater than normal only in the diabetics with AN on all occasions tested (Table 2). Basal IRG concentration (49.4 ±

TABLE 2
Plasma glucose (mmol/L) and glucagon (pmol/L)

	Acute ITT					ITT after 12-h insulin				
	FPG (basal)	PG (nadir)	IRG (basal)	IRG (peak)	IRG (increment)	FPG (basal)	PG (nadir)	IRG (basal)	IRG (peak)	IRG (increment)
Diabetics with AN										
RB	5.6	3.0	29	44	15	5.9	1.5	35	90	55
SH	3.4	1.6	53	66	13	4.5	1.4	51	67	16
CT	10.0	2.9	55	51	−6	5.9	2.8	34	47	13
WR	11.5	2.7	56	55	−1	4.7	1.8	55	63	8
CL	9.2	3.7	54	65	11	6.2	2.3	68	140	72
Mean	7.9‡	2.8‡	49.4†‡	53.6	6.8‡	5.4	2.0*	48.6†‡	82*‡	32.8*
SEM	1.5	0.3	5.1	3.4	3.9	0.3	0.2	6.4	16.3	12.9
Diabetics without AN										
JL	5.8	2.8	21	57	36	4.1	1.4	25	138	113
DG	15.3	8.9	9	15	6	5.3	0.9	12	20	8
PR	4.6	2.4	14	37	23	4.2	1.5	14	56	32
WN	8.3	3.0	19	34	15	5.1	1.7	17	52	35
WG	6.3	3.0	18	47	29	6.2	1.7	26	55	29
Mean	8.1‡	4.0‡	16.2	38	21.8†	5.0	1.5*	18.8	64*	43.4
SEM	1.9	1.2	2.1	7.0	5.2	0.4	0.1	2.8	16.6	18.0
Normal controls										
Mean	4.6	1.7	20.5	46.6	26.3					
SEM	0.52	0.02	5.2	10.9	6.7					

AN = autonomic neuropathy. ITT = insulin tolerance test (0.2 U/kg i.v.). FPG = fasting plasma glucose. IRG = immunoreactive glucagon (30K).

* Significantly different after 12-h insulin infusion.

† Significantly different between groups of diabetics.

‡ Significantly different from normal controls.

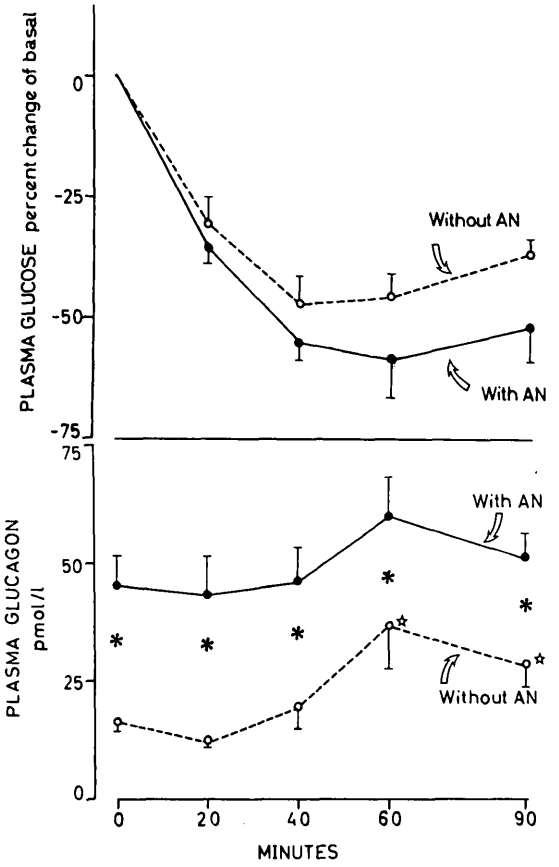

FIGURE 1. Plasma glucose and IRG responses to 0.2 U/kg insulin given at time 0 in diabetics with (N=5) and without (N=5) AN (autonomic neuropathy). Asterisk indicates significant rise above basal and star significant differences between the two groups.

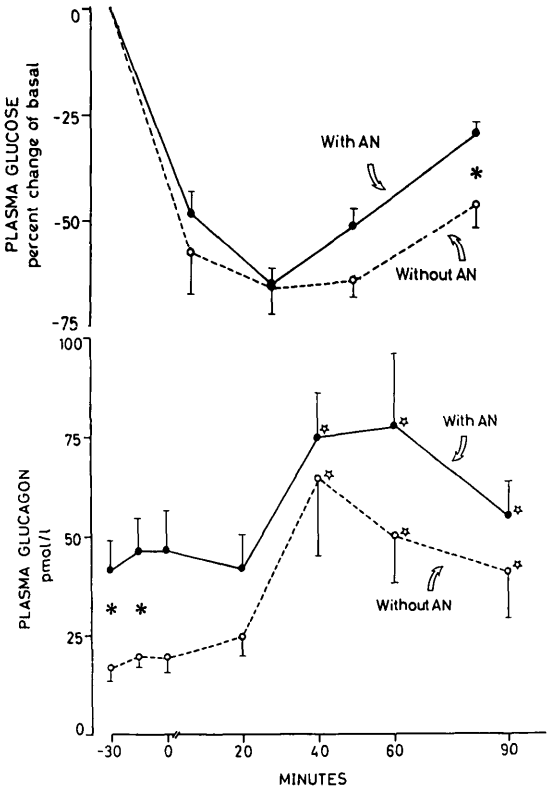

FIGURE 2. Plasma glucose and IRG responses to 0.2 U/kg insulin after a 12-h insulin infusion in diabetics with (N=5) and without (N=5) AN (autonomic neuropathy). Asterisk indicates significant difference between the two groups.

5.1) did not change after 12 h of either insulin infusion (48.6 ± 6.4) or gastric aspiration (50 ± 6.3 pmol/L). In diabetics without AN, the IRG concentration was 16.2 ± 2.1, and 12 h of insulin infusion did not change the mean concentration (18.8 ± 2.8) (Table 2) nor did gastric aspiration (20.8 ± 3.1 pmol/L).

In the 10 selected MODs, glucose responses to insulin injection, expressed as percent change from basal, are shown in Figure 1. Fasting and nadir glucose concentrations were slightly lower in diabetics with AN than in the diabetics without AN (Table 2). In the patients with AN, however, the nadir in glucose concentration occurred later and there was an impaired rise after the initial fall.

With acute administration of insulin (Figure 1), significant rises in plasma IRG concentrations were seen at 60 and 90 min in the patients without AN. In contrast, the patients with AN, though starting at higher levels of IRG, had a poor response to insulin administration. The mean maximum increment in IRG concentration was significantly impaired in MODs with AN compared with MODs without AN, in whom the responses were similar to those of controls (Table 2).

Insulin injection, following the overnight insulin infusion, resulted in a more rapid fall and a greater degree of hypoglycemia in both groups, but, now, in diabetics with AN, blood glucose rose significantly after the nadir (Figure 2). Data for absolute glucose values are given in Table 2.

Despite the fall in fasting plasma glucose, fasting plasma IRG concentrations were similar to those before the initial test (Table 2). After prolonged insulin infusion, an IRG response to acute insulin administration occurred in diabetics with AN, who had a significantly greater increment above basal (Table 2). However, the greater increments after the 12-h insulin infusion appeared to be related to the greater degree of hypoglycemia in individual instances (Table 2). The IRG increment was greater in four of five patients without AN, but this was not significant. However, peak plasma IRG concentrations (Table 2) were significantly greater in both groups compared with those before the prolonged insulin infusion. In both groups (Figure 2), plasma IRG concentrations rose significantly at 40 and 60 min, and, although IRG levels were declining at 90 min, they were still significantly above basal.

The rise in blood glucose concentration following ingestion of the mixed meal was impaired in diabetios with AN, but there were no significant differences in the insulin responses (Figure 3).

In contrast to responses to insulin-induced hypoglycemia, the IRG responses to the mixed meal were exaggerated in both groups of diabetics compared with those in controls, although there was an initial delay and a persistent late elevation in IRG concentration in diabetics with AN (Table 3).

DISCUSSION

There is considerable evidence that the autonomic nervous system plays a regulatory role in glucagon secretion. By electronmicroscopy, cholinergic and, possibly, adrenergic nerve endings on the surface of the alpha cell have been demonstrated.[30,31] The role of the parasympathetic

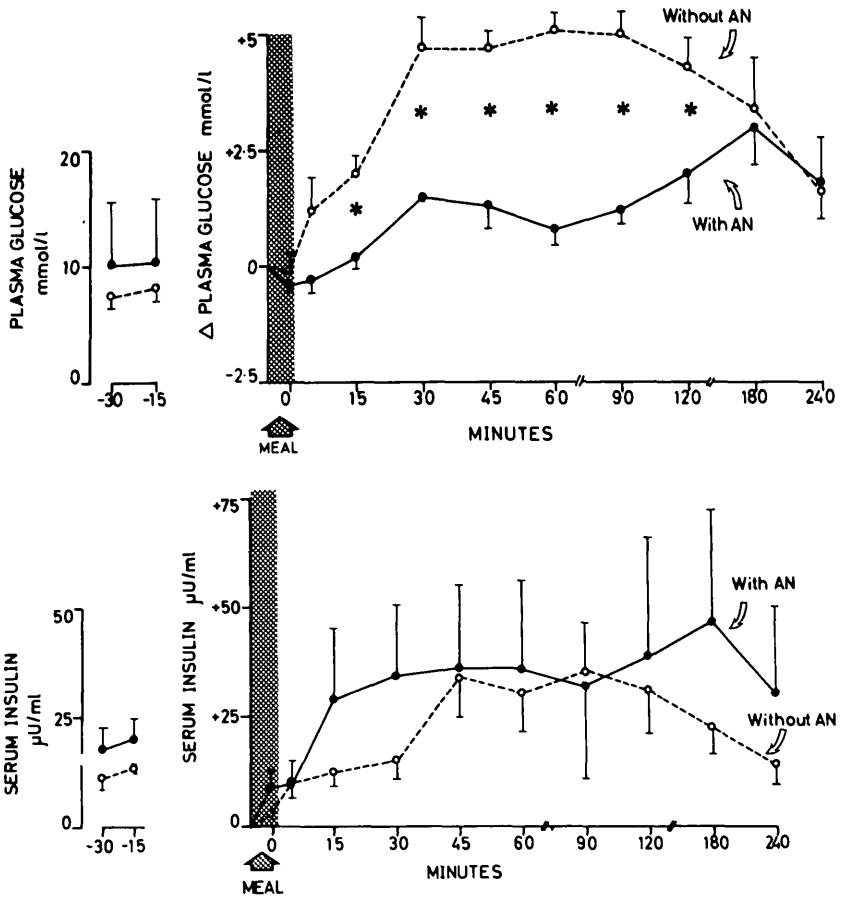

FIGURE 3. Basal plasma glucose (top) and serum insulin (bottom) concentrations and plasma glucose and serum insulin responses to a mixed meal in diabetics with (N=5) and without (N=5) AN (autonomic neuropathy). Asterisk indicates significant differences between two groups.

nervous system in glucagon release has been well demonstrated. In the unconscious calf, electrical stimulation of the peripheral end of the thoracic vagus nerve resulted in a rapid rise in plasma glucagon.[32] Atropine lowered basal glucagon levels in the calf and diminished the glucagon response to insulin-induced hypoglycemia.[32] In man, atropine lowered the basal glucagon levels and reduced the glucagon rise after intravenous arginine,[21] and truncal vagotomy in man significantly impaired the glucagon response to insulin-hypoglycemia, but it did not alter basal glucagon levels.[21] The sympathetic nervous system also plays a role in glucagon secretion. Electrical stimulation of the splanchnic nerves in the unconscious calf produced a rapid rise in plasma glucagon levels.[33] Epinephrine, norepinephrine, and a variety of stresses[34–39] have been shown to stimulate glucagon secretion.

In our study, fasting plasma IRG concentrations were similar in MODs and in normal controls. However, elevated basal IRG concentrations were found in MODs with AN on three separate occasions—in the early morning before the acute administration of insulin, in the early morning before eating a mixed meal, and in the early morning after normalization of fasting plasma glucose by overnight insulin infusion. In all situations, fasting glucose concentrations were similar in the two groups studied. This would seem to suggest that AN in maturity-onset diabetes is associated with elevated absolute basal IRG concentrations. Although IRG concentrations were normal in MODs without AN, they were inappropriately high for the fasting blood glucose level, as has been described in maturity- and juvenile-onset diabetes.[7–14] It has been suggested that the hyperglucagonemia in MODs could result from insulin deficiency, impair-

TABLE 3
Plasma Glucagon (pmol/L) responses to a mixed meal (mean ± SEM)

Time (min)	Basal −30	−15	0	5	15	30	45	60	90	120	180	240
Diabetics with AN	50†‡	54†‡	56.5†	65.7*†	67.4†	75.8†	70.8†	67.8*†	69.4*†	73.3*†‡	77.4*†‡	79.9*†‡
(N = 5)	6.3	6.6	8.7	10.3	10.7	15.1	14.8	9.1	12.3	12.2	17.1	20.3
Diabetics without AN	22.2	23.7	34.3*	48.7*†	49.8*†	44.1*†	45*	38.9*†	35.9*†	32.8*	36.6*	30.9*
(N = 5)	4.7	4.7	8.3	15.5	11.4	10.3	12.7	8.3	5.2	5.4	5.3	5.9
Normal controls	16.8	16.6	17.9	17.5	21.7*	22.5*	21.9*	21.3*	21.9*	22.7*	24.6*	25.1*
(N = 12)	3.0	2.9	4.1	3.9	4.4	5.4	5.2	4.7	4.6	4.2	4.3	4.8

AN = autonomic neuropathy.
* Significant change from basal.
† Significantly different from controls.
‡ Significantly different between groups of diabetics.

ment of the action of insulin, or an abnormality of carbohydrate metabolism in the alpha cell.[11] Our findings may suggest that AN may in some way contribute to the basal alpha cell dysfunction.[12] The raised IRG concentrations may be related, however, to altered clearance and not to hypersecretion, but we have no data to determine which of these possibilities was operative in our patients.

Insulin-induced hypoglycemia activates both the sympathetic[40] and parasympathetic nervous systems[41] and is a potent stimulus for glucagon secretion.[42] Impaired responses have been described in a number of situations in diabetes. Gerich reported that there was no glucagon response to insulin-induced hypoglycemia in six JODs, in whom details of control and the presence of AN were not indicated.[43] Impaired glucagon responses to hypoglycemia produced by insulin infusion were reported by Reynolds[44] in unstable diabetics, and the possibility of AN as a cause was raised but not defined in his patients. Maher et al.[45] found impaired glucagon responses in nine insulin-requiring probable JODs with symptomatic AN, in five of whom diabetes' control was unstable. We studied MODs and observed an impaired glucagon response after the acute administration of insulin in our patients with AN compared with diabetics without AN, who had a twofold increase in IRG levels.

Overnight insulin infusion normalized fasting glucose concentrations, and diabetics with AN responded to acute administration of insulin, which may have been related to a greater degree of hypoglycemia. It would seem that 12 h of insulin administration sensitized the alpha cell to acutely administered insulin by an undefined mechanism, which could be alterations in intracellular metabolism, changes in paracrine secretion, e.g., somatostatin,[17] or modification of receptor availability.[46] The last seems unlikely, as it has been shown in other tissues that insulinization decreases receptor binding[46] of insulin.

In contrast with the impaired responses to acute insulin administration without prior prolonged insulin infusion, we found similar maximum responses to a mixed meal in patients with and without AN that were, nevertheless, exaggerated compared with those of controls. Müller et al.[47] found that abnormal IRG secretion occurred in MODs and JODs after ingestion of either carbohydrate or protein meals, and Gerich et al.[48] reported excessive IRG and glucose responses to mixed meals in insulin-requiring JODs. This would suggest that factors other than the nerve supply are responsible for the IRG responses to the mixed meal. These factors may represent constituents of the meal or gastrointestinal hormones released during the ingestion of fat or protein.

Delayed gastric emptying[49] is a well-known complication of diabetic AN; thus, overnight gastric aspiration was done to prevent a slow "leak" from the stomach in the diabetics with AN that might have resulted in falsely elevated basal IRG levels by continuous stimulation of pancreatic or extrapancreatic IRG. However, basal IRG levels were not altered in either group by the aspiration. This suggests that delayed gastric emptying and retention of food in the stomach is not a major factor in raising fasting glucagon concentrations in the diabetics with AN.

We have found raised fasting IRG concentrations in MODs only in the presence of AN. This was not correctable by prolonged insulin infusion or by gastric aspiration. We suggest, therefore, that undiagnosed defective autonomic innervation may be an underlying pathogenetic factor in absolute fasting hyperglucagonemia in some MODs. Impaired responses to insulin-hypoglycemia are corrected by prolonged insulin infusion and adequate hypoglycemia, which cannot, therefore, be a result of autonomic neuropathy per se. Responses to a mixed meal were unaffected by the presence of neuropathy, suggesting that nonneural mechanisms may mediate the meal-induced IRG release.

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