Lack of Glucagon Response in Glucose Counter-Regulation in Type 1 (Insulin-Dependent) Diabetics: Absence of Recovery After Prolonged Optimal Insulin Therapy

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Summary. Mild hypoglycaemia was induced using an artificial pancreas in five normal subjects (from 5.00 ± 0.15 to 2.83 ± 0.15 mmol/l) by infusing 28 mU/m^2 per min soluble insulin for 60 min. Six Type 1 (insulin-dependent) diabetic patients were stabilized for 14 h using an artificial pancreas. They were then rendered hypoglycaemic (from 4.94 ± 0.09 to 2.89 ± 0.11 mmol/l) by infusing 28 mU/m^2 per min plus $16 \pm 3.8 \text{ mU/min}$ insulin for 60 min. Before the study, the diabetic patients were in optimal blood glucose control (mean blood glucose 6.72 ± 0.11 mmol/l over the previous 14–20 days; HbA_{1c} $8.3 \pm 0.1\%$). During the insulin infusion test, blood glucose decrement was slower in the diabetic patients than in the control subjects. The blood glucose nadir was delayed in the diabetics until 75 min compared with 55 min in the control subjects. Blood glucose recovery rate in the diabetic subjects was severely impaired. In Type 1 diabetes, the counter-regulatory hormonal response to insulin induced hypoglycaemia is similar to that of non-diabetics, except for that of glucagon, the blunted response of which is not reversed by prolonged optimisation of blood glucose control. This impaired response of the A cell does not seem to be a consequence of insulin deficiency.

Key words: Catecholamines, cortisol, glucagon, glucose counter-regulation, growth hormone, hypoglycaemia.

induced hypoglycaemia in diabetics are less numerous in the literature and contrasting results have been reported. Glucagon response to hypoglycaemia has generally been found blunted or suppressed in diabetics [5–12]. The response of catecholamines [12], cortisol and growth hormone [5, 8, 12] in diabetics has generally been reported to be similar to that of normal subjects. However, Saccà et al. [9] have described a blunted response of noradrenaline to hypoglycaemia in diabetics, and Campbell et al. [10] a suppressed response of cortisol and growth hormone in 50% of their diabetic patients.

Previous studies have generally investigated diabetics with long-term hyperglycaemia. In these subjects the chronically high blood glucose levels could alter the counter-regulatory mechanisms. A study of insulin-induced hypoglycaemia arising in diabetics with long-term near-normal blood glucose levels might clarify on the real mechanisms of the hormonal counter-regulatory response.

We have investigated the pattern of hormonal counter-regulatory response to a moderate, insulin-induced blood glucose fall in diabetics examined in conditions as similar as possible to those of non-diabetics, i.e. in normoglycaemia. In particular, we have attempted to determine whether the well known blunted response of glucagon to hypoglycaemia in diabetics could be normalised by a previous period of prolonged normoglycaemia.

Subjects and Protocols

Informed written consent was obtained from five normal volunteers and six diabetic subjects.

The normal subjects were aged between 24–32 years (27 ± 1.4 years) and were within 10% of ideal body weight [Metropolitan Life Insurance Tables, 1959]. None had a family history of diabetes or an abnormal glucose tolerance test. No subject was taking medi-

A number of studies have clearly established the primary role of glucagon [1] and catecholamines [2, 3] and the non-essential role of cortisol and growth hormone [3, 4] in counteracting insulin-induced hypoglycaemia in normal subjects. Studies investigating the counter-regulatory hormonal response to insulin-

Table 1. Clinical features of diabetic patients

Case (no.)	Sex	Age (years)	Diabetes duration (days) ^a	% ideal body weight	Body surface area (m ²)	Immuno-reactive C-peptide (ng/ml)		Mean daily plasma glucose (mmol/l)		Total daily insulin dose (units)				Circulating ketoamine-HbA ₁ [22] (%)	
						basal	90 min ^b	a	b	a		b		a	b
										Solu-ble	Lente insulin	Solu-ble	Lente insulin		
1	M	39	1800	95	1.77	0	0.40	7.39 ± 0.72	6.44 ± 0.39	4	20	10	24	8.7	7.8
2	F	36	2730	92	1.60	0	0.40	6.94 ± 0.61	6.56 ± 0.44	10	22	16	30	9.1	8.5
3	M	29	30	88	1.52	0	0.15	8.67 ± 0.39	6.00 ± 0.44	0	10	6	16	9.4	8.5
4	M	18	15	97	1.84	0	0.15	6.11 ± 0.72	6.17 ± 0.50	14	30	16	30	8.4	8.4
5	M	23	420	108	1.51	0	0.25	7.56 ± 0.50	6.56 ± 0.33	12	32	14	32	8.5	7.8
6	M	24	30	96	1.68	0	0.25	8.06 ± 0.50	6.28 ± 0.39	8	20	14	28	9.6	8.2

a = on admission; b = immediately before the insulin infusion test (14–20 days after admission)

^a On admission

^b After a mixed meal

cations at the time of the study nor showed intercurrent illness. A diet with at least 250 g carbohydrate/day was recommended during the week before the study. After overnight fasting, the normal subjects were connected through a double-lumen catheter to an artificial endocrine pancreas (Biostator, Miles Laboratories, Elkhart, Indiana, USA) for continuous blood glucose monitoring. Between 10.30 and 11.00 h an infusion of soluble insulin (Actrapid, Novo Industri, Copenhagen, Denmark) was started and continued at a constant rate (28 mU/m² body surface area/min) for 60 min by Biostator. Blood glucose monitoring was continued up to 120 min. One litre 0.9% saline was infused over 2½ h (from –30 to 120 min) via an indwelling catheter placed in an antecubital vein of the free arm. This access was used for blood sampling for hormone analysis.

The diabetic group consisted of six Type 1 (insulin-dependent) diabetics, the clinical features of whom are summarized in Table 1. No patient had symptoms of peripheral or autonomic neuropathy and in all patients the deep-breathing test [13], motor and sensory nerve conduction velocity and fundoscopic examination were normal (except for case 1, who showed a slight reduction of sensory nerve conduction velocity). No patient was taking medication other than insulin at the time of the study nor showed intercurrent illness. Diabetics admitted to the protocol were selected from patients attending our metabolic unit because they showed good blood glucose control over the period of observation (14–20 days) while on daily multiple insulin injections. Table 2 summarizes the indices of blood glucose control determined over 14–20 days (mean 18 ± 1 days) before the test. During this period venous blood was drawn daily for measurement of plasma glucose whilst fasting, and 22 ± 1.7 times 90 min after meals. Glycaemic profiles (plasma glucose on fasting, 90 min after breakfast, 30 min before and 90 min after lunch and dinner, and at midnight) were carried out once or twice per week (4 ± 0.4 over 14–20 days) along with the measurement of circulating ketoamine-HbA₁. The M-value [14] was calculated as modified by Service et al. [15] week by week from all the blood glucose values of subjects. No symptoms of hypoglycaemia occurred during the fortnight before the test. On the day before the test, Lente insulin (Novo Industri) was omitted, and only soluble insulin was injected before each meal. Patients were connected to the Biostator 1–1½ h after dinner, between 18.30 and 19.00 h on the day before the test. At this time blood glucose was 6.56 ± 0.32 mmol/l (range 5.44–7.39 mmol/l). Blood glucose values were maintained at preselected values (4.44 mmol/l) with the computer based device for insulin infusion. Minute-by-minute infusion rates

were based on blood glucose determinations (4/min by glucose oxidase method) on continuously withdrawn venous blood (50 ml/24 h). The mean blood glucose level during the feedback programme was 5.17 ± 0.02 mmol/l as calculated by a mean of blood glucose levels every 10 min. Total insulin infused over 16 h of feedback was 19 ± 2 U. Insulin infused over the 5 h before the test was 4.9 ± 1.1 U. From 10.30 to 11.00 h of the test-day, the feedback programme was stopped and an insulin infusion at a constant rate for 60 min started. Blood glucose monitoring continued during the insulin infusion and for the next 60 min. The rate of insulin infusion was the same as in normal subjects (28 mU/m² per min) though augmented by a supplementary dose (16 ± 4 mU/min) as calculated from the mean basal infusion rate, which was necessary to keep blood glucose normal over the 5-h period before the test. One litre 0.9% saline was infused over 2½ h from –30 to 120 min as in the control subjects.

Analytical Methods

In all subjects blood was drawn for measurement of glucagon, adrenaline, noradrenaline, growth hormone and cortisol. Plasma glucagon was determined by radioimmunoassay with 30K antiserum [16]. This system can measure 20 pg/ml with greater than 99% confidence. Plasma growth hormone was also determined by radioimmunoassay [17]. Plasma catecholamines were determined by a sensitive and specific fluorimetric method, as previously reported [18]. Plasma cortisol was measured by Mattingly's fluorimetric method [19]. Plasma glucose was determined in diabetics, before the insulin infusion test, by Beckman glucose analyzer (Beckman Instruments, Fullerton, California). During the test blood glucose was measured by Biostator in all normal and diabetic subjects. In normal subjects, insulin was determined according to the method of Yalow and Berson [20], as modified by Herbert et al. [21]. The circulating ketoamine-HbA₁ was determined chromatographically after incubation of red cells in 0.9% saline at 37 °C for 6 h, as recently described [22]. Immuno-reactive C-peptide was determined before and 90 min after a mixed meal (297 Kcal, 19% proteins, 38% lipids, 43% carbohydrate) according to the method of Beischer et al. [23]. The sensitivity of the assay method is 0.15 ng/ml with greater than 99 % confidence.

Table 2. Indices of glucose control of diabetic patients during the 14–20 day period of observation before the insulin infusion test

	Days 1–7		Days 8–14		Days 15–20	
Fasting plasma glucose (mmol/l)	5.67 ± 0.17	(7 ± 0)	5.50 ± 0.17	(7 ± 0)	5.61 ± 0.22	(3.7 ± 0.9)
Post-meal (90 min) plasma glucose (mmol/l)	7.94 ± 0.06	(11 ± 0.6)	7.44 ± 0.11	(7 ± 0.6)	7.44 ± 0.06	(3.8 ± 0.8)
Mean daily plasma glucose from glycaemic profile (mmol/l)	6.89 ± 0.22	(1.7 ± 0.2)	6.67 ± 0.17	(1 ± 0.3)	6.28 ± 0.11	(0.8 ± 0.2)
M-value	10.4 ± 0.7	(23 ± 1)	5.9 ± 0.7	(17 ± 1.3)	5.7 ± 0.3	(10 ± 2.1)
Circulating ketoamine-HbA _{1c} [22] (%)	8.84 ± 0.10	(1.7 ± 0.2)	8.30 ± 0.10	(1 ± 0.3)	8.25 ± 0.10	(0.8 ± 0.2)

Results expressed as mean ± SEM; number of determinations/profiles shown in parentheses

Statistical Methods

We determined the area above or under the curve of blood glucose and counter-regulatory hormones by including the interval between zero time and the time at which basal value were again obtained. An index was calculated as the ratio between the area and the minutes considered. All results are expressed as mean ± SEM. The results of the concentration of blood glucose and all counter-regulatory hormones in normal subjects and in diabetic patients were compared at each time with the mean of the values at –15 and 0 min by the Wilcoxon signed rank sum test [24]. The Wilcoxon rank sum test for group comparison was used to analyse the difference of the indices (ratio of area to minutes) between diabetic and control subjects. Correlations between the index of blood glucose and that of all counter-regulatory hormones were calculated by the method of the least squares [24].

Results

Normal Subjects

Plasma insulin levels ranged from 44 to 51 mU/l (mean 49 ± 1.3 mU/l) during insulin infusion. Blood glucose decreased significantly at 15 min (4.67 ± 0.16 mmol/l) as compared with basal levels (5.00 ± 0.15 mmol/l, $p \leq 0.05$) and reached the nadir at 55 min (2.83 ± 0.15 mmol/l). Blood glucose levels returned to the pre-infusion levels at 105 min (4.83 ± 0.11 mmol/l, $p = \text{NS}$ compared with basal). The rate of decrease (from 0 to 55 min) and increase in blood glucose (from 55 to 105 min) were very close (0.0389 ± 0.0028 versus 0.0400 ± 0.0022 mmol/min, $p = \text{NS}$). A concomitant and significant increase in plasma glucagon (basal 82 ± 7 pg/ml, peak at 75 min 177 ± 12 pg/ml), adrenaline (basal 33 ± 2 pg/ml, peak at 65 min 299 ± 65 pg/ml), noradrenaline (basal 170 ± 6 pg/ml, peak at 55 min 539 ± 77 pg/ml) was evident at 30 min ($p \leq 0.05$ versus basal) and remained statistically significant up to 75 min for adrenaline, and 105 min for noradrenaline and glucagon. The increase in plasma growth hormone (basal 1.0 ± 0.2 ng/ml, peak at 75 min 30.6 ± 7.2 ng/ml) and in plasma cortisol (basal 8.0 ± 1.2 µg/dl, peak at 90 min 21 ± 1.2 µg/dl) were delayed compared with that of glucagon and catecholamines (Fig. 1). A significant correlation was

found between the degree of blood glucose decrement and the increase in glucagon, catecholamines and cortisol, but not growth hormone (Table 3). On the other hand, no correlation was found between the blood glucose level at its nadir and the counter-regulatory hormone peaks.

Fig. 1. Blood glucose and plasma counter-regulatory hormone levels before, during and after a constant IV insulin infusion for 60 min.

Left panel: five normal subjects infused insulin at 28 mU/m^2 per min.

Right panel: six Type 1 diabetic patients infused the same insulin dose as in normal subjects. The pre-test insulin infusion (basal infusion rate by Biostator needed to keep blood glucose normal over the five previous hours, $16 \pm 3.8 \text{ mU/min}$) was continued during the test.

(●: $p \leq 0.05$ versus basal; ○: $p = \text{NS}$ versus basal)

Table 3. Correlation coefficients between the index (calculated as area/min ratio) of blood glucose and of plasma counter-regulatory hormones in five normal subjects and in six diabetic patients during an IV insulin infusion for 60 min

	Normal subjects		Diabetic subjects	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Glucagon	0.88	<0.025	0.28	NS
Adrenaline	0.84	<0.05	0.77	<0.05
Noradrenaline	0.81	<0.05	0.87	<0.01
Growth hormone	0.50	NS	0.68	NS
Cortisol	0.89	<0.025	0.96	<0.0025

NS: not significant

Diabetic Patients

Basal blood glucose levels (4.94 ± 0.09 mmol/l) were not statistically different from those of normal subjects (5.00 ± 0.15 mmol/l, $p = \text{NS}$). Blood glucose decreased significantly at 30 min (4.28 ± 0.21 mmol/l, $p < 0.05$ versus basal) and reached its nadir at 75 min (2.89 ± 0.11 mmol/l), which was not statistically different from that of normal subjects (2.83 ± 0.15 mmol/l). One hour after the end of insulin infusion (at 120 min) blood glucose levels were still significantly lower than the pre-infusion values (3.83 ± 0.18 mmol/l, $p < 0.05$). The rate of blood glucose decrease (from 0 to 75 min) was significantly lower than in normal subjects (0.0272 ± 0.0022 versus 0.0389 ± 0.0028 mmol/min, $p < 0.05$). The rate of blood glucose recovery from the nadir to 120 min was about twofold slower than in normal subjects (0.0206 ± 0.0022 versus 0.0400 ± 0.0022 mmol/min, $p < 0.05$). The index of blood glucose was 9% higher in diabetics than in normal subjects (1.21 ± 0.11 versus 1.09 ± 0.12 mmol l^{-1} min $^{-1}$). Basal values of all counter-regulatory hormones did not differ statistically from those of controls. Catecholamine response was similar to that in controls and was significant at 30 min (adrenaline basal 31 ± 2 pg/ml, peak at 65 min 299 ± 38 pg/ml; noradrenaline basal 166 ± 5 pg/ml, peak at 60 min 524 ± 45 pg/ml). Catecholamine increase closely correlated with blood glucose decrement as in controls (Table 3). The overall glucagon response in the diabetics was blunted and no peak clearly evident. In contrast to normal subjects, in whom the glucagon increase was simultaneous with that of catecholamines at 30 min, glucagon levels in the diabetics at 30, 45, 55, and 60 min were not significantly different from the basal levels. A statistically significant increase in glucagon was observed at 65 min (111 ± 14 versus basal 84 ± 4 pg/ml, $p < 0.05$). However, in contrast to normal subjects, in whom the statistical significance of glucagon increase was persistent up to 105 min (time at which blood glucose returned to ba-

sal values), in diabetics the increase in glucagon was no more significant at 75 and 105 min, despite the nadir of blood glucose at 75 min. In contrast to catecholamines, the index of glucagon was significantly lower in diabetics than in normal subjects (23 ± 2 versus 40 ± 4 pg ml $^{-1}$ min $^{-1}$, $p < 0.05$) and did not correlate with blood glucose decrement (Table 3). The defective glucagon response correlated neither with the duration of diabetes, nor with residual pancreatic B cell function. However, in subjects who had had diabetes for 5 years or more (cases 1 and 2) there was virtually no response, while two cases with duration of diabetes for one month (cases 3 and 6) had a glucagon response peaking at 65 min. The increase in plasma growth hormone (basal 1.3 ± 0.2 ng/ml, peak at 105 min 23.2 ± 6.5 ng/ml) and in plasma cortisol (basal 9 ± 1 µg/dl, peak at 105 min 21 ± 2 µg/dl) were delayed compared with that of catecholamines, as observed in normal subjects (Fig. 1). The indices of cortisol and growth hormone in diabetics were similar to those in normal subjects. Cortisol index, but not growth hormone, significantly correlated with blood glucose decrement, as in normal subjects (Table 3).

Discussion

At present, a growing number of subjects are treated with intensive conventional insulin regimens and artificial devices on a long-term basis. In these subjects blood glucose is often kept very close to the normal range, but the risk of hypoglycaemia may be higher than with less intensive therapeutic regimens. Therefore we have investigated the mechanisms of glucose counter-regulation in diabetics with prolonged normalisation of blood glucose levels.

The overall dose of insulin infused in our normoglycaemic diabetics during the test provoked a slow and moderate decrement in blood glucose which was never below 2.89 mmol/l. This type of study allowed a detailed analysis of the rate of blood glucose decrement and recovery as well as of the counter-regulatory response of different hormones.

Although the insulin infusion was carried out with exactly the same protocol in the normal subjects as in the diabetic patients, the pattern of the blood glucose curve during and after the test was different in the two groups, except for the absolute value of the minimal blood glucose level reached. In the diabetics the blood glucose decrement was slower, the nadir markedly delayed and the blood glucose recovery impaired compared with normal subjects. While the first two of these differences may be explained by the delayed onset of the injected insulin action in diabetics [25, 26] possibly due to circulating anti-insulin antibodies, the third might depend on the different hor-

monal counter-regulatory response to blood glucose decrement observed in diabetics.

In diabetics, the patterns of counter-regulatory hormone response to insulin-induced blood glucose fall were exactly the same as in normal subjects with the exception of glucagon, the response of which was blunted despite the prolonged period of blood glucose optimization.

The significant increase in both noradrenaline and adrenaline observed in the diabetics is fully in line with the report of Santiago et al. [12]. On the other hand, Saccà et al. [9] did not observe any significant increase in noradrenaline during a fall of blood glucose from 12.33 ± 0.39 to 3.28 ± 0.28 mmol/l (rate 0.06 mmol/min), while the increase in adrenaline was significant. The adrenaline and noradrenaline response in our diabetic patients was very similar to that of the normal subjects and closely correlated with blood glucose decrement, suggesting that both catecholamines equally and significantly respond to hypoglycaemia in diabetics. The response of cortisol and growth hormone was delayed compared with that of catecholamines and was similar in diabetics and normal subjects, as reported in the majority of previous studies [5, 8, 12, 27, 28].

Glucagon response to insulin-induced blood glucose fall was significantly impaired in all our diabetics. This defective A cell response, first observed by Gerich et al. [5], has been reported in all the studies investigating diabetics with long-term high blood glucose levels [8, 9, 11]. A similar blunted glucagon response to hypoglycaemia has been reported by Ensinck and Kanter [29] in four Type 1 diabetics 24h after glucoregulation by Biostator, and by Ohneda et al. [30] in seven diabetics investigated after conventional treatment. In our diabetics, despite the prolonged period of previous blood glucose optimization, the A cell response to the insulin-induced blood glucose decrement did not approach the normal range, but remained clearly blunted. This suggests that the defective glucagon release to hypoglycaemia in diabetics is not merely the consequence of insulin deficiency. It is well known, on the other hand, that the diabetic A cell is characterized by another abnormality, i.e. the sustained glucagon secretion which is inappropriate to the ambient glucose concentration and to IV arginine [31]. This defect may be reversed in part or in total by short- or long-term appropriate insulin treatment [32–34]. Therefore, in contrast to the above-mentioned impaired glucagon response to hypoglycaemia, it is generally accepted that hyperglucagonaemia, as well as other hormone [35] and metabolite [36] abnormalities in diabetes mellitus, is a secondary event closely related to insulin deficiency.

Although there was no correlation either with diabetes duration or with residual pancreatic B cell func-

tion, the A cell abnormality was more pronounced in diabetics of long standing, suggesting that it may appear soon after the onset of diabetes and progress during the course of the illness. At present, the cause of the impaired glucagon response to hypoglycaemia in diabetics is unknown. Initially, Gerich et al. [5] suggested a selective impairment of the A cell glucoreceptor in Type I diabetes. The later hypothesis of autonomic neuropathy [7, 8] affecting the control of glucagon release in diabetics seems no longer tenable after the definitive demonstration that glucagon response to hypoglycaemia is independent of vagal [37] and adrenergic control [38–40]. Very recently, however, Madsbad et al. [41] have found that the residual glucagon response to hypoglycaemia is greater in Type I diabetics with pancreatic B cell reserve and without autonomic neuropathy compared with other subjects without B cell reserve with or without neuropathy. These data would suggest that a physiological relationship exists between A, B and D cells for a prompt and efficient glucagon response to hypoglycaemia. Moreover, a super-sensitivity of the A cells to the suppressive effects of the insulin administered has been suggested in diabetes (R.H. Unger, personal communication).

In conclusion, the blunted glucagon response in normoglycaemic diabetics, in whom the other counter-regulatory hormones respond normally, could well be the cause of their impaired recovery from hypoglycaemia. However, the possible role of the concomitant prolonged activity of injected insulin cannot be excluded.

Acknowledgements. The authors gratefully acknowledge Dr. John E. Gerich for reviewing the manuscript and for his help in discussing the results obtained. We wish to thank Mrs. N. Pannaccio, Mr. F. Alunni, Mr. F. Cimarelli, Mr. G. Cipiciani, Mr. M. Frattegiani, Mr. R. Pippi for their excellent technical assistance. This work was supported by the Italian National Research Council (C.N.R., grant No. 80.00393.04/115).

Presented in part at the 41st Meeting of the American Diabetes Association (Cincinnati, 14–16 June 1981) and at the 17th Meeting of the European Association for the Study of Diabetes (Amsterdam, 15–18 September 1981).

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Received: 16 March 1981
and in revised form: 5 October 1981

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