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Failure of Indomethacin to Affect Arginine-Induced C-Peptide and Glucagon Release in Insulin-Treated Diabetics

Major Role of Residual B Cell Function in Conditioning the Magnitude of the Blood Glucose Rise After Intravenous Arginine

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Summary. Fourteen insulin-treated diabetics were submitted to an arginine infusion test performed with either 11.7 or 5.85 mg kg^{-1} min^{-1} arginine monohydrochloride infused during 40 min with or without previous oral administration of a low (75 + 50 mg) or a high (75 mg + 3 mg/kg) dose of indomethacin. Blood glucose, plasma non-esterified fatty acids, insulin, C-peptide and glucagon were determined at regular intervals before, during and after the arginine infusion. These parameters were totally unaffected by the two doses of indomethacin both in the basal state and during the arginine infusions at the two loads tested. Eight subjects had a basal C-peptide level above 0.07 pmol/ml and a mean (± SEM) maximal rise of 0.21 ± 0.04 pmol/ml during the arginine infusion, whereas the remaining six patients had virtually zero values throughout the tests. The arginine-induced plasma glucagon rise was similar for the two rates of arginine infusion; the sum of the increments in plasma glucagon averaged 877 ± 120 and 647 ± 92 pg/ml ($p > 0.1$) for the high and low rates of arginine infusion, respectively. The magnitude of the blood glucose rise appeared independent of the amount of arginine infused. Confirming previous reports, we found that the blood glucose rise after arginine was three to four times higher in subjects without C-peptide than in subjects with C-peptide. The mean glucagon response did not differ significantly between subjects with or without C-peptide. Thus, residual B cell function determines the magnitude of the blood glucose rise but not the glucagon response after intravenous arginine.

Key words: Insulin-treated diabetics, intravenous arginine, insulin, glucagon, C-peptide, indomethacin, prostaglandins.

Exogenous prostaglandins stimulate glucagon secretion *in vitro* [13, 25] and *in vivo* in animals [27] and in man [8]. Moreover, previous studies from our laboratory using isolated guinea-pig islets incubated *in vitro* support the view that intra-insular synthesis of prostaglandins is involved in the stimulus-secretion coupling of pancreatic A cells [17–21].

Since suppression of glucagon secretion may be considered as a therapeutic goal of diabetic management, at least under certain conditions [14], it is of interest to investigate the possible effect of inhibitors of prostaglandin synthesis in diabetic subjects. The present work was therefore designed to investigate arginine-induced glucagon secretion in insulin-treated diabetic subjects before and after the administration of moderate or high doses of indomethacin. The influence of indomethacin on residual insulin secretion was also assessed by measuring C-peptide plasma levels during the arginine infusions. Indeed, Robertson and his group [2, 22, 26] have reported that blocking the synthesis of prostaglandins by sodium salicylate intravenous infusion improved the insulin response to glucose in Type 2 (insulin independent) diabetics. In addition we attempted to correlate the magnitude of the blood glucose rise induced by arginine infusion with the insulin-secretory reserve and the amplitude of the glucagon rise.

Subjects and Methods

Subjects

After approval of the protocol by the University of Liège Human Investigation Ethical Committee, informed consent was obtained from 14 insulin-treated diabetics. Relevant clinical data are presented in Table 1. All subjects were admitted to the metabolic ward several days before the investigation in order either to initiate insulin therapy, to change the insulin regimen or to improve the degree of control. All studies were performed supine at

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Table 1. Clinical characteristics of the patients studied

	Patient	Sex	Age (years)	Height (cm)	Ideal body weight (%)	Duration of diabetes (years)	Duration of insulin therapy (years)	Ketonuria[a]	Insulin[b] dose (U/day)
Group 1	1	M	54	170	119	16	3	0	15
	2	M	41	164	95	W	D	0	28
	3	M	63	173	96	14	D	0	42
	4	F	44	170	126	15	15	+	72
	5	F	26	155	112	21	21	+	58
	6	M	42	177	87	17	17	+	52
Group 2	7	M	20	180	91	W	D	0	56
	8	M	25	171	83	W	D	0	26
	9	M	20	184	83	3	D	+	44
	10	M	54	169	86	7	D	+	42
	11	M	61	176	82	W	D	+	66
	12	M	24	176	101	16	16	+	60
	13	F	28	160	112	18	18	+	56
	14	F	26	164	89	12	12	+	58

W: A few weeks D: A few days (6–10)
[a] History of ketosis in the absence of adequate insulin therapy
[b] All patients but one (patient 9) received two insulin injections per day

08.00 h after a 12 h overnight fast. Insulin therapy was omitted on the morning of the test; thus, the last insulin injection was given 26 or 14 h before the test, depending on the frequency of insulin therapy.

Tests

Two groups of diabetic patients were studied. Group 1 comprised six patients who were submitted twice to an arginine infusion of 11.7 mg kg^{-1} min^{-1} via an antecubital vein with the aid of a peristaltic pump over a period of 40 min. The first test was performed under basal conditions and the second when the patients had received 25 mg indomethacin orally every 8 h the day before and 50 mg indomethacin 2 h before starting the arginine infusion.

Group 2 comprised eight other patients who were submitted twice to an arginine infusion of 5.85 mg kg^{-1} min^{-1} administered over a period of 40 min. Here again, the first test was performed under basal conditions and the second after indomethacin administration. This group of patients received 3 × 25 mg indomethacin the day before, 2 mg/kg body weight of indomethacin 2 h before and 1 mg/kg indomethacin immediately before starting the arginine infusion. Blood samples were withdrawn from the opposite antecubital vein before (−30, −20 and zero min), during (5, 10, 20, 30, 40 min) and after (55, 70 and 90 min) the arginine infusion.

Measurements

Blood glucose was measured by the hexokinase method adapted to the Technicon autoanalyzer. Plasma non-esterified fatty acids (NEFA) were estimated according to Dole and Meinertz [4]. Plasma insulin was measured [10] in subjects treated with insulin for less than 10 days and in those with no detectable circulating anti-insulin antibodies, determined by the method of Christiansen [3]. C-peptide was determined by the method of Heding [11] after precipitation of proinsulin bound to insulin antibodies with 2 ml of 95% ethanol added to 0.5 ml of plasma. The sensitivity of the assay was 0.03 pmol/ml of plasma. Plasma glucagon was determined according to Luyckx [16] using 30 K Unger's antiserum and ^{125}I-glucagon obtained from New England Nuclear (Boston) as tracer. Plasma indomethacin was assayed by high performance liquid chromatography [29].

Statistical Analysis

Statistical analysis was performed using the paired and unpaired Student's *t* tests. Regression lines and correlation coefficients were calculated using standard programmes on a Hewlett Packard desk calculator 9810 A.

Results

Basal Values

Basal levels of blood glucose, plasma NEFA and glucagon before and after indomethacin administration are depicted in Table 2. Blood glucose and plasma NEFA were similarly increased in both groups and were not significantly influenced by the dose of indomethacin. Basal plasma levels of glucagon were in the normal range for our assay method [15, 16] and not significantly affected by indomethacin administration. Eight of the 14 patients (three from group 1 and five from group 2) had a basal C-peptide level above 0.07 pmol/ml. C-peptide was undetectable in the plasma of the remaining patients both in the basal state and during the arginine infusion tests. As shown in Table 3, the mean (± SEM) basal C-peptide levels in these eight patients averaged 0.21 ± 0.04 pmol/ml before and 0.20 ± 0.05 pmol/ml after indomethacin (NS).

Table 2. Basal values of blood glucose, plasma NEFA and glucagon in two groups of insulin-treated diabetics before and after indomethacin administration. Results are expressed as mean ± SEM ($n = 6$ in both groups)

	Group 1 Indomethacin low dose ($n = 6$)		Group 2 Indomethacin high dose ($n = 6$[a])	
	Before	After	Before	After
Blood glucose (mmol/l)	14.9±1.8	15.2±2.7	15.3±1.1	16.5±1.3
Plasma NEFA (μmol/l)	1108±135	1215±118	1266±166	1176±117
Plasma glucagon (pg/ml)	78±41	102±39	64±26	68±29

[a] Only six out of the eight patients are included in this table.
Patient 13 was tested only under control conditions and did not undergo the second test. Patient 14 was not included because of unusually high plasma glucagon levels in the basal state (300–600 pg/ml) due to the presence of big plasma glucagon

Table 3. C-peptide and insulin levels in response to arginine infusion before and after indomethacin administration

	C-Peptide (pmol/ml)						Insulin (mU/l)	
	Basal level[c]		Responses to Arginine					
			Maximal increase		Sum of increments 0–90 min		Maximal increase in plasma insulin	
	Before Indomethacin	After Indomethacin	Before Indomethacin	After Indomethacin	Before Indomethacin	After Indomethacin	Before Indomethacin	After
Group 1								
1	0.40	0.35	0.25	0.16	1.22	0.71	6.1	5.5
2	0.16	0.12	0.15	0.29	0.61	1.67	5.6	8.6
3	0.32	0.50	0.14	0.68	0.45	2.20	8.0	6.5
Group 2								
7	0.14	0.12	0.18	0.17	0.67	0.86	3.6	2.3
8	0.22	0.22	0.28	0.29	1.39	1.24	6.0	6.0
9	0.09	0.04	0.08	0.08	0.51	0.45	0.9	0.8
10	0.15	0.09	0.11	0.18	0.47	0.94	0.6	1.3
11	0.19	0.18	0.25	0.10	1.20	0.58	5.8	4.7
mean[a,b]	0.21	0.20	0.18	0.24	0.82	1.08	4.6	4.5
SEM	0.04	0.05	0.03	0.07	0.14	0.21	0.9	1.0

[a] Patients 4, 5, 6, 12, 13, 14 had no detectable C-peptide levels under basal conditions or during arginine infusion
[b] Since the mean values calculated separately for groups 1 and 2 were not significantly different, the mean of the eight subjects is given
[c] Each value represents the mean of three determinations (−30, −20 and 0 min) before arginine infusion

Arginine Infusion: C-Peptide and Insulin Response

Plasma insulin levels were able to be measured in three subjects in group 1 and five patients in group 2 (Table 3). These eight patients were those who had detectable plasma levels of C-peptide (Table 3). The insulin "reserve" was evaluated in these eight patients by calculating the maximal increase in plasma levels of insulin during the arginine infusion and also by calculating the sum of increments in plasma levels of C-peptide at 5, 10, 20, 30, 45, 55, 70, 90 min of the arginine tests (Σ 0–90).

The maximal plasma insulin response to arginine was 4.6 ± 0.9 mU/l ($n = 8$) without and 4.5 ± 1.0 mU/l after indomethacin (Table 3). A rise in plasma C-peptide levels ranging from 0.08 to 0.28 pmol/ml (mean: 0.18 ± 0.03) was observed in all eight patients in response to arginine; the magnitude of this increase was not significantly influenced by previous administration of indomethacin (mean: 0.24 ± 0.07 pmol/ml; Table 3). The overall C-peptide response to arginine (Σ 0–90) did not differ significantly before and after indomethacin averaging 0.82 ± 0.14 and 1.09 ± 0.21 pmol/l respectively (Table 3).

Arginine Infusion: Dose-Effect Relationship to Plasma Glucagon Response

Patients in group 1 (mean weight 64.6 ± 3.7 kg) received 11.7 mg kg^{-1} min^{-1} of arginine (mean 30.2 g) IV over 40 min. The dose of arginine in group

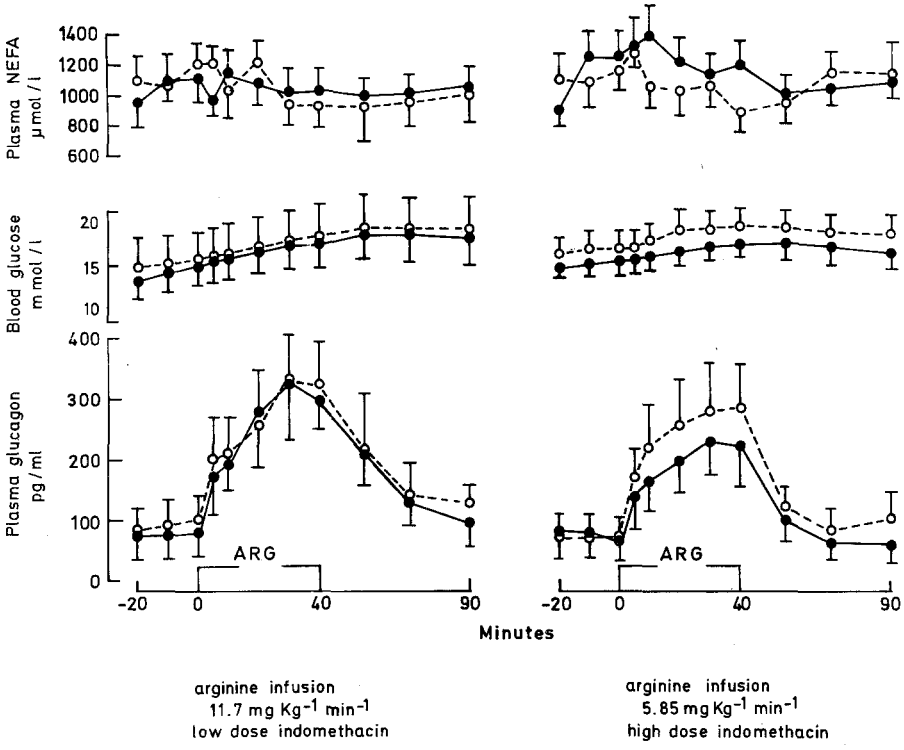

Fig. 1. Influence of arginine infusion on blood glucose, plasma NEFA and glucagon concentrations in two groups of insulin-treated diabetics. Arginine infusion rates (ARG): left panel: 11.7 mg kg^{-1} min^{-1}; right panel: 5.85 mg kg^{-1} min^{-1}. ●—● control test; ○----○ after indomethacin administration. Results are expressed as mean ± SEM ($n = 6$ in both groups)

2 (mean weight 59.8 ± 2.6 kg) was 5.85 mg kg^{-1} min^{-1} (mean 14.0 g) over 40 min.

In group 1, with the larger dose of arginine, plasma glucagon rose from a basal value of 78 ± 41 pg/ml to a maximal level of 324 ± 90 pg/ml at 30 min (Fig. 1). The overall glucagon response expressed as the sum of increments in plasma glucagon at 5, 10, 20, 30 and 40 min (Σ 0–40) averaged 877 ± 120 pg/ml.

In group 2, with the lower dose of arginine, plasma glucagon rose from 64±26 to 237 ± 50 pg/ml at 30 min and 232 ± 62 pg/ml at 40 min (Fig. 1). The mean sum of increments in plasma glucagon in this group of patients was 647 ± 92 pg/ml. Neither the maximal nor the sum of increments in plasma glucagon were significantly different between the two groups, despite the difference in arginine dose.

Arginine Infusion: Lack of Effect of Indomethacin on the Glucagon Response and on Plasma NEFA Concentrations

As illustrated by Figure 1 (left panel, group 1), administration of the low dose of indomethacin did not modify the kinetics or the amplitude of the arginine-induced increase in plasma glucagon. With indomethacin treatment, the overall glucagon response (Σ 0–40) averaged 882 ± 164 pg/ml and was similar to that of the control test (877 ± 120 pg/ml, see above).

In group 2 where the patients received the high dose of indomethacin, the arginine-induced increase in plasma glucagon was slightly but not significantly *higher* than that obtained in the corresponding control tests (Fig. 1, right panel). The values of (0–40) were 916 ± 188 and 647 ± 92 pg/ml respectively (NS). Plasma NEFA concentrations were unaffected by the arginine infusions whether or not indomethacin was previously given (Fig. 1).

Arginine-Induced Blood Glucose Rise: Correlation with C-Peptide Plasma Levels

In both groups of patients, arginine-induced rises in blood glucose were similar before and after indomethacin treatment (Fig. 1). The sum of the increments in blood glucose between 5 and 90 min was calculated. A strongly positive correlation was found between the glucose increments of the two tests performed in each subject ($r = 0.922$ $p < 0.001$). Thus, the rise in blood glucose was not significantly affected by previous indomethacin administration.

The magnitude of the sum of the increments in blood glucose was clearly related to the presence or absence of C-peptide and appeared to be independ-

ent of the amount of arginine infused. Indeed, the mean sum of the increments in blood glucose of subjects with C-peptide was 6.8 ± 1.9 mmol/l (two tests per patient; $n = 6$) in group 1 and 6.8 ± 1.1 mmol/l ($n = 10$) in group 2 patients. A much higher blood glucose increase was recorded in subjects without C-peptide: 22.9 ± 2.8 mmol/l ($n = 10$) in group 1 and 26.3 ± 2.9 mmol/l ($n = 5$) in group 2 patients ($p < 0.005$ versus subjects with C-peptide). No such correlation was found between the sums of increments in plasma glucagon (Σ 0–40, see above) and those in blood glucose ($r = 0.021$, $p > 0.1$). Finally, no correlation was found between arginine-induced increments in glucagon and those in C-peptide. In fact, the mean glucagon response in subjects with C-peptide and those without did not differ significantly: 851 ± 107 and 805 ± 32 pg/ml, respectively.

Plasma Levels of Indomethacin

Plasma concentrations of indomethacin were determined in five patients of the second series, i. e. in the subjects receiving the higher dose of the drug. Samples were taken 120, 60 min and immediately before starting the arginine infusion as well as at the end of the infusion (40 min). Values (nmol/ml) were as follows: −120 min 0.7 ± 0.2; −60 min 12.8 ± 3.9; 0 min 10.0 ± 1.8 and 40 min 17.3 ± 2.5. Thus, the drug given at −120 min and again at 0 min was rapidly absorbed from the gut so that the mean plasma concentration was already 10 nmol/ml at the start of the arginine infusion.

Discussion

The 14 diabetic patients included in the present study were undoubtedly insulin-requiring at the time of the study. Eight of them showed evidence of residual B cell activity, whereas the remaining six did not.

Hendriksen et al. [12] have proposed that a plasma level of C-peptide higher than 0.07 pmol/ml is an index of metabolically significant endogenous insulin reserves and Gonen et al. [9] have shown that patients with fasting C-peptide concentrations above 0.1 pmol/ml have significantly better metabolic control than did those with lower values. In this study of 14 subjects, seven had C-peptide levels above 0.1 pmol/ml, one was between 0.07 and 0.1 pmol/ml (0.085) and six had virtually zero values. The seven latter cases, as well as two subjects with C-peptide levels above 0.1 pmol/ml were ketosis-prone. Such a group of patients appeared suitable for evaluating any possible influence of acute indomethacin administration on the metabolic (blood glucose and plasma NEFA) and hormonal (insulin, C-peptide, glucagon) responses to an arginine infusion. For this purpose, we selected two experimental conditions, (1) a low dose of indomethacin and the usual rate of arginine infusion (11.7 mg kg^{-1} min^{-1}) and (2) a high dose of indomethacin and a 50% lower rate of arginine infusion (5.85 mg kg^{-1} min^{-1}). Blood glucose, plasma NEFA, C-peptide, insulin and glucagon were totally unaffected by the two doses of indomethacin, both in the basal state and during the differing rates of arginine infusion. This observation is in agreement with the results of a similar study performed in healthy volunteers [33] but are in sharp contrast with those of studies *in vitro* which showed that indomethacin abolishes arginine-induced glucagon secretion by isolated guinea-pig islets [18, 21]. A possible explanation for this discrepancy between the studies *in vivo* and *in vitro* is the fact that plasma concentrations of indomethacin in the present study were in the range of 10 nmol/ml and about 90% of the drug in the circulation is bound to plasma albumin and thus biologically unavailable. In contrast, the concentration *in vitro* was higher (100 nmol/ml) and the experiments were conducted in the presence of a very low albumin concentration (2 g/l) in the incubation medium. According to Ekstrand et al. [5] the therapeutic plasma levels of indomethacin are in the range of 0.3 to 3.0 nmol/ml, thus slightly lower than those reached in the patients of group 2 and very likely similar to those reached in the patients of group 1. Moreover, indomethacin not only inhibits cyclo-oxygenase activity and prostaglandin synthesis [32], but also reduces the activity of some enzymes involved in degradation of prostaglandins [24], as well as that of phosphodiesterase [6], the latter effect leading to increased intracellular concentrations of cyclic AMP. The dose-effect relationship for these various actions of indomethacin *in vivo* remains to be determined.

The metabolic role of prostaglandins and of the inhibitors of their synthesis in man remains an unsolved problem. Micossi et al. [23] reported that aspirin (but not ibuprofen or ketoprofen) stimulated insulin and glucagon secretion in normal and diabetic subjects, whereas Giugliano et al. [7] found that acetylsalicylic acid did not modify basal or arginine stimulated glucagon concentrations in insulin-requiring diabetics. In normal subjects, acetylsalicylic acid did not alter glucagon response to glucose, arginine [31] or tolbutamide [30]. Thus, most clinical studies failed to demonstrate an effect of inhibitors of prostaglandin synthesis on glucagon secretion and this contrasts with several clinical investigations dealing with insulin secretion. Chen and Robertson [2] reported that infusion of sodium salicylate in

hyperglycaemic, Type 2 (insulin independent) diabetics restored acute insulin release and augmented the second phase of insulin secretion in response to glucose. Such an effect cannot be considered in our patients with insulin-requiring diabetes and minimal insulin reserves since their C-peptide response to arginine was not improved by indomethacin (present results) or by aspirin [7].

Our results allow interesting conclusions regarding the mechanisms for an arginine-induced increase in blood glucose. It appears that arginine-induced hyperglycaemia is relatively independent of the amount of arginine infused in the dose range 14–30 g. On the contrary, the rise in blood glucose is critically dependent upon the presence or absence of a minimal insulin secretory reserve since, for a given arginine load, the rise in blood glucose was three to four times lower in subjects with C-peptide when compared with diabetics without endogenous insulin secretion. This observation is in agreement with a previous report by Shima et al. [28]. Comparing two groups of insulin-requiring patients submitted to an IV arginine test, these authors found that the arginine-induced rise in blood glucose was greater in patients without any C-peptide response when compared with diabetics who had a significant rise in C-peptide during the test. As in our study, the increase in plasma glucagon was similar in the two groups. However, definite conclusions could not be drawn by these authors who mentioned that their C-peptide assay system was affected by non-specific factors particularly proinsulin. Such interference was not present in our study, since C-peptide determinations were made after an ethanol precipitation step. Finally, our results support the recently reported conclusion that in normal man, the arginine-induced rise in blood glucose results primarily from a glucagon-mediated enhancement of hepatic glucose production and not from an arginine-derived glucose synthesis [1]. However, the fact that, for a given rise in plasma glucagon, the rise in blood glucose is higher in C-peptide deficient patients supports the concept that insulin is a major regulator of glucose production by the liver when arginine is administered.

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