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Inverse relation between amylin and glucagon secretion in healthy and diabetic human subjects

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Abstract

Background The role of amylin, which is cosecreted together with insulin by the pancreatic B-cells, in the pathogenesis of type-2 diabetes is still unclear. To elucidate a possible relation between amylin and glucagon we directly evaluated the respective prehepatic secretions following administration of a 75-g oral glucose load (OGL) in humans.

Materials and methods We studied six healthy controls (C), six obese, insulin resistant subjects (O) and six patients with type 2 diabetes (D). Catheters were placed in the femoral artery and hepatic vein according to the hepatic vein catheterization technique. Splanchnic blood flow was assessed by infusion of indocyanine-green dye. The measured variables were analyzed by a general circulatory model for calculation of prehepatic secretion.

Results The total amount of released glucagon was not different between the respective groups (20·5 ± 2·3 in C, 27·7 ± 5·1 in O and 27·9 ± 5·4 μg/4 h in D). When considered as the difference from the fasting profile, however, glucagon secretion was reduced by 3·5 ± 14% in C, 25 ± 12% in O and increased by 36 ± 21% in D ($P = 0·051$, D vs. C). Amylin secretion was increased in O (1·10 ± 0·15) vs. C (0·63 ± 0·05, $P < 0·05$) and D (0·24 ± 0·10 nmol, $P < 0·01$). Following glucose administration, glucagon secretion significantly inversely correlated with secretion of amylin ($r = –0·6$, $P < 0·01$), but not with that of insulin ($r = –0·23$, $P = 0·36$).

Conclusions The inverse correlation between amylin and glucagon secretion suggests that amylin modulates glucagon secretion following oral glucose administration. This study proves for the first time a role of endogenous amylin in the regulation of glucose homeostasis.

Keywords Amylin, circulatory model, glucagon, OGL, postprandial state.
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Introduction

Glucose homeostasis is regulated by balanced interplay between the absorption of glucose from the gut, splanchnic glucose uptake, secretion of pancreatic B-cell hormones and the uptake of glucose in target cells. In healthy subjects, following ingestion of carbohydrates, insulin secretion increases while glucagon levels are suppressed, thus promoting uptake of glucose in skeletal muscle and fat cells and decreasing hepatic glucose production [1]. A second B-cell hormone, amylin [2], constitutes the main component of amyloid deposits found in the pancreas of type-2 diabetic patients [3]. It is a 37 amino acid peptide, which is stored and released together with insulin in response to nutrient stimuli [4]. Amylin secretion is increased in parallel with that of insulin in obese, insulin-resistant subjects and is decreased in type 2 diabetic patients [5]. Additionally, amylin slows gastric emptying [6]. As administration of amylin has been shown to decrease food intake in rats [7] it has further been speculated that amylin might act as a satiety factor. Recent evidences from animal experiments on the infusion of amylin as well as results from studies with the amylin agonist pramlintide in patients with type-1 diabetes suggest that amylin influences postprandial glucose levels by decreasing glucagon secretion [8,9]. As the circulating levels of amylin or pramlintide in these studies

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were supra-physiological and not timely related to the secretion of insulin, the role of endogenous amylin on postprandial glucose regulation in man remains a matter of speculation.

The aim of this study was to directly evaluate endogenous secretion of amylin and glucagon following oral glucose administration (OGL) in control and diabetic subjects and to elucidate their dynamic interrelationship. We used the hepatic vein catheterization technique to obtain the secretion of the respective pancreatic hormones, which were exploited by a circulatory model.

Methods

Subjects

Six male nondiabetic (C, age 44·7 ± 4·4 years, BMI 26·5 ± 0·9 kg m^{-2}), six male obese, nondiabetic (O, age 44·7 ± 2·4 years, BMI 35·3 ± 1·2 kg m^{-2}) and six type 2 diabetic subjects (D, five males, one female, age 50·5 ± 5·3 years, BMI 30·8 ± 1·1 kg m^{-2}) participated in the study. In the diabetic subjects, diabetes duration was 7·7 ± 2·7 years, HbA1c was 8·4 ± 0·9%. Any antidiabetic medication (sulphonylureas) was withdrawn 3 weeks before the experiments. The ethnic background of the subjects investigated was Caucasian. All patients had normal renal function, none of the diabetic subjects presented with microalbuminuria. Three of the obese and four of the diabetic subjects were on antihypertensive treatment (ACE-inhibitors, low-dose diuretics). All subjects were admitted 3 days before the respective study to the San Diego VAMC SDTU, and consumed a weight maintenance diet containing 55% carbohydrate, 30% fat and 15% protein. None of the nondiabetic subjects had a positive family history for diabetes or was taking any medication known to affect glucose metabolism. The purpose, nature, and potential risks of the study were explained in detail to all subjects before obtaining their written consent. The study protocol was reviewed and approved by the Human Subjects Committee of the University of California San Diego. All studies were performed at 08:00 h after a 10–12-h overnight fast.

Glucose clamp study

The glucose clamp was performed as described previously under euglycaemic conditions to quantitatively measure glucose uptake [10]. The insulin infusion rate was 120 mU M^{-2} min^{-1} for 3 h, plasma glucose was maintained at the desired euglycemic level throughout the study by monitoring the glucose level at 5-min intervals and adjusting the infusion rate of a 20% dextrose solution using a servo-control negative principle. Thus, plasma glucose and insulin levels were kept constant while the glucose infusion varied and the rate of glucose uptake assessed by the concomitant administration of 3-(^{3}H) glucose served as a direct measurement of insulin effectiveness.

Hepatic vein catheterization and OGL

This technique has been already reported in detail [11], and it is only briefly summarized here. Under local anaesthesia a 5 French Teflon catheter (Hedtronic, Danvers, MA, USA) was introduced into the femoral artery to position the tip at the level of the inferior end of the sacroiliac joint. A 6·5 French polyethylene catheter (Hedtronic) was advanced from the femoral vein via the inferior vena cava into the right-sided hepatic vein. One millilitre of contrast medium was injected to visualize the tip of the catheter and to ensure that it was positioned in an area of adequate blood flow. Hepatic blood flow was estimated by a primed-continuous infusion of indocyanine green [12], the infusion of which was started via an antecubital vein 75 min before glucose ingestion, and was continued throughout the study. Blood was sampled simultaneously from the artery and the hepatic vein at 10-min intervals starting 45 min after the beginning of the green dye infusion. At time zero, the subjects ingested 300 mL of a 75-g glucose solution over 5 min. Arterial and hepatic venous blood was sampled at 15-min intervals to determine the concentration of glucose, C-peptide, glucagon, amylin and the indocyanine green concentration for 4 h after glucose ingestion. Hepatic plasma flow was calculated by dividing the green dye infusion rate by the arteriohepatic venous dye concentration difference. Hepatic blood flow was estimated by dividing hepatic plasma flow by 1 – haematocrit.

Assays

Glucose was measured with an automated glucose analyser (Yellow Springs Instrument Co., Yellow Springs, OH). Insulin was assayed by a double antibody RIA according to the method of [13]. The antibody did not detect IGF-II, recognized IGF-I at ≤ 5% efficiency and had 10–12% cross-reactivity with proinsulin. C-peptide [14], glucagon [15] and amylin [16] were measured as previously described. The amylin antibody did not cross-react with the glycosylated peptides (amylin-like peptides). Indocyanine green was analyzed by spectrophotometer after precipitation with sodium-desoxycholate [17].

Data analysis

Whole body kinetics of the pancreatic products was described with a circulatory model, which includes the main processes involving the liver. In particular, this organ is represented as a compartment with inputs from the portal vein (pancreatic secretion) and the hepatic artery, and output in the hepatic vein. As a second process of possible substrate disappearance from the liver, degradation in the hepatocytes (hepatic extraction) is considered. As the overall system can be assumed in a quasi-steady state given the slow dynamics of the OGL, the mass flux of peptide across the liver was described as the steady-state equation:

$$\text{outflow} = \text{inflow} + \text{secretion} - \text{extraction} \quad (1)$$

This relationship can be applied to all the substances under study: C-peptide, amylin and glucagon. For C-peptide it is known that only a negligible part is degraded in the liver [18], therefore equation 1 becomes:

$$CPv\ HBF = CPa\ HBF + CPS(t) \quad (2)$$

where CPv and CPa are the measured C-peptide time-dependent concentrations in the hepatic vein and in the artery, respectively; HBF is the measured hepatic blood flow, and CPS(t) is the C-peptide secretion rate. The only unknown is CPS(t), which can be thus calculated. As CPS(t) represents also insulin secretion, we refer to both C-peptide and insulin secretion with the same meaning.

Amylin is cosecreted with C-peptide and insulin [4,19], but not equimolarly, thus a constant cosecretion factor is likely during an OGL [20]. It has also been shown that amylin is cleared by the liver to a much lower extent than insulin [21,22], and even a zero hepatic extraction is plausible [23]. Taking these elements into account, equation 1 becomes for amylin:

$$AMv\ HBF = AMa\ HBF + \sigma\ CPS(t) \quad (3)$$

where AMv and AMa are measured amylin concentrations in the hepatic vein and in the artery, respectively, and σ is the amylin/insulin cosecretion factor, the only unknown of equation 3 that allows reconstruction of amylin secretion. Equation 1 becomes for glucagon:

$$GNv\ HBF = GNa\ HBF + GNS(t) - GND(t) \quad (4)$$

where GNv and GNa are measured glucagon concentrations in the hepatic vein and in the artery, respectively. In this formula there are two unknowns: GNS(t), glucagon secretion and GND(t), glucagon degradation in the liver. Equation 4 cannot be solved unless there exists another relationship between the unknowns. From other studies it is known that liver degradation of glucagon is one-fourth of the hormone entering the liver [24], thus:

$$GND(t) = 0{\cdot}25\ [GNa\ HBF + GNS(t)] \quad (5)$$

which, after substitution in equation 4, yields:

$$GNS(t) = HBF\ [(GNv/0{\cdot}75) - GNa] \quad (6)$$

allowing the calculation of glucagon secretion. Systemic clearances of the three compounds were calculated as the ratio of the time integral of the secretion rate to that of the concentration in the artery, which is equal to the mixed venous blood concentration. This procedure has been previously detailed [11].

Calculations and statistical analysis

Areas-under-the-curves were calculated using the trapezoidal rule. Integrating the single processes and then dividing by the 240-min observation period provided the average values of secretion and clearance. All data are presented as means ± SEM unless otherwise designated. All statistical comparisons between different groups were performed by ANOVA or the unpaired *t*-test. Changes from baseline within any given group were evaluated by the paired *t*-test.

Results

Glucose clamp study

Isotopically determined glucose disposal rate (GDR) was $8{\cdot}52 \pm 0{\cdot}43$ mg kg^{-1} min^{-1} in C, $5{\cdot}61 \pm 0{\cdot}32$ mg kg^{-1} min^{-1} in O ($P \leq 0{\cdot}05$ vs. C) and $4{\cdot}95 \pm 0{\cdot}94$ mg kg^{-1} min^{-1} in D ($P \leq 0{\cdot}05$ vs. C), indicating insulin resistance in obese and diabetic subjects.

Oral glucose load

Basal glucose was the same in the control (C) and the obese (O) subjects ($5{\cdot}4 \pm 0{\cdot}2$ mmol L^{-1} and $5{\cdot}6 \pm 0{\cdot}1$, respectively, $P = 0{\cdot}5$), while it was significantly higher in the diabetic (D) patients ($11{\cdot}2 \pm 1{\cdot}5$, $P = 0{\cdot}004$). During the OGL, glucose levels were not different between C and O, while D showed a marked hyperglycaemia. The time courses of the *arterial* concentrations of the measured pancreatic hormones before and following administration of the oral glucose load are reported in Fig. 1. C-peptide levels showed an elevated variability, being highest in O and almost not stimulated in D. Amylin levels paralleled those of C-peptide in the respective groups; glucagon exhibited a tendency to suppression. Similar patterns, but higher values, have been observed for the concentrations in the hepatic vein (not shown). The inter-subject variability of the concentration levels of every single peptide was comparable among the different groups if normalized with respect to the average values of each group. In particular, the percent coefficients of variation were, on average, 15·6 for C-peptide, 25·8 for insulin, 19·6 for amylin and 16·5 for glucagon, and was not different among groups.

Amylin

The time course of the amylin secretion rate as reconstructed by the model is shown in Fig. 2. The three patterns were statistically different as demonstrated by the relative areas-under-the-curve that yielded the total amount of amylin released in 240 min by the B-cell (Table 1). Amylin clearances did not differ among the three groups (Table 1) showing that the changes in amylin concentrations are solely the result changes in secretion. The amylin/C-peptide cosecretion factor was similar in the two insulin-resistant states ($0{\cdot}0031 \pm 0{\cdot}0048$ in O and $0{\cdot}0024 \pm 0{\cdot}0047$ in D, $P = 0{\cdot}3$), but lower than in C ($0{\cdot}0051 \pm 0{\cdot}0045$, $P < 0{\cdot}015$).

Glucagon

During OGL, glucagon levels were suppressed in C and O, regardless of the different basal levels, with a nadir between

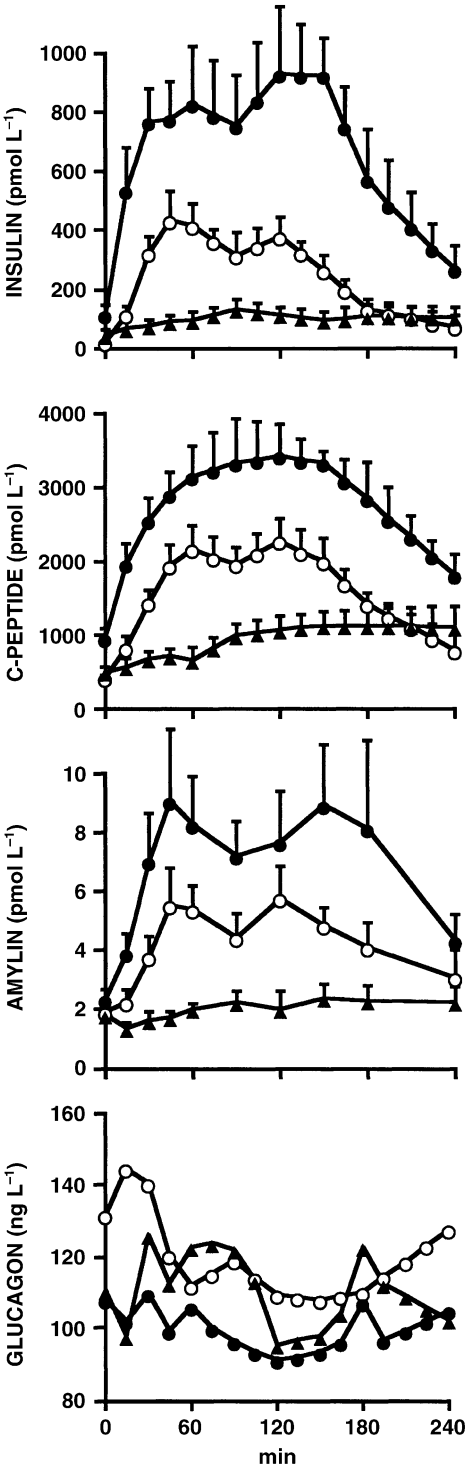

Figure 1 Measured arterial concentrations (mean ± SE) of the pancreatic products before and following oral administration (75 g) of glucose. Control (○), obese (●) and diabetic subjects (▲) (six subjects per group). As glucagon values in the three groups were similar, error bars have been omitted for better readability (mean ± SD of the standard error was 23 ± 2 ng L^{-1} for control, 14 ± 6 for obese and 15 ± 3 for diabetic subjects).

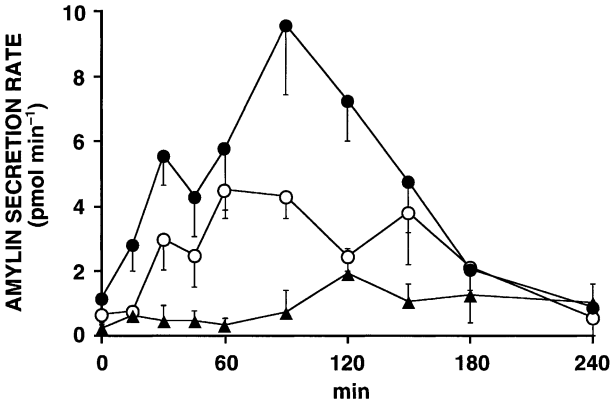

Figure 2 Amylin secretion rate (mean ± SE) during oral glucose load as calculated by the circulatory model of equation 3. Control (○), obese (●) and diabetic subjects (▲) (six subjects per group).

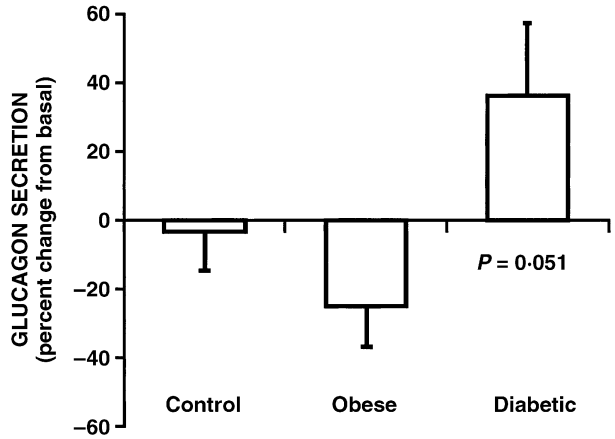

Figure 3 Percent variation of glucagon secretion, as calculated by the circulatory model of equation 6, with respect to fasting levels during oral glucose load in the study groups (six subjects per group).

60 and 180 min and a tendency to return to basal levels thereafter. This pattern might reflect the time required for absorption of the glucose load. In D, glucagon levels only slightly decreased between 120 and 180 min. Overall glucagon secretion resulting from these concentration patterns, expressed as the total amount of hormone released by the A-cell, was not different in the three groups (Table 1). However, given the similar clearance and the quite wide range of basal secretion levels (Table 1), it was more informative to calculate the dynamic secretion, i.e. the integrated change from basal. As shown in Fig. 3, an almost unchanged postprandial dynamic glucagon secretion resulted in C (–3·5 ± 14%), a decrease was observed in O (–25 ± 12%), while in D the glucagon secretion rate was increased (36 ± 21%), reaching borderline significance vs. C ($P = 0{\cdot}051$). When glucagon secretion was correlated with the insulin and amylin concentrations, both total and suprabasal area-under-the-curve, no significant relationship was detected. However, when glucagon secretion was correlated with the dynamic

Table 1 Measured values of systemic concentration and calculated kinetic parameters of the pancreatic hormones amylin and glucagon during OGL with hepatic vein catheterization

	Control	Obese	Diabetic	C vs. O	C vs. D	O vs. D
Basal concentration values						
Amylin (pmol L^{-1})	1·9 ± 0·2	3·8 ± 1·5	1·8 ± 0·4	$P = 0·236$	$P = 0·873$	$P = 0·231$
Glucagon (pg mL^{-1})	132 ± 22	105 ± 12	111 ± 10	$P = 0·308$	$P = 0·398$	$P = 0·729$
Area-under-the-curve during OGL						
Amylin (pmol mL^{-1} min^{-1})	1·0 ± 0·1	1·8 ± 0·4	0·5 ± 0·2	$P = 0·134$	$P = 0·002$	$P = 0·017$
Glucagon (ng mL^{-1} min^{-1})	28·5 ± 5·3	22·1 ± 3·4	25·9 ± 2·6	$P = 0·334$	$P = 0·667$	$P = 0·397$
Basal hormone secretion						
Amylin (pmol min^{-1})	0·69 ± 0·29	1·16 ± 0·38	0·24 ± 0·11	$P = 0·353$	$P = 0·177$	$P = 0·044$
Glucagon (ng min^{-1})	97·7 ± 12·4	186·6 ± 52·8	101·5 ± 28·7	$P = 0·058$	$P = 0·438$	$P = 0·085$
Total hormone secretion						
Amylin (nmol)	0·63 ± 0·05	1·10 ± 0·15	0·24 ± 0·10	$P = 0·015$	$P = 0·006$	$P = 0·001$
Glucagon (μg)	20·5 ± 2·3	27·7 ± 5·1	27·9 ± 5·4	$P = 0·134$	$P = 0·134$	$P = 0·258$
Metabolic clearance rate						
Amylin (L min^{-1})	0·61 ± 0·06	0·75 ± 0·12	0·44 ± 0·10	$P = 0·289$	$P = 0·179$	$P = 0·064$
Glucagon (L min^{-1})	0·82 ± 0·11	1·26 ± 0·20	1·28 ± 0·20	$P = 0·083$	$P = 0·066$	$P = 940$

OGL, oral glucose load.

secretions of amylin and insulin (from C–peptide), i.e. their changes from basal, a significant correlation between the secretion of glucagon and that of amylin ($r = -0·6$, $P = 0·008$), but not with that of insulin ($r = -0·2$, $P = 0·4$) was found (Fig. 4). When the subject with very high insulin secretion was excluded, the correlation was even weaker.

Discussion

By combining hepatic vein catheterization and a circulatory mathematical model this study provides the direct quantification of the secretion and clearances of amylin and glucagon and their interrelationships. We have evaluated these parameters directly by the hepatic vein catheterization technique under dynamic conditions, i.e. during an OGL, which is the most physiological glucose tolerance test. Besides confirming hypersecretion of amylin in the obese subjects compared with the control subjects, we observed that dynamic glucagon secretion declined following oral glucose ingestion in the control and obese subjects in contrast to an increase in the diabetic patients. The most interesting result was the significant correlation between changes in amylin secretion and the suppression of glucagon secretion following oral glucose ingestion, while no correlation between insulin secretion from C-peptide and glucagon suppression was observed.

Moreover, a relationship between amylin and glucagon has been hypothesized from studies investigating the dynamics of amylin or its agonist pramlintide after their administration [8,9,25]. However, only the results of the present study confirm for the first time the role of endogenous amylin in the modulation in dynamic conditions of postprandial glucagon secretion in human subjects and therefore a physiological role for amylin in glucose homeostasis, possibly through this modulation of glucagon secretion. Another aspect worth considering is that the relationship of amylin and glucagons secretion (Fig. 4) seems to be valid in general. In fact, the three groups are not clustered and therefore it can be assumed that there is no dependency on the particular pathophysiological condition.

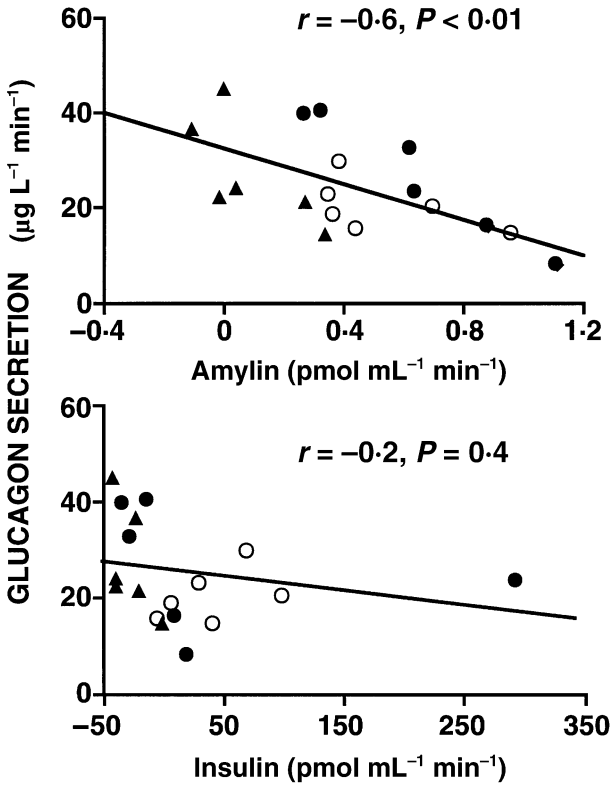

Figure 4 Relationships between glucagon secretion and changes with respect to basal of insulin from C-peptide and amylin. Control (○), obese (●) and diabetic subjects (▲). Statistics in the Results section.

The putative mechanism by which amylin regulates postprandial glucagon secretion, however, is not clear. While in the isolated perfused rat pancreas, amylin failed to exert a glucagonostatic effect [26], a recent study described that amylin totally inhibits insulin, glucagon and somatostatin secretion following arginine stimulation [27]. Alternatively, the effect of amylin on glucagon secretion could be mediated through the autonomic nervous system analogous to the vagally mediated effect of amylin on gastric emptying [28]. In this context it is of interest that binding of amylin has been demonstrated in the area postrema in the hindbrain of the rat involving the dorsal motor nucleus of the vagus [29]. Taken together, these data fit well in the suggested model that amylin might act as a third pancreatic hormone by slowing down gastric emptying and reducing glucagon secretion and consequently hepatic glucose output following carbohydrate ingestion.

The interplay of insulin and glucagon determines the amount of net glucose production. Glucagon acts through glycogenolysis and gluconeogenesis and is the primary determinant of the blood glucose level in the postabsorptive state. Following carbohydrate ingestion, glucagon levels have been shown to decline in healthy subjects thus reducing hepatic glucose output by ~50% [30]. In patients with type-2 diabetes, however, postprandial glucagon secretion is not suppressed [31]. These observations were confirmed by our results that indicated that the modelling approach we have used is a reliable method. The only assumption we have made is the 25% hepatic degradation of glucagon. As our data cannot allow a reliable estimation of this process (see equation 4), the evidence obtained by other studies indicates that this seems to be an accepted value [24]. Our results showed that arterial glucagon levels declined from 60 to 180 min following glucose ingestion in healthy subjects. Cumulative glucagon secretion over a period of 240 min, when glucose absorption is assumed to be completed [32], showed a very small decrease in the control subjects. It was, however, suppressed to a higher extent in the obese subjects, while it was increased in the diabetic patients. The combination of compromised insulin secretion and absent decrease in glucagon secretion will ultimately result in full maintenance of hepatic glucose output and thus contribute to postprandial hyperglycemia in diabetic patients.

The present study also quantified amylin and glucagon dynamics during the OGL. To the best of our knowledge, their secretion and clearance in control, obese and type-2 subjects were directly quantified for the first time by investigating arterio-venous differences at the hepatic level. To this aim, a circulatory model was exploited. For amylin, an absent hepatic extraction was assumed based on previous findings [23]. The secretion rate of amylin paralleled that of insulin and was decreased in the diabetic and increased in the obese compared with the control subjects. The model also quantified the metabolic clearance rate of amylin, which was not different in the three groups, showing that the concentration patterns in periphery and at the liver level solely result from changes in secretion and not in degradation. Also the total clearance rates of glucagon were not different, indicating again the fundamental role of pancreatic secretion in modulating the systemic levels of the hormone.

In conclusion, we have observed that in diabetic patients the dynamic secretion of glucagon was increased following oral glucose administration and this might contribute to postprandial hyperglycemia. The negative correlation between the dynamic secretion of glucagon and amylin provides evidence for the first time that endogenous amylin might be involved in the regulation of glucagon secretion and thus of postprandial glucose control in humans.

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