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Renal Elimination of Islet Amyloid Polypeptide

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Six healthy volunteers showed significantly higher plasma islet amyloid polypeptide levels following an oral glucose tolerance test compared to fasting levels. The urine IAPP concentration before and after the OGTT was comparable to that in plasma. Reverse phase HPLC and radioimmunoassay analysis of urine samples revealed a single IAPP-immunoreactive peak. Before hemodialysis, the plasma levels of IAPP and C-peptide, but not of insulin, were significantly elevated in eight fasting patients with chronic renal failure, compared to eight healthy matched control subjects. After hemodialysis, there was a tendency for decreased IAPP levels compared to before dialysis. In summary, elevated levels of plasma IAPP were found in patients with chronic renal failure and the peptide is eliminated by hemodialysis. Furthermore, immunoreactive IAPP is normally present in the urine. These results suggest that IAPP is, at least in part, renally eliminated from the plasma by excretion (glomerular filtration and/or tubular secretion).

Islet amyloid polypeptide (IAPP), consisting of 37 amino acid residues, is the major protein component of amyloid deposits found in human insulinomas [1], pancreatic islets of the diabetic cat [2,3], and pancreatic islets of type II diabetic patients [3,4]. The peptide shares approximately 50 % homology with calcitonin gene-related peptide (CGRP) [2,5], which is an alternative product of the calcitonin gene. Secretion of IAPP occurs together with insulin from the same β-cell secretory granules of the pancreatic islets [6-8].

There are several reports about the physiological and pathophysiological role of IAPP, suggesting its involvement in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM). In vitro and in vivo IAPP has been shown to inhibit both basal and insulin-stimulated glucose uptake as well as glycogen synthesis in skeletal muscles [9-11]. High doses of IAPP have also been reported to restrain insulin secretion in a number of studies [12,13]. In humans, elevated plasma levels of IAPP have been found in patients with diabetes associated with pancreatic cancer [14].

The biological elimination of IAPP has not yet been fully characterized [15]. Only a limited amount of information has been obtained from animal studies, and there are only a few reports available from human studies [15,16]. These studies have shown increased plasma IAPP levels in patients with chronic renal failure, indicating that IAPP at least in part might be excreted and /or metabolized by the kidneys.

The aim of the present study was to further elucidate the role of the kidneys for the elimination of IAPP analyzing the possible presence and nature of IAPP in urine of healthy volunteers. Furthermore, the plasma levels and the hemodialysis clearances of IAPP, insulin and C-peptide were investigated in patients with chronic renal failure.

MATERIALS AND METHODS

Subjects and Study Design

Study I. Eight patients (male/female = 7/1, age 68.8±4.0 years, range 50-82, body mass index 23.3±0.7 kg/m^2) with severe chronic renal failure, all of whom were treated with 4 hours of hemodialysis 3 times per week, were investigated. After a 12-hour fast, blood samples for the analysis of IAPP, insulin, C-peptide, creatinine, urea and glucose were taken from the AV-fistula immediately before and after 2 hours of dialysis. On the later occasion, samples were drawn both proximal and distal of the dialysis filter (F50 - Polysulfonemembrane, Fresenius AG, Bad Homburg, Germany) via two separate dialysis needles for the calculation of clearance of the investigated substances. Eight healthy volunteers matched for sex (male/female = 7/1), age (66±3.3, range: 56-78) and BMI (Body mass index) (22.6±1.1) served as controls. There were no indications of renal disease or diabetes mellitus in this group, or any personal or family history of such diseases.

Study II. Six male healthy volunteers (age 29±1.8, range 25-37) underwent an oral glucose tolerance test (OGTT) (75 g) after an overnight fast. Blood was drawn at 0 and 45 min for the measurement of IAPP. Urine was obtained at 0 and 120 min also for the analysis of IAPP.

All subjects gave informed consent to participate in the respective studies. The study was approved by the local ethics committee.

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Assays

Plasma (1 ml) and urine (10 ml) samples were extracted on C-18 Sep-Pak cartridges as described in detail elsewhere [17], dried and reconstituted in 1 ml assay buffer prior to the IAPP radioimmunoassay [17]. Insulin and C-peptide were analysed on unextracted plasma by the use of commercial kits from Pharmacia, Uppsala, Sweden. To determine the circulating concentrations of glucose, urea and creatinine, samples were routinely analysed by the Laboratory for Clinical Chemistry, University Hospital, Linköping, Sweden. Plasma clearance for IAPP, insulin, C-peptide, creatinine and urea over the dialysis filter was calculated according to the established formula: $F\ (C_1 - C_2)/C_1$, where F is the plasma flow, C_1 the concentration proximal and C_2 distal of the filter.

Reverse Phase High-Performance Liquid Chromatography

One C-18 Sep-Pak-extracted urine sample (10 ml) from one healthy volunteer was analyzed by reverse phase high-performance liquid chromatography (HPLC) (LKB, Bromma, Sweden). Prior to chromatography, the dried sample was reconstituted in 1 ml of assay buffer and centrifuged for 5 min at 2500 rpm. In two consecutive experiments 200 μl of the supernatant was applied to a TSK gel ODS-120 T (4.6 $\times$ 250 mm) C-18 column (LKB). A 30 min linear gradient of 40-53% solvent B (see below) was used at a flow rate of 0.7 ml/min. Solvent A was 0.1% trifluoroacetic acid in water and solvent B 30% of solvent A in acetonitrile. The effluent was monitored at 226 nm. Fractions (500 μl) were collected and dried in a Savant Speedvac SC-100 vacuum centrifuge (Techtum Lab, Umeå, Sweden) and reconstituted in 500 μl assay buffer prior to analysis for IAPP-immunoreactivity in the RIA. Standard human IAPP ("Amylinamide", Peninsula, Merseyside, England) was used as control.

Statistical Methods

All data are presented as mean ± SEM. The statistical significance of differences between group means was assessed by Student's *t* test or the Mann-Whitney U test, p values less than 0.05 being considered significant.

TABLE 1

Basal Plasma Levels of IAPP, C-peptide, and Insulin in 8 Patients with Chronic Renal Failure (CRF) (n = 8) before and after 2 Hours of Dialysis and in Healthy Control Subjects (n = 8) (mean ± SEM)

	Plasma concentration		
	CRF patients		
Parameter	Before dialysis	After dialysis	Control subjects
Urea (mmol/l)	19.1 ± 1.5#	10.0 ± 1.0	n.d.
Creatinine (μmol/l)	690 ± 52.9#	390.6 ± 37.5	n.d.
Glucose (mmol/l)	4.7 ± 0.2#	3.5 ± 0.1	n.d.
IAPP (pmol/l)	12.8 ± 3.0✪	8.8 ± 1.7	6.4 ± 0.6
C-peptide (pmol/l)	2690 ± 300*†	1638 ± 294	654 ± 96
Insulin (pmol/l)	67.4 ± 12.7	39.3 ± 4.5	72.5 ± 16.9

Note. n.d., not determined.

# $p < 0.001$ vs. after dialysis; ✪$p < 0.05$ vs. control subjects; *$p < 0.05$ vs. after dialysis; †$p < 0.001$ vs. control subjects.

TABLE 2

Hemodialysis Clearances of Urea, Creatinine, IAPP, C-peptide, and Insulin in 8 Patients with Chronic Renal Failure (mean ± SEM)

Parameter	Clearance (ml/min)	Number of patients	Molecular weight (Da)
Urea	131.8 ± 6.4	7	60.6
Creatinine	103.3 ± 6.1	7	113.1
IAPP	66.1 ± 18.8	5	3901
C-peptide	40.3 ± 8.2	7	3617
Insulin	27.1 ± 5.1	8	5750

RESULTS

Creatinine and Urea in Plasma and Blood Glucose

The plasma levels of creatinine and urea in the 8 patients with chronic renal failure were before hemodialysis 690±52.9 μmol/l and 19.1±1.5 mmol/l, respectively (Table 1). After 2 hours of dialysis both levels had decreased significantly (390.6±37.5 μmol/l and 10.0±1.0 mmol/l; $p < 0.001$ in both cases). The blood glucose concentration was normal in all subjects, but before dialysis the levels were significantly higher compared to after 2 hours of dialysis (4.7±0.2 vs. 3.5±0.1 mmol/l; $p < 0.001$).

Basal Plasma Levels of IAPP, Insulin, and C-peptide

The basal levels (before dialysis) of plasma IAPP in the fasting patients with renal failure were significantly higher than in the control subjects (12.8±3.0 vs. 6.4±0.6 pmol/l; $p < 0.05$) (Table 1). The basal plasma C-peptide levels were also significantly elevated in the patients, compared to the control group (2690±300 vs. 654±96 pmol/l; $p < 0.001$), while there were no differences in the insulin levels between the two groups (67.4±12.7 vs. 72.5±16.9 pmol/l; ns).

Plasma Levels of IAPP, Insulin, and C-peptide after Dialysis

After 2 hours of dialysis there was a tendency to decrease of plasma IAPP from 12.8±3.0 to 8.8±1.7 pmol/l ($p = 0.05$ ns) (Table 1). Both insulin and C-peptide levels were significantly lower after dialysis (39.3±4.5 and 1638±294 pmol/l, $p < 0.05$ in both cases), compared to basal levels.

Hemodialysis Clearance

The clearance (ml/min) of the different investigated substances over the dialysis filter was: IAPP: 66.1±18.8, insulin: 27.1±5.1, C-peptide: 40.3±8.2, creatinine: 103.3±6.1, urea 131.8±6.4. In Table 2 the hemodialysis clearance values and molecular weights of

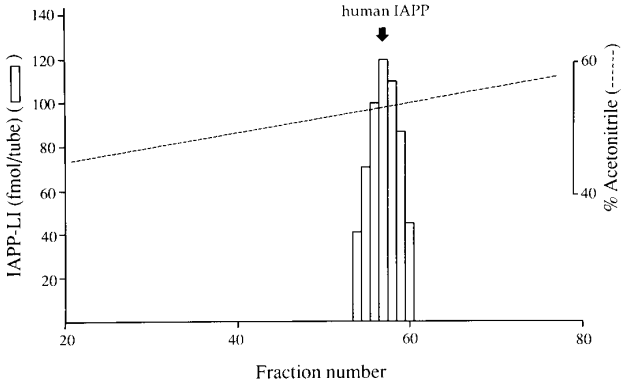

FIG. 1. Plasma and urine IAPP levels in 8 healthy volunteers before (Pre) and after (Post) an oral glucose tolerance test (75 g). * $p < 0.05$.

IAPP, C-peptide, insulin, creatinine and urea are given.

IAPP in Plasma and Urine during OGTT

The six healthy subjects that underwent the OGTT showed significantly elevated plasma levels of IAPP 45 min after the glucose load, compared to fasting levels (11.5±2.0 vs. 7.1±1.2 pmol/l; $p < 0.05$) (Fig. 1). IAPP immunoreactivity was found in the urine after overnight fasting in all six subjects (15.0±4.9 pmol/l, range: 4.7-35.6). Two hours after the glucose load the urine contained 9.1±3.4 pmol/l. The total volume of urine was not measured, however.

Nature of Immunoreactive IAPP in Urine

Reverse phase HPLC of urine extracts revealed that the immuno-reactivity eluted in a single peak, appearing simultaneously with authentic human IAPP (Fig. 2). No other IAPP immunoreactive peaks appeared.

DISCUSSION

The present investigation demonstrates increased plasma levels of IAPP in hemodialysis patients with severe chronic renal failure, compared to healthy volunteers. This is in accordance with previous studies [15,16,18,19], suggesting an important role of the kidneys for the filtration and/or metabolism of IAPP. By the use of whole-body autoradiography, Stridsberg et al. [20] have shown a high uptake of ^{125}I-IAPP in the cortex of the rat kidney after i.v. injections of ^{125}I-IAPP. They also found some radioactivity in the renal pelvis and ureter, implying a renal excretion of IAPP. It was not established, however, whether this radioactivity corresponded to processed or intact IAPP.

After 2 hours of hemodialysis there was a tendency to a decrease in the plasma IAPP levels in the patients compared to pre-dialysis levels. This is in accordance with a previous study by Ludvik et al. [16], which showed a significant decrease in circulating IAPP levels after hemodialysis for a longer period (4 hours) in a larger number of patients (20) with chronic renal failure. In the present study, there seemed to be a relationship between the molecular weights and the hemodialysis clearances for creatinine, urea, IAPP, C-peptide and insulin. IAPP appears, however, not to be metabolized exclusively by the kidneys, but also by the liver. This is supported by a study by Sowa et al. [11], in which infusion of IAPP via the portal vein in vivo in dogs was shown to reduce the effect of the peptide on insulin-stimulated glucose metabolism, compared with infusion via a peripheral vein.

Prior to hemodialysis, the plasma levels of C-peptide, but not of insulin, were elevated in the patients with impaired renal function, which seems reasonable since C-peptide is mainly excreted by the kidneys, while insulin to the greatest extent is metabolized in the liver [15,21,22]. Plasma insulin levels have in previous studies been reported to be either unchanged [15] or elevated [23] in patients with chronic renal failure, compared to healthy control subjects. Surprisingly, the plasma insulin levels in the present study had decreased significantly 2 hours after hemodialysis in the patients with impaired renal function, despite the low hemodialysis clearance. This could be explained by a decreased β-cell secretion during the hemodialysis as a result of the significantly reduced blood glucose levels in the fasting patients.

The hypothesis that IAPP is excreted by the kidneys was supported by our discovery of IAPP-like immunoreactivity in human urine. Previous investigators have

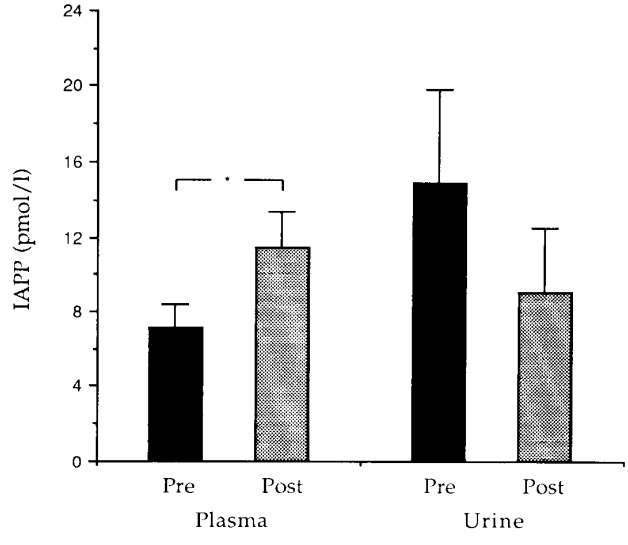

FIG. 2. IAPP-like immunoreactivity (vertical bars) in reverse phase HPLC fractions originating from urine extract from a healthy volunteer. The elution position of authentic human IAPP is indicated.

failed in their attempt to demonstrate IAPP in the urine [15,19], most likely due to different extraction procedures. van Hulst et al. [19], argued that possible urinary IAPP levels might be under the detection limit of their assay or that the IAPP might be processed by the kidney in such a way that the antibodies used in the assay no longer recognizes the peptide. In the present report, however, the concentration of IAPP in the morning urine was comparable or even higher than the fasting plasma levels of the same healthy volunteers. Furthermore, our HPLC experiments on extracts from human urine confirm that the IAPP-immunoreactivity measured in our assay is identical to authentic, intact IAPP.

The physiological importance of IAPP for the development of non-insulin-dependent diabetes mellitus (NIDDM) is not clear. IAPP has been shown to cause insulin resistance both in vitro [9,24]; and in vivo [10,11], but only when using supraphysiological concentrations. Elevated fasting plasma IAPP levels have been seen in patients with pancreatic carcinoma and impaired glucose tolerance or diabetes [14]. There are reports showing impairment of insulin action in patients with renal disease [25,26]. Therefore, chronically increased circulating levels of IAPP, such as seen in patients with chronic renal failure, might have an impact on insulin action. Ludvik et al. [16], however, did not find insulin resistance or decrease in insulin release in such individuals. On the other hand, in a recent investigation by de Koning et al. [27], a higher prevalence of islet amyloid was demonstrated in non-diabetic patients with end-stage renal failure (ESRF), compared with non-diabetic control subjects without ESRF.

In conclusion, the present study demonstrates elevated plasma IAPP levels in patients with severe chronic renal failure and that the peptide is in part eliminated during hemodialysis. Furthermore, significant amounts of IAPP were found in urine from healthy volunteers. These results suggest that IAPP, at least in part, is cleared from plasma by renal excretion (glomerular filtration and/or tubular secretion).

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