Increased plasma levels of islet amyloid polypeptide in patients with primary hyperparathyroidism

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Amylin, also named islet amyloid polypeptide (IAPP), is a protein that is processed and released from pancreatic β-cells in parallel with insulin. Islet amyloid polypeptide is currently studied with regard to a role for insulin resistance in non-insulin-dependent diabetes. To elucidate a possible function of IAPP for impaired glucose tolerance in primary hyperparathyroidism (pHPT), we studied plasma IAPP levels during an oral glucose tolerance test (OGTT) in seven pHPT patients before and 8 weeks after surgery and in six healthy subjects. The B-glucose level of the patient groups was 4.34 ± 0.12 mmol/l before and 3.97 ± 0.16 mmol/l after surgery (NS), while the serum level of insulin was significantly higher before (16.9 ± 2.8 mIU/l) than after (8.9 ± 1.9 mIU/l) the operation (p < 0.05), indicating a moderately increased insulin resistance in pHPT. The basal plasma levels of IAPP were significantly higher in pHPT patients before than 8 weeks after surgery (9.71 ± 1.05 and 4.30 ± 0.82 pmol/l, respectively; p < 0.01). When compared to the plasma IAPP level of the controls at 1.80 ± 0.38 pmol/l, pHPT patients had higher IAPP values both before (p < 0.01) and at 8 weeks after (p < 0.05) operation. There was a significant correlation between the serum levels of insulin and plasma levels of IAPP in pHPT patients before (r = 0.87, p < 0.01) as well as 8 weeks after surgery (r = 0.69, p < 0.05). The area under the curve for IAPP during OGTT in pHPT patients was 1872.4 ± 187.7 pmol-min/l, which is significantly higher than after surgery (1010.8 ± 93.7 pmol-min/l) (p < 0.05) and compared to the area for the controls at 840.3 ± 49.9 pmol-min/l (p < 0.01). In conclusion, pHPT is associated with an increased plasma level of IAPP, correlated to the serum insulin level, but persistently higher than in controls also 8 weeks after surgery. Possibly, increased IAPP levels can have a role for impaired glucose tolerance in pHPT. The hyperparathyroid state might have a specific role for the release of this peptide, otherwise closely connected to insulin secretion.

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Primary hyperparathyroidism (pHPT) is known to be associated with disturbances in glucose metabolism (1, 2) and an increased prevalence of diabetes mellitus (3). The pathophysiological mechanism is still unclear but a decreased peripheral insulin sensitivity has been demonstrated, reflected by high serum insulin concentrations in pHPT patients (4). In addition to HPT, other factors of importance for insulin resistance may also be present in these patients, but it has recently been demonstrated that insulin resistance is present in pHPT in the absence of hypertension and obesity and that overt impaired glucose tolerance is more likely to develop in those pHPT patients who have a reduced beta-cell function (5). However, so far, no consistent association has been demonstrated between insulin sensitivity and parathyroid hormone (PTH), calcium or phosphate levels (5), making these variables unlikely to be of major direct importance for impaired glucose uptake per se in pHPT.

The overall pathophysiological mechanism for insulin resistance is still unclear but some interest has been focused recently on islet amyloid polypeptide (IAPP), also named amylin (6). This is a 37-amino-acid protein that has a 43–46% homology to calcitonin gene-related peptide (7). Ilet amyloid polypeptide has been demonstrated to be stored in pancreatic beta-cells and to be released in parallel with insulin. It has also been suggested to modulate basal and stimulated glucose uptake in skeletal muscle, leading to insulin resistance (6). Furthermore, IAPP may also have a role in calcium metabolism via an inhibiting effect on osteoclast function that is stronger than that of calcitonin gene-related peptide but weaker than that of calcitonin (7), and also by promoting intestinal calcium absorption (8).

Patients with pHPT might have an increased level of IAPP because this disease is followed by hyperinsulinemia. Furthermore, increased levels of calcitriol, as is seen in pHPT, have been reported to stimulate insulin and therefore probably IAPP secretion (9).
A demonstration of increased levels of IAPP in pHPT could be of interest for the understanding of insulin resistance and vascular disease in these patients. To explore this possibility we investigated circulating IAPP and insulin levels during an oral glucose tolerance test in patients with pHPT before and 8 weeks after parathyroid surgery and compared these data to healthy subjects.

**Methods**

**Patients and controls**

Six females and one male patient with hypercalcemia, judged to be due to pHPT, participated after informed consent. The pre-operative diagnosis of pHPT was supported by persistently increased serum levels of intact parathyroid hormone (PTH) in the presence of hypercalcemia that were not explained by any other disease. The age of the group ranged from 36 to 78 years (median age 67 years). Body mass index (BMI) ranged from 19.1 to 32.0, with the mean (± SD) BMI = 24.8 ± 4.5.

The male patient was treated with enalapril for mild hypertension. Three of the female patients also had hypertension, treated with beta-blockers and diuretics. One woman was euthyroid on thyroxine substitution after surgery and radiiodine therapy for hyperthyroidism several years earlier. The other two patients were otherwise healthy.

The mean (± SD) systolic and diastolic blood pressures at presentation were 154 ± 28 and 89 ± 10 mmHg, respectively.

All patients underwent parathyroid exploration. Primary HPT was confirmed in each patient and the ionized serum calcium level was normalized in all patients after the operation. Six otherwise healthy and normotensive subjects, five women and one man, served as controls. The age of the controls ranged from 43 to 60 years (median age 50 years). The BMI ranged from 20.6 to 25.2, with mean (± SD) BMI = 23.5 ± 1.9 (NS vs the patient group).

**Oral glucose tolerance test (OGTT)**

A standard 75-g OGTT was performed in the morning after 6 h of fasting. Blood samples for glucose (B-glucose), insulin and IAPP were drawn at 0, 15, 30, 45, 60, 75, 90 and 120 min. The IAPP samples were drawn in tubes with 400 KIU/ml aprotinin (Trasylol: Bayer, Leverkusen, Germany). Plasma and serum were stored at −20°C and analyzed for IAPP and insulin at one later occasion.

The OGTT investigation was performed before surgery and repeated 8 weeks after the operation.

**Laboratory analysis**

The plasma IAPP concentration was measured using an assay as described previously (10). Briefly, the assay is based on rabbit anti-human IAPP antiserum (Peninsula, Merseyside, UK) with labeled rat/mouse IAPP (Amersham, Solna, Sweden) as tracer and detects changes between adjacent samples equivalent to 1 pmol/l plasma. Before assay, each plasma sample was extracted on a Sep-Pak C18 cartridge and the eluate was lyophilized and reconstituted in assay buffer.

Serum insulin was analyzed by radioimmunoassay (11). The detection limit was 3 mlU/l and the intra- and interassay coefficients of variation were 5% and 8%, respectively. The reference value for fasting subjects was < 25 mlU/l (the 97.5% percentile for 30 healthy subjects with normal fasting blood glucose level).

Intact parathyroid hormone (PTH) was analyzed by an N-tact PTH assay (Incstar, Stillwater, MN): sensitivity = 0.13 pmol/l; interassay variation < 11%; intra-assay variation < 6%; reference range 1.0–5.0 pmol/l.

The plasma level of ionized calcium (i-Ca) was determined by an ion-selective electrode (Radiometer, Copenhagen, Denmark) and corrected to pH 7.40; reference range 1.17–1.29 mmol/l.

**Statistics**

In the results, the values are presented as means ± SEM. Difference between means was evaluated by the Wilcoxon rank order test and correlations between variables by the method of least squares. The degree of change for the variables studied during the OGTT was estimated from the area under the curve (AUC) in each patient.

The value for the AUC was calculated from the product of the concentration of the variable studied (f) against time according to the formula: 

\[ T \text{ (AUC)} = 15 \text{ minutes (between blood sampling)} \times [0.5f_1 + f_2 + \ldots + f_{n-1} + 0.5f_n]. \]

The difference between the AUC before and after surgery and between patients and controls was evaluated by the Wilcoxon rank order test.

**Results**

**Basal levels**

Plasma i-Ca was 1.52 ± 0.04 (SEM) mmol/l before and 1.23 ± 0.02 mmol/l after surgery (N = 7, p < 0.01). In the control group, plasma i-Ca was 1.23 ± 0.01 mmol/l (N = 6) p < 0.01 compared to patients before surgery but not significantly different when compared to the patients after surgery.

The serum level of intact PTH decreased in each patient. The mean value was 10.4 ± 3.0 pmol/l before and 5.0 ± 1.1 pmol/l after operation (p < 0.05). The
value of the control group (3.1 ± 0.3 pmol/l) was significantly lower than the value for the patient group before surgery (p < 0.01) but not different from that after surgery.

There was no significant difference between basal B-glucose level in the patient group before and after surgery (4.34 ± 0.12 and 3.97 ± 0.16 mmol/l, respectively) nor between the postoperative B-glucose level and the value of the control group at 3.78 ± 0.08 mmol/l, which, however, was significantly lower than the value of the patient group before surgery (p < 0.05) (Fig. 1).

For the patient group, the basal serum insulin level was significantly higher before surgery (16.9 ± 2.8 mIU/l) compared to after the operation (8.9 ± 1.9 mIU/l, p < 0.05). Compared to the value of the control group at 8.7 ± 1.0 mIU/l, the mean insulin level of the pHPT patients was significantly higher before (p < 0.05) but not after surgery (Fig. 1).

The plasma IAPP level of the pHPT patients was significantly higher before than 8 weeks after surgery (9.71 ± 1.05 pmol/l and 4.30 ± 0.82 pmol/l, respectively;
Fig. 3. Relationship between circulating basal levels of serum insulin and islet amyloid polypeptide (IAPP) in seven patients with primary hyperparathyroidism before (top: \( r = 0.87, \ p < 0.01 \)) and after surgery (bottom: \( r = 0.69, \ p < 0.05 \)).

\( p < 0.01 \) and significantly higher than the value of the control group at 1.80 ± 0.38 pmol/L, both before (\( p < 0.01 \)) and 8 weeks after (\( p < 0.05 \)) surgery (Figs. 1 and 2).

There was a significant correlation between the basal serum insulin and plasma IAPP values before (\( r = 0.87, \ p < 0.01 \); Fig. 3A) as well as 8 weeks after the operation (\( r = 0.69, \ p < 0.05 \); Fig. 3B).

There was no significant correlation between the basal values for intact PTH, i-Ca or glucose on the one hand and the IAPP values on the other.

**Data from the OGTT**

The glucose AUC was 673.3 ± 53.9 mmol·min/l before and 710.6 ± 37.5 mmol·min/l 8 weeks after operation (NS) and 566.4 ± 37.8 mmol·min/l in the control group. The value for the control group was not significantly different compared to patients before operation but lower than after surgery (\( p < 0.05 \)).

The insulin AUC was 11655 ± 2850 mlU·min/l before and 8053 ± 1243 mlU·min/l 8 weeks after surgery (NS). Compared to the value of the control group (4773 ± 599 mlU·min/l) the insulin AUC for the patient group was significantly higher than before (\( p < 0.05 \)) but not 8 weeks after the operation.

The IAPP AUC for the patient group was significantly higher before than 8 weeks after surgery (1872.4 ± 187.7 and 1010.8 ± 93.7 pmol·min/l, respectively; \( p < 0.05 \)). The IAPP AUC for the control group (840.3 ± 49.9 pmol·min/l) was significantly lower than the value for the patient group before surgery (\( p < 0.01 \)) but not different from the postoperative patient IAPP AUC (Fig. 4).

There were no significant correlations between the patient PTH levels before surgery on the one hand the glucose, insulin and IAPP AUC response on the other.

In the control group, a significant correlation was noted between the glucose AUC and the insulin AUC data (\( r = 0.86, \ p < 0.01 \)). No such correlation could be demonstrated in the patients.

**Discussion**

The major finding in the present study was the demonstration of increased plasma levels of the newly
discovered IAPP, not only during basal conditions but also during an OGTT in patients with pHPT. As expected from earlier data on patients with pHPT, we found a biochemical profile consistent with a moderate insulin resistance in our patients, having increased basal levels of insulin and an enhanced insulin release during an OGTT but without frank diabetes mellitus. This is well in accordance with earlier and recent studies on the impaired glucose uptake in patients with pHPT (5).

Islet amyloid polypeptide is supposed to be processed and released in parallel with insulin from the islet beta-cells in pancreas (6). In agreement with this, we found the basal levels of serum insulin and plasma IAPP to be correlated significantly in our pHPT patients both before and after parathyroid operation. Furthermore, stimulated release of insulin and IAPP during the OGTT were increased compared to controls before but not after surgery for pHPT. These data support the connection between IAPP and insulin release in pHPT patients. These findings also suggest a common stimulus for the release of insulin and IAPP, notably glucose. In our control group, however, a positive significant correlation was observed between the AUCs for glucose and insulin but not between the glucose and IAPP AUCs during the OGTT. Therefore, an additional mechanism might influence the regulation of IAPP release. The mechanism for the enhanced release of IAPP in pHPT as well as for the persistence of an increased IAPP level 8 weeks after surgery for pHPT remains unclear. An increased level of PTH is known to be present in some patients after successful surgery for pHPT (12). There was, however, no correlation between the PTH and IAPP levels in our present study.

The role of IAPP for an impaired glucose uptake has been studied repeatedly in recent years. According to animal studies, IAPP may play a role for insulin resistance in rats (13–15) and dogs (16), while its importance for glucose metabolism in humans is currently being investigated. Islet amyloid polypeptide has been proposed to be a pathogenic factor in non-insulin-dependent diabetes mellitus (17) and an inverse correlation has been reported between the IAPP/insulin ratio and glucose disposal in obese man (18), while in another study, no correlation could be found between an insulin sensitivity index and IAPP release (19). In a recent study, IAPP could not be demonstrated to impair insulin sensitivity during a hyperinsulenic euglycemic clamp in man (20). Thus, although there are data supporting a role for IAPP in insulin resistance (6), the final role of IAPP in pathogenesis of non-insulin-dependent diabetes mellitus in man remains to be settled.

The mechanism for the decreased insulin sensitivity in pHPT is unclear and no consistent correlations have been found between insulin sensitivity and PTH, calcium and phosphate levels (5). Hypercalcemia (21, 22) and increased levels of calcitriol (23) may enhance insulin secretion, while hypercalcaemia (21, 22) along with hypophosphatemia (24) may reduce insulin sensitivity. Parathyroid hormone has been reported to suppress insulin secretion from the beta-cells (25, 26) and to antagonize the insulin action in pHPT (27); the latter finding was not detected in other studies (28, 29). So far, it therefore seems unclear which of these factors are most important for an impaired glucose uptake in pHPT. Furthermore, hyperinsulinemia in patients with long-standing pHPT has been reported not to be normalized in all subjects, although the blood pressure level decreased soon after parathyroid surgery, suggesting that factors of importance for the impaired glucose tolerance will only improve partially soon after surgery (21). In addition, postmenopausal women with pHPT have been reported to have higher body weight and total body fat mass and also an increased proportion of android fat (30). The demonstration of increased IAPP levels in pHPT in our patients opens the possibility that this peptide might be a link between pHPT and insulin resistance and thereby towards the impact of pHPT on vascular disease. The persistence of increased IAPP levels 8 weeks after surgery would also be consistent with a previous report that intestinal absorption of calcium may remain enhanced in some patients also after surgery for pHPT (31).

In conclusion, the present study has demonstrated that pHPT is associated not only with hyperinsulinemia but also with increased levels of IAPP, a protein that can have a role for impaired glucose uptake in man in addition to a role in calcium metabolism, including bone turnover and promotion of intestinal calcium uptake. The present data may also support the possibility that PTH release can have a specific role for the regulation of this peptide, otherwise closely connected to insulin secretion. Further studies on this issue seem to be of great interest.

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References


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