Impaired Insulin Sensitivity and Insulin Secretion in Hemodialysis Patients With and Without Secondary Hyperparathyroidism

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Introduction

Until now, studies in the uremic rats and humans, have indicated that insulin resistance, reduced beta cell secretion and glucose intolerance leading to dyslipidemia were associated with hyperparathyroidism[1-3]. It was observed that hyperparathyroid-induced increase in intracellular free calcium concentration (Ca^{2+}i) could be responsible for beta-cell dysfunction[1]. As we know, changes in the concentration of intracellular calcium have a central role in a various cellular processes, from growth and development to hormone secretion. Increase in Ca^{2+}i are likely to be sustained by Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCC) or non-selective cation channels (NSCC), depending on the cell type[5,6]. Raised Ca^{2+}i levels trigger insulin secretion. Squires et al. reported that increasing extracellular calcium ions (Ca^{2+}o) increases insulin secretion from human pancreatic islets, but, that transient increase in insulin secretion was followed by a Ca^{2+}concentration-dependent and prolonged inhibition of secretion[7,8]. This study demonstrated another, CaR (calcium sensing receptor)-mediated inhibitory mechanism, which may be an important autoregulatory mechanism in the control of insulin secretion.

A rightward shift of the calcium set-point and an increase of the minimum secretion rate have been found in secondary hyperparathyroidism, indicating abnormal calcium sensing by parathyroid cells. Several studies have shown significant reductions in the levels of expression of CaR mNA and/or protein in such parathyroid gland to normal parathyroid tissue[9]. It is considered that various candidate genes, vitamin D receptor (VDR) gene-, CaR gene- and PTH gene- polymorphism might contribute to differences in deterioration of secondary hyperparathyroidism[10,11]. The complications associated with chronic secondary hyperparathyroidism are numerous and include "classic effects" of PTH excess on kidney, bone, cardiovascular and erythropoetic system, as well as effects on other "non classic" targets, such as some components of endocrine system (pancreas, adrenal cortex, testis and pituitary gland). The treatment of secondary hyperparathyroidism with calcimimetics, new agents which potentiate the effects of Ca^{2+}o on the CaR, besides reducing PTH synthesis and secretion, could make possible the identification of secondary effects of Ca^{2+}o on tissues not involved in Ca^{2+} homeostasis, such as pancreatic islets[12].

Patient population

Overall number of subjects involved in study was 70. Twenty seven of them were stable, end-stage renal patients (17 males and 10 females), on chronic HD program - 4 hours three times weekly, with HD duration more than six months. Exclusion study criteria included history of diabetes mellitus, malignancy and cardiac and vascular failure. The causes of end-stage renal disease were glomerular disease, chronic pyelonephritis, polycystic renal disease, urolithiasis and hypoplastic kidney in 11, 7, 4, 4 and one patient, respectively. They were never submittied to total or subtotal parathyreoidectomy nor received pulse intravenous high-dose active vitamin D treatment. Control group consisted of 43 healthy individuals (23 males and 20 females) without renal failure, diabetes or any serious cardiorespiratory disease.

Design of study

The study was organized as randomized cross-sectional trial. Each patient was treated with dialyzers containing membranes of cuprofan and polysulfone, and bicarbonate dialysates with calcium concentration of 1.75mmo/l. Patients received calcium carbonate or aluminium hydroxide as phosphate binders. Oral active vitamin D (1,25-dihydroxyvitamin D3) was administered to patients at dosages 0.25 to 0.5 μg/d, if hyperphosphatemia was less than 1.8 mmol/L. With higher serum iP concentration, phosphate binder therapy was only intensified. Serum intact parathyroid hormone level (iPTH) measurement revealed relative hypoparathyroidism (iPTH <200 pg/ml) in 9 (33.3%)(Gr1) hyperparathyroidism (iPTH≥200 pg/ml) in 18 (66.6%)(Gr2) of 27 subjects, with mean age of 53.7±8.3 and 49.5±7.0 years respectively. The HD duration did not differ beteween two groups (67±15 months in Gr 1 and 79 ±38 months in Gr 2). Mean age of control group (Gr 3) was 50.3±9.6 years.

Methods

Blood samples were obtained during midweek, after 12hrs fasting and just-before-dialysis, for the following variables: serum glucose, insulin, C-peptide, iPTH, total serum proteins, albumine, BUN, creatinine, serum Ca^{2+} and inorganic phosphate (iP), which were measured by standard labora-

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tory methods. iPTH was measured using radioimmunoassay method (CIS Biointernational) and insuline and C-peptide were measured using an radioimmunoassay method (INEP Zemun, Belgrade). The intra-assay and inter-assay coefficients of variation of all analytical assays were <.6% and < 8%, respectively. Estimates of pancreatic beta-cell function and relative insulin resistance were calculated from fasting insulin and glucose concentration using Homeostatic Model Assessment score [HOMA BETA(%)= fasting insulin (mU/L) x 20/fasting serum glucose (mmol/L)-3.5 and HOMA IR ($mU \cdot L^{-1}$)= fasting insulin (mU/L) x serum glucose (mmol/L)/22.5]. Anthropometric measurements were done by one observer. The percentage of body fat mass and fat-free mass was estimated from skinfold measurements. Ultrasonography of enlarged parathyroid glands was conducted with an Acuson 128 Computed Sonography System using a 7.5MHz linear array transducer.

Statistical analysis

Data are presented as means±SD. Differences between groups were evaluated by unpaired two-tailed Student's t tests and one-way ANOVA with Bonferroni or Tukey multiple comparison post-test. Linear regression models were used to explore relationship between HOMA BETA or HOMA IR and other variables.

Results

The clinical characteristics of the participating subjects are reported in Table 1.

Table 1. Clinical and biochemical characteristics of relative hypoparathyroid (iPTH<200pg/ml) and hyperparathyroid (iPTH≥200pg/ml) HD patients

	iPTH<200pg/ml	iPTH≥200pg/ml	p	Control	p
N	9	18		43	0.00
Mean age	53.6±8.3	49.5±7.0	ns	48.2±6.5	ns
Dialysis duration	67.1±14.7	78.9±38.2	ns		
BMI	23.3±2.1	22.6±3.1	ns	23.5±2.3	ns
Albumin	38.0±5.0	38.3±3.6	ns	41.1±2.1	0.01
Creatinine	635.5±179.8	625.5±187.6	ns	94.5±6.4	0.00
Glucose	5.0±0.9	5.2±0.7	ns	4.5±0.4	0.02
Insulin	15.2±5.5	20.1±7.8	ns	9.8±4.4	0.00
C-peptide	2.18±1.0	2.25±0.9	ns	0.91±0.3	0.00
HOMA IR	3.2±1.3	4.8±2.4	0.05	1.7±0.8	0.00
HOMA BETA	391.6±667.7	266.9±121.4	ns	167.5±139.7	0.02
Ca^{2+}	1.10±0.9	1.17±0.1	ns		
iP	1.80±0.3	1.89±0.4	ns		
Ca^{2+}x iP	5.1±0.8	5.2±0.7	ns		
iPTH	102.8±31.2	876.0±447.5	0.00		

There were no differences for age, sex, and HD duration between groups.When we compared the clinical profiles, besides significantly higher serum iPTH, insulin resistance (HOMA IR) was significantly, but only borderline, higher (p=0.054) in Gr2, and insulin secretion (HOMA BETA) did not differ significantly between groups. Mean values of HOMA IR (3.3±1.3 in Gr1, 4.8±2.4 in Gr2, 1.7±0.8 in Gr3) and glucose (5.0±1.0mmol/l in Gr1, 5.2±0.8mmol/l in Gr2, 4.6±0.4mmol/l in Gr3) of our HD patients were significantly higher than the values of the control group. There were no differences for fasting serum glucose, Ca^{2+}, iP and Ca x P product, BUN, creatinine and the indexes of body fatness, nutrition status and protein intake (skin folds, BMI, serum protein, albumin and nPCR) between groups. The HOMA BETA was higher in HD patients, but significantly in Gr2, only. There was significant negative correlation between HOMA IR and HD duration, as well as creatinine, but there was no correlation with other parameters in Gr1. HOMA IR correlated directly with serum iPTH, only in Gr2 (r=0.565, p=0.02). Some extent of correlation between HOMA IR and iPTH existed in Gr1, but negative and not significant. Within Gr3, HOMA IR correlated directly with BMI (r=0.45, p=0.002) and WHR (r=0.63,p=0.000). HOMA BETA inversely correlated with Ca x iP product in Gr1 (r= -

0.689, p=0.04). There was significant negative correlation, only between HOMA BETA and age (r= -0.594, p=0.01) and not significant with serum Ca^{2+}, in Gr 2. Serum iPTH positively correlated with serum Ca^{2+} (r= 0.489, p=0.03) and enlarged parathyroid glands volume (r= 0.556, p= 0.04) in Gr2, also.

Discussion

Insulin sensitivity and secretion has been investigated in subjects with end-stage renal failure (ESRF), and hyperinsulinemia with reduced insulin sensitivity and hyperglycemia were confirmed [1,2]. Since pure insulin level in ESRF represents the result of both secretion and elimination, because of reduced renal insulin clearance in ESRF patients, and reduced hepatic insulin clearance as in other subjects with insulin resistance, the presence of hyperinsulinemia is hall mark of those patients. We have also included measurement of C-peptide, as we reported earlier, whose value correlated excellently with serum insulin and/or homeostatic model of beta cell function, HOMA BETA. We have found reduced insulin sensitivity, in both group of patients, but the difference between them was of only borderline significance. In patients with relative hypoparathyroidism the index of insulin resistance correlated inversely only with HD duration and creatinine level. In group with secondary hyperparathyroidism reduced insulin sensitivity also existed, even in greater measure, and we have confirmed relationship between insulin resistance index and serum iPTH level. Our present findings of reduced insulin sensitivity in patients with secondary hyperparathyroidism corresponded with the results of other investigators[2,10,13]. Obesity, difference in body mass index, waist/hip ratio, or in the protein and albumin levels were excluded in both groups. Therapeutic and environmental factors, such as dietary calcium, vitamine D, calcitriol use and physical acitivity, were similar in both groups of our ESRF patients. Some studies had shown diminished expression of vitamin D receptors, particularly in hyperplastic parathyroid glands, induced by the action of low molecular weight substance in uremia[13]. The study of Kauzcki–Willer reported efect of biological active vitamine D on insulin sensitivity of periferal tissue, independent of PTH secretion[10]. Non-significant correlation of insulin sensitivity and serum PTH in group with relative hypoparathyroidism may be a consequence of small number of participants, better mineral and hormone homeostasis, or some other mechanism that might independently affect insulin sensitivity in relatively hypoparathyroid ESRF patients. One could speculate that expression of vitamine D receptor and Ca-sensing receptor may regulate PTH level and insulin sensitivity at the same time, independently[9]. The presence of oxidative stress as a consequence of higher plasma glucose and higher non-esterified free fatty acids (NEFA), could take part in mechanisms involved in insulin resistance of both groups of HD patients and correction of vitamin D depletion in secondary hyperparathyroidism has been followed by the reductions in NEFA concentrations and insulin resistance[11].

Beta cell function differed significantly from control subjects in hyperparathyroid group only. It was likely as compensatory effect to increased insulin resistance in the same patients. Index HOMA BETA was higher in relatively hypoparathyroid patients, but not significantly. Etiology of beta cell dysfunction in ESRF is not clear, but it is believed that overactivation of the CaR inhibits basal and nutrient-stimulated insulin secretion [4,5] In our patients, in relatively hypoparathyroid group, index HOMA BETA correlated negatively only with Ca^{2+} x iP product, but in hyperparathyroid group HOMA β correlated non-significantly with serum Ca^{2+}. Higher level of serum glucose, which was present in both groups of patients, could be accused for glucose toxicity, and is potential modulator of insulin secretion, too. Early correction of vitamine D depletion has been shown to restore both insulin secretion and normoglycemia in dialysis patients developing glucose intolerance[11]. So, we could suppose that low level of active vitamin D and/or high extracellular Ca^{2+}, or high Ca^{2+}x iP product were crucial in beta cell dysfunction in those patients. As we know extracellular Ca^{2+} influx through VDCC, raises Ca^{2+}i levels and triggers insulin secretion. So, negative correlation of beta cell secretion and serum ionized Ca and Ca^{2+}x iP product, assesed in HD patients, represents CaR-mediated inhibitory auto-regulatory mechanism which protects beta cells from overstimulation.We have demonstrated preserved beta cells function in both groups of our patients, which implies relatively good sensitivity of CaR in beta cells to extracellular calcium. On the other hand, in parathyroid cells of hyperparathyroid patients we have expected abnormal calcium sensing.

In conclusion, the present study has demonstrated the presence of higher level of serum insulin and insulin resistance in HD patients, as assessed by the calculation of HOMA IR score. The patients with severe hyperparathyroidism have shown greater level of insulin resistance, and we have established the relationships between insulin resistance and iPTH level. Serum iPTH correlated directly with serum Ca^{2+}, only in hyperparathyroid patients. Beta cell function was overexpressed in both groups, but did not differ significantly from the control group. It seems that parathyroidectomy might restore insulin sensitivity and partly beta cell function, in our hyperparathyroid patients. The number of patients in relatively hypoparathyroid group of HD patients is too small for any conclusion.

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