Role of secondary hyperparathyroidism in the genesis of hypertriglyceridemia and VLDL receptor deficiency in chronic renal failure

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Role of secondary hyperparathyroidism in the genesis of hypertriglyceridemia and VLDL receptor deficiency in chronic renal failure. Recent studies have revealed marked down-regulation of hepatic lipase (HL), lipoprotein lipase (LPL) and very low density lipoprotein-receptor (VLDL-R) expressions in animals with chronic renal failure (CRF). Acquired deficiency of these proteins, which together play an important role in catabolism of triglyceride-rich lipoproteins, is involved in the pathogenesis of CRF hypertriglyceridemia. Down-regulation of HL and LPL expressions in CRF can be completely reversed by parathyroidectomy (PTx), suggesting the role of excess parathormone (PTH). However, the role of hyperparathyroidism in the pathogenesis of CRF-induced VLDL-R deficiency has not been investigated before, and was studied here. To this end, VLDL-R mRNA (Northern analysis) and VLDL-R protein (Western analysis) of the fat pad and soleus muscle were compared in CRF (5/6 nephrectomized) rats, CRF animals with PTx (CRF-PTx) and sham-operated control animals. The CRF animals exhibited marked hypertriglyceridemia coupled with significant reductions in skeletal muscle and adipose tissue VLDL-R mRNA abundance and protein mass. Parathyroidectomy resulted in a significant, but partial, amelioration of CRF hypertriglyceridemia. However, in contrast to its effect on HL and LPL expressions, PTx did not improve VLDL-R expression, suggesting a PTH-independent mechanism for the latter abnormality. The differential effect of PTx on HL and LPL on the one hand and VLDL-R on the other can, in part, account for partial as opposed to complete correction of the associated hypertriglyceridemia with PTx in the CRF animals.

Very low density lipoprotein-receptor (VLDL-R) is a member of the large low density lipoprotein (LDL) receptor family, which despite substantial structural homology, is distinctly different from the LDL receptor with respect to its ligand binding properties and tissue distribution. Accordingly, VLDL-R is primarily expressed in skeletal muscle, heart and adipose tissue, and specifically binds and internalizes Apo E-containing lipoproteins, but does not bind LDL particles. In contrast, LDL receptor is specifically binds and internalizes Apo E-containing lipoproteins, primarily expressed in the liver, adrenal glands and gonads and binds lipoproteins through Apo-B100 in LDL particles or Apo E but does not bind LDL particles. In contrast, LDL receptor is specifically binds and internalizes Apo E-containing lipoproteins, primarily expressed in the liver, adrenal glands and gonads and binds lipoproteins through Apo-B100 in LDL particles or Apo E but does not bind LDL particles. The role of the large low density lipoprotein-receptor (LDL-R) family, which despite substantial structural homology, is distinctly different from the LDL receptor with respect to its ligand binding properties and tissue distribution. Accordingly, LDL-R is primarily expressed in skeletal muscle, heart and adipose tissue, and specifically binds and internalizes Apo E-containing lipoproteins, but does not bind LDL particles. In contrast, LDL receptor is specifically binds and internalizes Apo E-containing lipoproteins, primarily expressed in the liver, adrenal glands and gonads and binds lipoproteins through Apo-B100 in LDL particles or Apo E but does not bind LDL particles. The role of hyperparathyroidism in the pathogenesis of CRF-induced VLDL-R deficiency has not been investigated before, and was studied here. To this end, VLDL-R mRNA (Northern analysis) and VLDL-R protein (Western analysis) of the fat pad and soleus muscle were compared in CRF (5/6 nephrectomized) rats, CRF animals with PTx (CRF-PTx) and sham-operated control animals. The CRF animals exhibited marked hypertriglyceridemia coupled with significant reductions in skeletal muscle and adipose tissue VLDL-R mRNA abundance and protein mass. Parathyroidectomy resulted in a significant, but partial, amelioration of CRF hypertriglyceridemia. However, in contrast to its effect on HL and LPL expressions, PTx did not improve VLDL-R expression, suggesting a PTH-independent mechanism for the latter abnormality. The differential effect of PTx on HL and LPL on the one hand and VLDL-R on the other can, in part, account for partial as opposed to complete correction of the associated hypertriglyceridemia with PTx in the CRF animals.

METHODS

Animal models

Male Sprague-Dawley rats weighing 225 to 250 g were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN, USA). They were housed in a climate-controlled, light-regulated facility with 12-hour day (~500 lux) and 12-hour night (<5 lux) cycles. The animals were fed regular rat chow (Purina Mills, Brentwood, MO, USA) and water ad libitum. They were then randomly assigned to the following groups:
(1) Chronic renal failure group (CRF). The animals assigned to the CRF group were subjected to 5/6 nephrectomy by surgical resection, using a dorsal incision as described previously [12].

2. Chronic renal failure-parathyroidectomy group (CRF-PTx). Animals assigned to this group were first subjected to surgical parathyroidectomy (PTx) without removing the thyroid tissue. The procedure was carried out by electrocautery under a surgical microscope as described by Ni et al [14]. The success of the procedure was ascertained by demonstrating a fall in serum calcium concentration by at least 2 mg/dl below the baseline following PTx. The animals failing this test were excluded. In an attempt to dissect the effect of PTx from that of the associated hypocalcemia, the drinking water supply for these animals was supplemented with calcium gluconate at a concentration of 50 g/liter. This intervention was sufficient to restore normocalcemia despite parathyroid ablation in these animals as demonstrated by other investigators [14]. The animals were then allowed to recover for one week after, which they were subjected to 5/6 nephrectomy as noted above.

(3) Control group. The animals assigned to the control group were subjected to sham operation and were provided free access to food and water.

The study groups were observed for five weeks at which point they were placed in metabolic cages for a 24-hour urine collection. They were then sacrificed between the hours of 9 a.m. and 11 a.m. by exsanguination using cardiac puncture. Soleus muscle and suprasternal fat pad were harvested immediately, snap frozen in liquid nitrogen and stored at −70°C until processed. Total RNA was prepared from 1 g of tissue with RNAzol using the manufacturer’s recommended procedure (Tel-Test Inc., Friendswood, TX, USA) as previously described [8]. RNA concentration was determined from the absorbance at 260 nm using a spectrophotometer (Gene-Quat; Bio-Rad, Hercules, CA, USA). Twenty-five microgram aliquots of total RNA were denatured in 2.2 M formaldehyde at 65°C for 15 minutes and run on 1.0% agarose formaldehyde gel at 46 V for four hours. The separated RNA was transferred to the nylon membrane (Zeta probe; Bio-Rad) by capillary blotting in 6 × SSC buffer overnight and immobilized by UV irradiation (Ultraviolet Crosslinker; Fisher Scientific, Pittsburgh, PA, USA). The membrane was incubated at 65°C in a solution containing 5 × SSPE, 5 × Denhard’s, 1% SDS and 100 μg/ml salmon sperm DNA for two hours. The cDNA probe for rat glyceraldehyde phosphate dehydrogenase (GAPDH) (1.3 Kb PstI fragment) was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). The cDNA probe for VLDL-R was prepared as described above. The probes were labeled with [32P] dCTP (3000 Ci/mmol; New England Nuclear Inc., Boston, MA, USA) by the random primer method (Promega Inc., Madison, WI, USA). Hybridization was carried out at 60°C in a prehybridization solution with 32P labeled cDNA. The blots were washed twice in 2 × SSPE/0.5% SDS solution at room temperature, twice in 1 × SSPE/0.5% SDS solution at 37°C, and twice in 0.1 × SSPE/0.5% SDS solution at 65°C, for 15 minutes each. The washed blots were exposed to X-ray film (New England Nuclear Inc.) at −80°C for two to six hours for GAPDH and two to three days for VLDL-R. The autoradiographs were scanned with a laser densitometer (Molecular Dynamics, Sunnyvale, CA, USA) to determine relative mRNA levels. The values obtained for constitutively expressed GAPDH gene were used as an internal control.

Monoclonal antibody production

The hybridoma cell line, IgG-6A6, producing mouse monoclonal antibody recognizing the cytoplasmic domain of the VLDL-R from various tissues and species was purchased from ATCC. The hybridoma was grown in Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL Products, Gaithersburg, MD, USA) supplemented with 10% fetal calf serum (FCS). The cells were then subcultured and allowed to overgrow until cell death occurred. The supernatant containing the antibody was harvested and stored.

Western blot analysis

Muscle and fat cell plasma membranes were prepared as follows: frozen rat tissues were homogenized in 20 mM Tris-HCl (pH 7.5) containing 2 mM MgCl2, 0.2 mM sucrose, 5 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 10 μg/ml aprotinin and 3 μg/ml pepstatin A. The crude extracts were centrifuged at 3000 × g for 10 minutes at 4°C. The supernatant was then centrifuged at 35,000 × g for 30 minutes. The crude membrane preparations were washed with the above buffer and centrifuged at 35,000 × g for 45 minutes at 4°C. Protein concentration was
determined using a Bio-Rad Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). One-hundred microgram protein aliquots were size-fractionated on 4 to 12% Tris-glycine gel (Novex, San Diego, CA, USA) at 120 V for two hours. After electrophoresis, proteins were transferred to Hybond-ECL membrane (Amersham Life Science Inc., Arlington Heights, IL, USA). The membrane was incubated for one hour in buffer A (1 × PBS, 0.1% Tween-20 and 8% nonfat milk) and then overnight in the same buffer to which 1:10 dilution of the above antibody preparation were added. The membrane was washed once for 15 minutes and then twice for five minutes in 1 × PBS, 0.1% Tween-20 prior to a one hour incubation in buffer A to which diluted (1:1000) horseradish peroxidase-linked sheep anti-mouse IgG (Amersham) was added. The washes were repeated before the membranes were developed with chemiluminescent agents (ECL; Amersham) and subjected to luminography for one hour.

**Data analysis**

Analysis of variance (ANOVA) and Duncan’s multiple range test were performed in evaluation of the data. P values equal to or less than 0.05 were considered significant. Data are presented as mean ± SEM.

**RESULTS**

**General data**

As expected, the CRF animals showed a significant fall in creatinine clearance and a significant rise in arterial blood pressure. This was associated with a marked elevation of serum triglyceride concentration and modest increase in serum cholesterol level. Parathyroidectomy (PTx) led to a significant fall in serum triglyceride concentration from the high levels seen in CRF animals with intact parathyroid glands. However, PTx did not affect the serum triglyceride concentration prior to calcium gluconate supplementation. The results reported for lipoprotein lipase [12] and hepatic lipase [13] whose expressions markedly improved with PTx in the CRF animals did not improve with PTx, contrasting with the results reported for lipoprotein lipase [12] and hepatic lipase [13] whose expressions markedly improved with PTx in the CRF animals. Impaired VLDL-R mRNA abundance in the CRF animals did not improve with PTx, contrasting with our earlier studies [8]. In addition, fat tissue VLDL-R mRNA abundance (not studied before) was significantly reduced in the CRF animals. Impaired VLDL-R mRNA abundance in the CRF animals with surgical parathyroidectomy did not improve with PTx, contrasting with the results reported for lipoprotein lipase [12] and hepatic lipase [13] whose expressions markedly improved with PTx in the CRF animals. Data are depicted in Figures 1 and 2.

**Very low density lipoprotein-receptor mRNA abundance**

Compared to the control group, the CRF animals showed a marked reduction in the skeletal muscle VLDL-R mRNA abundance consistent with our earlier studies [8]. In addition, fat tissue VLDL-R mRNA abundance (not studied before) was significantly reduced in the CRF animals. Impaired VLDL-R mRNA abundance in the CRF animals did not improve with PTx, contrasting with the results reported for lipoprotein lipase [12] and hepatic lipase [13] whose expressions markedly improved with PTx in the CRF animals. Data are depicted in Figures 1 and 2.

**DISCUSSION**

The present study confirmed the earlier observation reported by our laboratory that CRF leads to marked down-regulation of skeletal muscle VLDL-R mRNA and protein mass. In addition, the present study revealed, for the first time, that VLDL-R deficiency in CRF is not confined to the muscle tissue that uses VLDL for energy production, but it also affects adipose tissue.
which serves as an energy storage facility. Thus, the effect of CRF on VLDL-R expression is diffuse and not tissue specific. Similar diffuse and depressive effects were previously noted with LPL in all tested tissues in the CRF animals [11]. This effect of CRF is distinct from those of the physiological regulators such as thyroid hormone, exercise, feeding and fasting states that modulate LPL expression in the fat and muscle tissues in the opposite directions. Thus, the effect of uremia on expression of these lipid regulatory factors represents a pathophysiological as opposed to a physiological phenomenon.

In contrast to its effect on LPL and hepatic lipase expression, PTx did not improve VLDL-R expression in the CRF animals. Thus, CRF-induced down-regulation of VLDL-R expression must involve a different mechanism(s) than that of LPL and HL expressions. Given the distinct structural and functional differences of VLDL-R from HL and LPL, it is not surprising that their dysregulation in CRF may involve different mechanisms.

Interestingly, while improving hypertriglyceridemia, PTx did not restore normal triglyceride concentration in the CRF animals. This partial, but not complete, amelioration of CRF-hypertriglyceridemia with PTx can be, in part, explained by its divergent action on HL and LPL on the one hand and VLDL-R on the other. Accordingly, correction of HL and LPL deficiencies by PTx can account for the partial reduction in plasma triglycerides in CRF-PTx animals. However, persistent VLDL-R deficiency in CRF-PTx animals can, in part, account for the residual hypertriglyceridemia in these animals. The authors wish to acknowledge that the present study of VLDL-R and earlier studies of LPL [12] and hepatic lipase [13] have specifically dealt with the effects of excess PTH on the gene expressions of three important proteins involved in VLDL and triglyceride catabolism. Consequently, several other important questions such as the possible effects of excess PTH on triglyceride and VLDL synthesis, VLDL—VLDL-R affinity and lipoprotein composition, have not been addressed in these studies and await future investigations.

According to a recent study, CRF is associated with depressed expression of receptors for parathormone (PTH—PTHrP receptor), angiotensin II (AT₁ receptor), vasopressin (V₁a receptor), which act through the G-protein-adenylcyclase-cAMP-dependent calcium signaling pathway [15]. The down-regulation of these receptors and the associated impaired calcium signaling in response to their respective agonists improved by either PTx or calcium channel blockade, which reversed the abnormal elevation of cytosolic [Ca²⁺] in the CRF animals. Thus, by raising the cytosolic [Ca²⁺], excess PTH plays a major role in down-regulation of several hormone receptors [15] and enzymes [12, 13] in CRF. However, based on the present data, excess PTH does not appear to be involved in down-regulation of VLDL-R in CRF. Further studies are required to discern the mechanism(s) responsible for depressed VLDL-R expression in chronic renal insufficiency.

ACKNOWLEDGMENT

The authors are grateful to Mr. Thomas Yuen for his generous support of this project.
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