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Research Article

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Abnormalities in Hepatic Lipase in Chronic Renal Failure

Role of Excess Parathyroid Hormone

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Abstract

Post-heparin hepatic lipase activity is reduced in chronic renal failure (CRF). This could be due to reduced synthesis, decreased activity, and/or impaired secretion of the enzyme. Further, the factor(s) responsible for such derangements are not elucidated. We examined hepatic lipase metabolism in normal, 6-wk-old CRF rats, CRF-PTX (parathyroidectomized) rats, and CRF and normal rats treated with verapamil (CRF-V, normal-V) using liver homogenate, hepatic cell culture for 8 h, and in vitro liver perfusion. The V_{max} of hepatic lipase in liver homogenate was significantly ($P < 0.01$) reduced and the K_m was significantly ($P < 0.01$) increased in CRF rats, but the values were normal in CRF-PTX, CRF-V, and normal-V rats. Culture of hepatic cells for 8 h was associated with an increase in hepatic lipase activity but the increment in CRF rats was significantly ($P < 0.01$) lower than that of normal, CRF-PTX, CRF-V, and normal-V rats. Both parathyroid hormone (PTH)-(1–84) and 1–34 inhibited the production of hepatic lipase in cultured cells from normal, CRF-PTX, CRF-V, and normal-V rats. The expression of the mRNA of the hepatic lipase was significantly reduced in CRF animals with the ratio between it and that of house keeping gene G3DPH being 15±3% compared to 40±1.3% in normal, 44±2.9% CRF-PTX, 44±5.4% in CRF-V, and 39±3.9% in normal-V rats. Infusion of heparin to the in vitro hepatic perfusion system increased the activity of hepatic lipase in the effluent in all groups of rat except in CRF animals. Infusion of PTH-(1–34) in dose of 10^{-6} M into the liver perfusion system inhibited the increase in post-heparin hepatic lipase activity. The data show that in CRF (*a*) the mRNA of hepatic lipase is downregulated, and hepatic lipase production, activity and release are impaired, (*b*) that this is due to the state of secondary hyperparathyroidism of CRF since both acute and chronic excess of PTH were associated with these abnormalities, (*c*) and that prevention of excess PTH by PTX of CRF rats or blocking the effect of PTH by treatment with verapamil corrected the derangement in hepatic lipase metabolism. (*J. Clin. Invest.* 1996. 97:2167–2173.) **Key words: parathyroid hormone • uremia • calcium channel blockers • calcium • hepatic lipase**

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Introduction

Chronic renal failure (CRF)[1] is associated with hyperlipidemia (1–5) due in major part to impaired removal of triglycerides from plasma (3–5). Both lipoprotein lipase and hepatic lipase are involved in the removal of triglyceride from plasma (6, 7). We have found that hepatic lipase activity after injection of heparin is reduced in CRF (5). This defect was apparently due to the rise in calcium content of the liver mediated by the state of secondary hyperparathyroidism of CRF (5). An increase in calcium content of the liver may reflect an elevation in cytosolic calcium ($[Ca^{2+}]i$) of hepatocytes. Indeed, CRF is associated with sustained elevation in $[Ca^{2+}]i$ of many cells (8), including hepatocytes (9), and the high $[Ca^{2+}]i$ is a major factor underlying cell dysfunction in CRF (8).

A decrease in the activity of hepatic lipase in CRF could be due to a decrease in the production of the enzyme, an inhibition of the enzyme activity, an impairment in its release from the liver, or to any combination of such potential derangements. These possibilities have not been fully elucidated.

Recent data have demonstrated that the elevation in $[Ca^{2+}]i$ downregulates the mRNA of many proteins such as the receptors of parathyroid hormone (PTH)-PTHrP, angiotensin II, or vasopressin in hepatocytes (10) and of PTH-PTHrP in kidney (10–12) and heart (13). It is theoretically possible that the elevation in $[Ca^{2+}]i$ of hepatocytes in CRF exerts a similar effect on the mRNA of hepatic lipase. Such a potential action could cause a decrease in the production of the enzyme.

The present study evaluated the effect of CRF on the mRNA of hepatic lipase, on the production of the enzyme by hepatocytes, the activity of the enzyme, and on its release by the liver. We also explored the effect of the state of secondary hyperparathyroidism of CRF on these parameters.

Methods

A total of 180 male Sprague Dawley rats weighing between 290 and 390 g (340±2.8 g) were studied. They were fed normal rat laboratory diet (Wayne Research Animal Diets, Chicago, IL) and allowed to drink water ad libitum. The diet contained 1.4% calcium, 0.97% phosphorus, and 4.4 IU of vitamin D per g. Studies were performed in five groups of animals: (*a*) normal rats, (*b*) rats with CRF of 6-wk duration, (*c*) normocalcemic parathyroidectomized CRF rats (CRF-PTX) of 6-wk duration, (*d*) CRF rats of 6-wk duration treated with verapamil (0.1 μg/g body wt), which was given subcutaneously twice a d from day 1 of CRF (CRF-V), and (*e*) normal rats treated with verapamil as described above for 6 wk (normal-V).

CRF was produced by a five-sixths nephrectomy; the animals underwent two-thirds nephrectomy of the right kidney through a flank incision, and 1 wk later, a left nephrectomy was done. PTX was per-

1. *Abbreviations used in this paper:* $[Ca^{2+}]i$, cytosolic calcium; CRF, chronic renal failure; PTH, parathyroid hormone; PTX, parathyroidectomized; V, verapamil.

formed by electrocautery, and the success of the procedure was ascertained by a decrease in plasma levels of calcium of at least 2 mg/dl. This procedure does not produce significant damage to the thyroid glands, which remain intact. The PTX rats were allowed to freely drink water containing 5% calcium gluconate. This procedure is adequate to normalize plasma calcium in the PTX rats. 7 d after PTX, the rats were subjected to five-sixths nephrectomy as described above. 2 d before the animals were killed, they were housed in metabolic cages, and two consecutive 24-h urine collections were obtained for the measurement of creatinine clearance. Animals were killed by decapitation on day 42 after the completion of the five-sixths nephrectomy in CRF rats (CRF, CRF-PTX, CRF-V) or after the beginning of the treatment with verapamil in normal rats.

Hepatocytes were isolated using a modification of the method of Seglen (14). The details of this procedure have been previously reported from our laboratory (15). The cells displayed well-preserved refringent shape with intact boundaries. There were no differences in the appearance of the hepatocytes obtained from normal and CRF animals as reported previously (9). Viability of the cells was > 90% as assessed by the trypan blue exclusion test.

Hepatocytes (2.5×10^6) from all groups of animals were suspended in an incubation media containing in mM; 5.4 KCl, 0.44 KH_2PO_4, 0.98 $MgCl_2$, 0.8 $MgSO_4$, 137 NaCl, 1.33 Na_2HPO_4, 1.33 $CaCl_2$, and 20 Hepes, 2 mg/ml of DL-myo-inositol 1 monophosphate, 10 mg/ml of BSA, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5% vol/vol heat-inactivated FCS, pH 7.4; the medium was supplemented with amino acids and vitamins as described by Soler et al. (16). The cell suspension was plated in a standard flat bottom, 96-well microplate (Dynatech Laboratories Inc., Alexandria, VA) and incubated at 37°C under O_2/CO_2 (19:1) atmosphere in an incubator (Forma Scientific Inc., Marietta, OH) with continuous shaking. Incubation was carried out in the presence and absence of 5 U/ml of heparin (ICN Biomedicals Inc., Irvine, CA), or various concentrations (10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M) of PTH-(1–84) or PTH-(1–34), 400 μM cAMP, or 100 μM epinephrine as a positive control. After 8 h of incubation, the cell suspension was centrifuged at 1,000 *g* for 10 min with a refrigerated centrifuge (Model PR-7000M; International Equipment Company, Needham, MA). The supernatant was removed and stored at −70°C. The pellet was resuspended in 150 μl of the incubation medium and homogenized by sonication with Braunsonic 1510 sonicator (B. Braun Instrument, Melsungen, Germany) and centrifuged at 1,000 *g* for 10 min and the supernatant was removed and frozen.

The activity of hepatic lipase in both the supernatant and the hepatocytes before and after 8 h of incubation was estimated in the presence of 1.5 μM of triolein, a concentration which provides for the V_{max} of the enzyme; the values are expressed as μmol/10^6 cells per 8 h.

In another study, the whole liver from all groups of animals was removed and weighed. They were then cut into small pieces and homogenized in 30 ml of a buffer containing in mM: 1.0 dithiothreitol, 1.0 EGTA, and 10 Hepes, and 0.25 M sucrose, pH 7.4, with a Dounce-type glass homogenizer (Wheaton Scientific, Millville, NJ) with a clearance of 0.34 mm, 20 times. The homogenate was centrifuged at 1,000 *g* for 10 min with a refrigerated centrifuge. The supernatant was removed for the determination of the hepatic lipase activity.

The assay of the hepatic lipase activity was done according to the method reported by Ehnholm and Kuusi (7). We first prepared the substrate for the assay as follows: radioactive tri (1-^{14}C) oleylglycerol (Amersham Corp., Arlington Heights, IL) was diluted in toluene to provide 2 Ci/ml; 5 ml of this solution was added to 5 ml of nonradioactive triolein (20 mg/ml) (Sigma Chemical Co. St. Louis, MO.) in conical tube, and the solvent was evaporated under nitrogen gas. The solution was then mixed three times with 3 ml of haptane (Sigma Chemical Co.), and solvent was evaporated under nitrogen gas between washes. To prepare substrate emulsion, 7.5 ml of 5% arabic gum solution was added to the tube. The microtip of the Braunsonic sonicator–cell disrupter was placed 0.5 cm below the surface of the mixture which was then sonicated in an ice bath for 4 min. After sonication, 5 ml of 10% BSA solution was added, and the tube was agitated with a vortex mixture. Adequate amount of the substrate (0.125, 0.250, 0.500, 0.750, 1.500, and 3.00 μM of triolein) was added to 250 μl of 0.2 M Tris HCl buffer, pH 8.5. The buffer contained 2.0 M NaCl, 3% vol/vol FCS heated for 60 min at 50°C. Also 50 μl of the supernatant from the liver homogenate was added to the mixture. The latter was incubated for 60 min at 37°C in water bath with constant shaking. The reaction was terminated by the addition of 3.5 ml of methanol/chloroform/haptane (145:125:100 vol/vol/vol). Oleate was extracted by the addition of 1.0 ml of 0.1 M borate/carbonate buffer, pH 10.5. The mixture was centrifuged for 15 min at 3,000 *g*. An aliquot of 1.0 ml of methanol fraction was aspirated and counted for radioactivity. Hepatic lipase activity was calculated as 1 mU which is equal to 1 μmol of oleate released/h per 1 g tissue.

In another set of experiments, liver perfusion studies were performed in all groups of animals. The chest was opened, and the aorta was cut and catheterized. Subsequently, the liver was perfused with 200 ml cold (4°C) oxygenated, calcium free Joklik media (Sigma Chemical Co.) supplemented with 10 mM Na Hepes, 10 mM glucose, 0.5% BSA, and 1 mM EGTA, pH 7.4, over 2 min. At the end of this procedure the liver was uniformly pale. Both superior and inferior vena cava were ligated, and a PE 20 tube was placed in the superior vena cava below the ligation. The liver was then removed and perfused through the vena cava catheter with warm (37°C) oxygenated, calcium Joklik medium at a rate of 15 ml/min. The effluent from the portal vein was collected. The first 50 ml of the Joklik medium were discarded. The perfusate then contained either 10^{-6} PTH-(1–34), heparin 5 U/ml, or both at variable sequences. The effluent was collected every 2 min and kept for assay of hepatic lipase. To stabilize the lipolytic activity, glycerol was added to the effluent to give a final concentration of 20%.

Total RNA was isolated from the liver by acid guanidinium thiocyanate-phenol-chloroform extraction described by Chomczynski and Sacchi (17) and later modified by them (18) using Trizol reagent. The yields of total RNA (mg/g tissue) from the liver of the various groups of animal were not significantly different (normal: 7.7±0.5; CRF: 6.7±0.74; CRF-PTX: 7.4±0.25; CRF-V: 7.5±0.46, and normal-V: 7.6±0.81).

The poly A^+ RNA of the liver was prepared from the total RNA according to the method of Ansubel et al. (19) as previously reported by us (10). The yield of poly A^+ RNA from total RNA was 3.7±0.37% in normal rats, 3.6±0.25% in CRF animals, 2.8±0.48% in CRF-PTX rats, 2.6±0.26% in CRF-V, and 2.8±0.42% in normal-V rats. These values were not statistically different.

pRHLG2-2 plasmid containing 1,640-bp insert for rat hepatic lipase CD1A in EcoR1 site of pGEM-2 vector (20) was kindly supplied by Dr. Michael C. Schotz of Veterans Administration Wadsworth Medical Center, Los Angeles, CA. The plasmid was cloned into competent DH5 α cells (Invitrogen Corp., San Diego, CA) in LB medium containing ampicillin. The isolated plasmid DNA was digested with the restriction endonuclease EcoRI, and the 1,640-bp fragment of the cDNA of hepatic lipase was recovered by gel electrophoresis.

An aliquot containing 30 ng of the fragment of the cDNA of the hepatic lipase was labelled with 5 μl of ^{32}P dCTP (10 mCi/ml; Amersham Corp.) using a Random Primed DNA Labelling Kit (Boehringer-Manneheim, Indianapolis, IN) and purified through G50 Sepharose (Sigma Chemical Co.).

Aliquot of 4 μg of poly A^+ RNA of the liver were placed in separate lanes of 1.2% agarose-formaldehyde gel and subjected to electrophoresis in 1× Mops buffer (20 mM Mops, 5 mM sodium acetate, and 10 mM EDTA, pH 7.4) at 100 V for 3 h. The separated poly A^+ RNAs were transferred to Hybond N^+ nylon paper (Amersham Corp.) with 20× SSC (1.5 M NaCl and 0.15 M sodium citrate, pH 7.5). The poly A^+ RNA was cross-linked to the membrane in a UV Strata Linker 1800 (Stratagene Corp., La Jolla, CA).

Prehybridization of the membrane was performed in 7% SDS, 1% polyethylene glycol, 2× SSPE (0.9 M NaCl, 40 mM NaOH, 50 mM NaH_2PO_4 and 5 mM EDTA, pH 7.4) for 2 h at 68°C (21). Subsequently, the membrane was hybridized for 16 h at 68°C with 10 ml of

Table I. Body Weight and Biochemical Parameters of All Groups of Animals

		Weight	Plasma Creatinine	Plasma Calcium	Plasma Phosphorus	Creatinine clearance	Serum PTH
	n	*g*		*mg/dl*		*μl/100 g body weight*	*pg/ml*
Normal	36	309±2.5	0.29±0.01	9.6±0.07	6.5±0.06	571±12	15±0.9
CRF	36	312±5.9	1.17±0.07*	9.5±0.09	7.0±0.09*	155±7.0*	71±4.6*
CRF-PTX	36	359±4.7*	1.00±0.05*	9.3±0.08	6.5±0.10	171±5.4*	14±1.4
CRF-V	36	351±4.3*	1.16±0.07*	9.5±0.12	7.0±0.12*	154±5.9*	80±7.3*
Normal-V	36	369±3.7*	0.29±0.01	9.5±0.09	6.5±0.06	556±12	17±1.4

Data are means±SE. *$P < 0.01$ versus other groups.

prehybridization solution plus the ^{32}P-labelled cDNA probes of the hepatic lipase (sp act 10^{-6} cpm/ml). The membrane was washed twice for 20 min each at 65°C with 1% SDS, 50 mM NaCl, and 12 mM EDTA. The membrane was then autoradiographed and analyzed by densitometric scanning (LKB Ultrascan IX; Bromma, Sweden). Northern blot analysis from the same poly A^+ RNA were performed using cDNA probe of G3PDH, (Clontech Laboratories Inc., Palo Alto, CA). The amount of mRNA of the hepatic lipase in the liver from animals with the various experimental conditions was normalized by calculating the ratio between the mRNA of the receptor and the G3PDH in the same tissue.

The measurement of calcium in plasma was made by Perkin Elmer atomic absorption spectrophotometer (model 503; Perkin Elmer Corp., Norwalk, CT) and those of plasma creatinine and phosphorus and urine creatinine by an autoanalyzer (Technicon Instrument Corp., Tarrytown, NY). The serum levels of PTH were determined by an ISN-PTH immunoassay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). This assay recognizes the amino-terminal fragment of PTH. The lowest detectable level is 3 pg/ml; the intraassay variation is 7.3%, and the interassay variation is 4%.

Bovine PTH-(1–84) (lot 10HO6661) and epinephrine were purchased from Sigma Chemical Co., and bovine synthetic PTH-(1–34) was obtained from Bachem (Torrance, CA). Bovine PTH-(1–84) was dissolved in 0.15 N acetic acid and synthetic bovine PTH-(1-34) in water and epinephrine was dissolved in DMSO and water.

Statistical analysis used paired or unpaired *t* test. Comparison between groups was assessed by ANOVA, and significance was determined with Bonferonni-Dunn test.

Results

Table I presents the body weights and the biochemical parameters of the five groups of animals studied. The body weight of CRF-PTX, CRF-V, and normal-V rats were modestly but significantly ($P < 0.01$) higher than those of normal or CRF animals. There were no significant differences in the concentrations of plasma calcium among the various groups of animals, but the concentrations of plasma phosphorus in CRF and CRF-V rats were modestly but significantly ($P < 0.01$) higher than in normal, CRF-PTX, and CRF-V rats. The CRF, CRF-PTX, and CRF-V animals had significantly ($P < 0.01$) higher levels of plasma creatinine and significantly ($P < 0.01$) lower values of creatinine clearance than in normal and normal-V rats. There were no significant differences in these two parameters among the three CRF groups of animals. The serum concentrations of PTH in CRF and CRF-V rats were significantly ($P < 0.01$) higher than those in normal, CRF-PTX, and normal-V animals.

Figs. 1 and 2 show that maximal velocity (V_{max}) and Michaelis constant (K_m) of hepatic lipase of liver homogenate. The V_{max} of the enzyme in CRF animals (121±5.8 μmol oleate/h per g tissue) was significantly ($P < 0.01$) lower than in normal rats (246±10.8 μmol oleate/h per g tissue). PTX of CRF rats or their treatment with verapamil prevented the impairment in

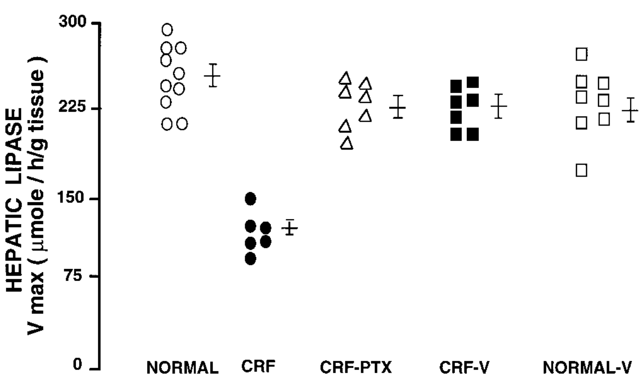

Figure 1. The V_{max} of hepatic lipase of liver homogenate from normal, CRF, CRF-PTX, CRF-V, and normal-V rats. Each data point represents one rat and brackets denote mean±1 SE. Values in CRF animals are significantly ($P < 0.01$) lower than those of the other groups of animals.

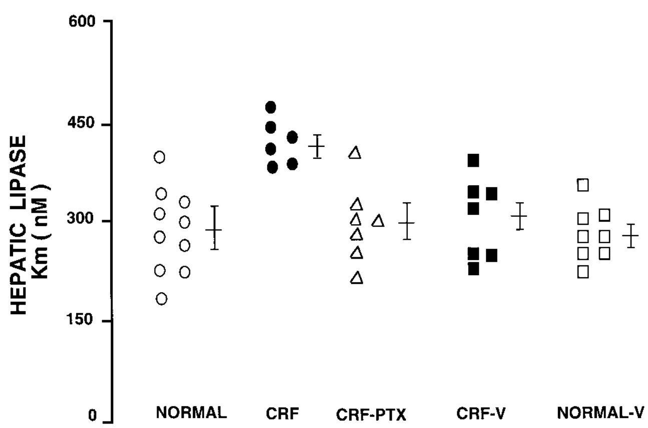

Figure 2. The K_m of hepatic lipase of liver homogenate from normal, CRF, CRF-PTX, CRF-V, and normal-V rats. Each data point represents one rat. Brackets denote mean±1 SE. Values in CRF animals are significantly ($P < 0.01$) higher than those of the other groups of animals.

nificant ($P < 0.01$) increment in the K_m of hepatic lipase (410±12 versus 270±24 nM in normal rats). Again PTX of CRF rats or their treatment with verapamil normalized the K_m of hepatic lipase (290±26 and 310±27 nM). Verapamil administration did not affect the K_m of the enzyme with the value being 280±18 nM.

Incubation of hepatocytes from normal rats for 8 h in the presence or absence of heparin was associated with increased activity (V_{max}) of the hepatic lipase in the supernatant. When the incubation was carried out in the absence of heparin and in the presence of PTH-(1–34) or PTH-(1–84), the activity of the enzyme was markedly ($P < 0.01$) reduced with 10^{-7} or 10^{-6} PTH (Fig. 3). Similar changes occurred with 10^{-9}–10^{-6} M PTH-(l–34). Similarly cAMP (400 μM) or 100 μM epinephrine caused a significant reduction in hepatic lipase activity in the supernatant as well as in hepatocytes (Fig. 3).

Table II depicts the data after the incubation of hepatocytes from normal, CRF, CRF-PTX, CRF-V, and normal-V for 8 h in the presence and absence of 10^{-6} M PTH-(1–84) with and without heparin. The hepatic lipase activity in both supernatant and cells after 8 h of incubation was significantly ($P < 0.01$) lower in hepatocytes from CRF animals than in the other four groups of rats, and the presence of PTH failed to reduce the activity of the enzyme in the CRF animals. In contrast, the activity of the enzyme in the supernatant of the studies with hepatocytes from CRF-PTX, CRF-V, and normal-V rats was not different from that observed with normal hepatocytes, and PTH-(1–84) did reduce the activity of hepatic lipase in these three group of animals. Similar phenomenon was observed in the hepatic lipase activity of the cells.

Fig. 4 depicts the results of the perfusion studies of liver from normal rats. The perfusion of the livers with heparin (5 U/ml) produced gradual and significant increment in the activity of hepatic lipase in the effluent reaching a peak by 12 min after the start of the heparin administration followed by a rapid decline almost to baseline values. The immediate reperfusion of these livers with heparin and 10^{-6} M PTH-(1–34) was not associated with an increase in hepatic lipase activity in the

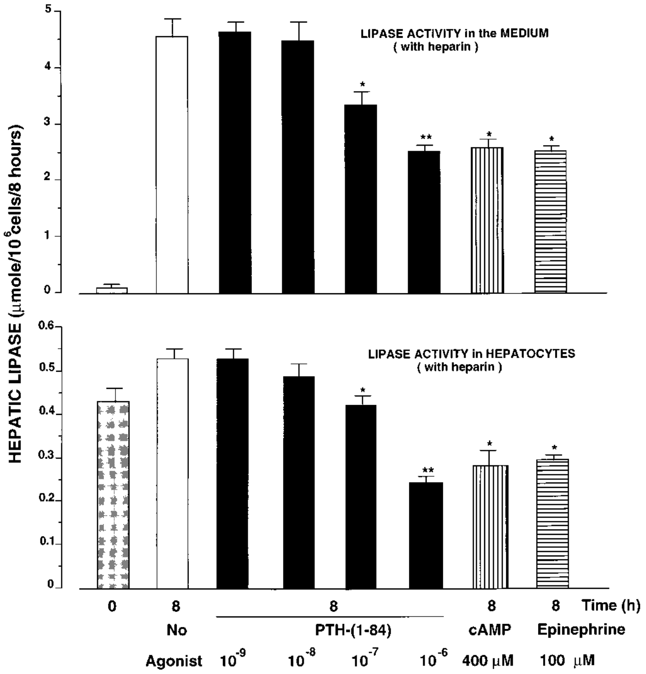

Figure 3. The activity of hepatic lipase after 8 h incubation of hepatocytes in the presence of heparin. The top panel depicts the values in the incubation media and the lower panel shows the values in the cells. Each column represents the mean of data obtained from seven to eight rats and brackets denote ±1 SE. *$P < 0.01$ versus no agonist, 10^{-9} and 10^{-8} M PTH; **$P < 0.01$ versus no agonist and 10^{-9}, 10^{-8}, and 10^{-7} PTH. Similar effects occurred with 10^{-9}–10^{-6} M PTH-(1–34). The data are not shown to maintain simplicity of the figure.

the V_{max} of hepatic lipase with the values being 221±6.7 μmol oleate/h per g tissue and 225±3.8 μmol oleate/h per g tissue, respectively. Treatment of normal rats with verapamil did not affect the V_{max} of hepatic lipase with the value being 223±12.2 μmol oleate/h per g tissue. CRF was also associated with a sig-

Table II. Effect of Incubation of Hepatocytes for 8 h in the Absence or Presence of PTH and Heparin on Hepatic Lipase Activity

		No heparin			5 U/ml heparin	
		No PTH	PTH - (1–84) 10^{-6} M		No PTH	PTH - (1–84) 10^{-6} M
	0	*8 h*	*8 h*	*0*	*8 h*	*8 h*
Hepatic lipase in supernatant (μmol/8 h/10^6)						
Normal (7)	0	4.5±0.32	2.5±0.14*	0	4.7±0.19	2.5±0.13*
CRF (8)	0	2.9±0.19‡	2.6±0.17‡	0	2.9±0.17‡	2.7±0.19
CRF-PTX (7)	0	4.4±0.18	2.5±0.18*	0	5.0±0.11	2.8±0.15*
CRF-V (8)	0	4.4±0.16	2.8±0.14*	0	4.4±0.15	2.8±0.07*
Normal-V (7)	0	4.6±0.23	2.3±0.14*	0	4.6±0.20	2.3±0.10*
Hepatic lipase in cells (μmol/8 h/10^6)						
Normal (7)	0.38±0.03	0.48±0.02	0.23±0.02*	0.43±0.02	0.52±0.01	0.24±0.01*
CRF (8)	0.17±0.01‡	0.28±0.01‡	0.25±0.02	0.18±0.01‡	0.27±0.01‡	0.25±0.02
CRF-PTX (7)	0.27±0.01	0.41±0.02	0.25±0.02*	0.27±0.02§	0.39±0.02	0.24±0.02*
CRF-V (8)	0.28±0.03	0.42±0.02	0.25±0.014*	0.29±0.07	0.40±0.01	0.23±0.01*
Normal-V (7)	0.36±0.02	0.46±0.01	0.26±0.014*	0.43±0.03	0.52±0.01	0.29±0.02*

Data are mean±SE, * $P < 0.01$ versus No PTH; ‡$P < 0.01$ versus other groups; §$P < 0.01$ versus normal and normal-V.

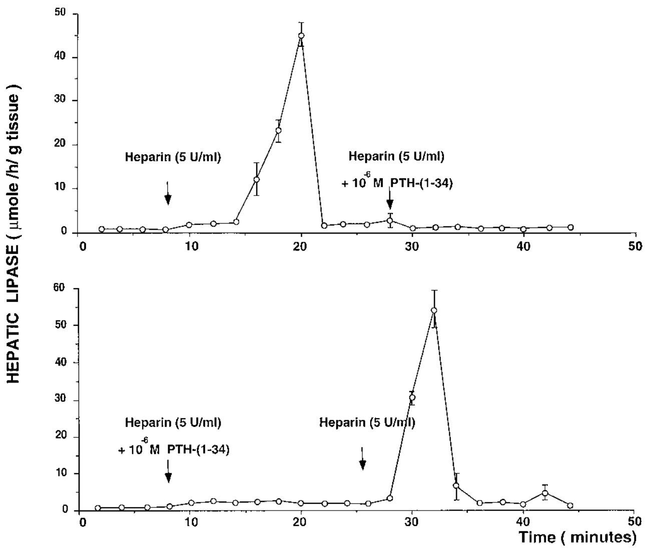

Figure 4. The activity of hepatic lipase in the effluent from the liver perfusion studies in normal rats. Each data point represents the mean data from six studies and brackets denote ±1 SE.

effluent. In another set of experiments where the livers were first perfused with heparin (5 U/ml) and 10^{-6} M PTH-(1–34), there was no increase in the hepatic lipase activity in the effluent over the 18 min of such perfusion. The immediate reperfusion of these livers with heparin (5 U/ml) alone produced a significant increment in hepatic lipase activity reaching a peak within 4 min followed by a decline toward baseline.

Fig. 5 depicts the results of the perfusion of the livers from CRF, CRF-PTX, CRF-V, and normal-V with heparin alone. The hepatic lipase activity in the effluent from livers of CRF animals was markedly reduced as compared to that obtained from livers of CRF-PTX, CRF-V, and normal-V rats. The values in the latter three groups were not different from values noted in normal animals.

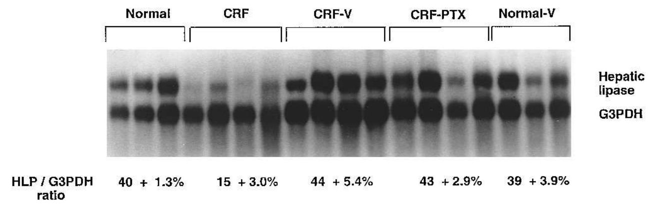

Figure 6. Northern blot analysis of the mRNA of hepatic lipase and G3DPH of liver from normal (lanes *1–3*), CRF (lanes *4–7*), CRF-V (lanes *8–11*), CRF-PTX (lanes *12–15*), and normal-V (lanes *16–18*) rats. Each lane contained 4 μg of poly A^+ RNA from different animals. The hybridization of the membrane containing poly A^+ RNA of the liver with hepatic lipase and G3DPH probes were done simultaneously. The exposure time of the auto radiographs was 48 h. The autoradiograph were scanned as described in Methods. The numbers provide the mean±1 SE of the density of the mRNA signal of hepatic lipase relative to that of G3DPH. The values in CRF are significantly ($P < 0.01$) lower than those in the other four groups of animals.

Fig. 6 depicts the expression of the mRNA of the hepatic lipase and G3PDH obtained from livers of the five group of animals studied. The ratio of the concentration of the mRNA of hepatic lipase to that of G3PDH in CRF rats (15.0±3%) was significantly lower than that of normal rats (40±1.3%), CRF-PTX animals (43±2.9%), CRF-V rats (44±5.4%), and normal-V animals (40±3.9%).

Discussion

The results of the present study demonstrate that CRF is associated with multiple disturbances in the metabolism of hepatic lipase including downregulation of the mRNA of the enzyme and impairment of hepatic lipase production, activity, and release. The data also show that these derangements are in major part due to the state of secondary hyperparathyroidism of CRF in that prior PTX of the CRF animals prevented these abnormalities in the metabolism of this enzyme.

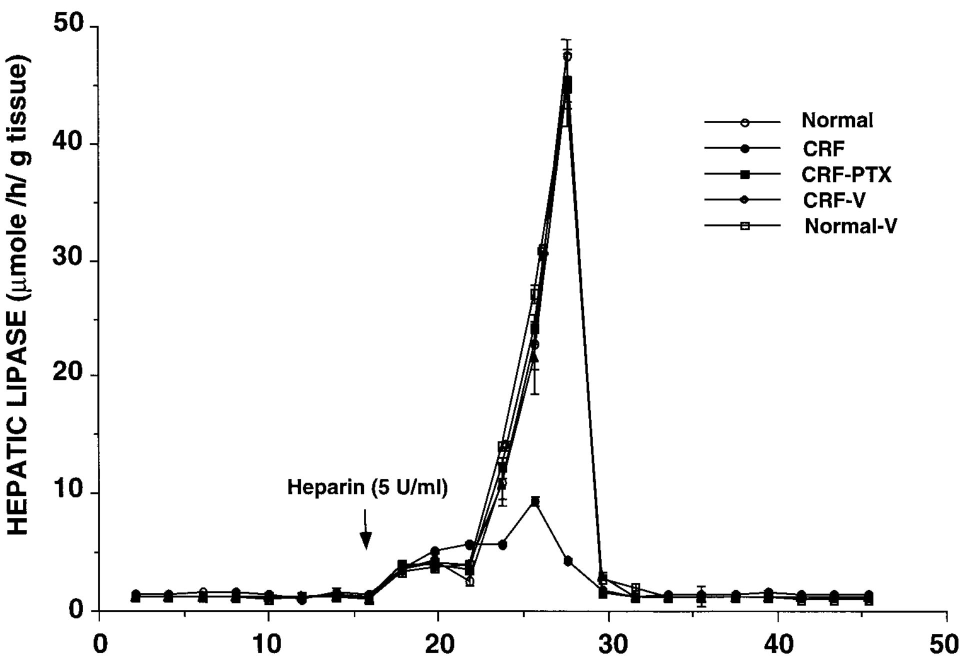

Figure 5. The activity of hepatic lipase in the effluent from liver perfusion studies in CRF, CRF-PTX, CRF-V, normal-V, and normal rats. Each data point represents the mean of data from 11–7 studies and brackets denote ±1 SE.

Chronic excess of PTH exerts its deleterious effect on cell function and metabolism through the hormone-mediated rise in the basal levels of $[Ca^{2+}]i$ (8). The chronic elevation of PTH, as in CRF, has been shown to cause a sustained elevation in $[Ca^{2+}]i$ of hepatocytes (9); it is therefore plausible to suggest that such an increase in $[Ca^{2+}]i$ is responsible for the derangements in hepatic lipase metabolism. Support for this notion is found in the observations obtained in CRF-V rats which have normal $[Ca^{2+}]i$ of hepatocytes (9) and normal hepatic lipase metabolism despite CRF and elevated levels of PTH.

It has been previously shown that the elevation in $[Ca^{2+}]i$ in hepatocytes in CRF is associated with downregulation of their mRNAs of the PTH-PTHrP, vasopressin (V1a), and angiotensin II (AT1) (10). The data of the present study provide another example where the elevation in $[Ca^{2+}]i$ is also associated with downregulation in mRNA of another protein, i.e., hepatic lipase. Indeed, the mRNA of the hepatic lipase in livers from CRF-V rats which have normal $[Ca^{2+}]i$ despite CRF and high serum PTH levels (9) is not downregulated. The mechanisms through which high $[Ca^{2+}]i$ exerts this affect are not, as yet, delineated. It could be due to impaired transcription or processing of the mRNA and/or an increase in its turnover. The observations of Yang and Tashjian (22) provided evidence that a rise in $[Ca^{2+}]i$ adversely affected the rate of gene transcription of thyrotropin-releasing hormone receptor in GH_4C_1 cells. Others have found that CRF is associated with increased degradation of albumin mRNA in liver of CRF rats (23). Thus, either one or both mechanisms may be at work to explain the reduction in the concentration of the mRNA of the hepatic lipase activity in CRF.

The data obtained after 8 h of incubation of hepatocytes showed that the activity of hepatic lipase in the hepatocytes of CRF animals and in their incubation media was significantly lower than that noted in the other four groups of animals. This phenomenon is again due to excess PTH in CRF and its effect on $[Ca^{2+}]i$ of hepatocytes in that the activity of the enzyme after 8 h of incubation of cells from CRF-PTX and CRF-V and of their media was not different from those obtained in hepatocytes and their media from normal or normal-V animals. Also, the exposure of hepatocytes from normal animals to PTH for 8 h was associated with reduced activity of the enzyme in these cells and in their media, further supporting the adverse effect of PTH on the activity of hepatic lipase. It is of interest that PTH caused a reduction in the enzyme activity of hepatocytes from CRF-PTX, CRF-V, and normal-V rats but not those from CRF animals. This difference is most likely due to downregulation of PTH-PTHrP receptors in the hepatocytes from CRF animals (10). The observation that treatment of CRF animals with verapamil blocked the effect of PTH on hepatic lipase but that the hormone did inhibit the activity of the enzyme in the cultured hepatocytes obtained from CRF-V animals seems contradictory. However, one must consider that in the in vivo setting, verapamil is always present in the blood of the CRF-V animals, while in the in vitro study verapamil was not present in the culture media. Since hepatocytes from CRF-V rats have normal $[Ca^{2+}]i$ (9) and a normal amount of mRNA of PTH-PTHrP receptor (10), it is reasonable to expect that the hormone may exert on their hepatocytes similar effects to those observed on normal hepatocytes.

The decreased activity of hepatic lipase after the 8 h incubation of hepatocytes from CRF rats could be due to decreased production of enzyme units and/or inhibition of the activity of each unit. Our demonstration that the mRNA of hepatic lipase in CRF is downregulated provide support for the notion that the synthesis of hepatic lipase is reduced. However, a definite answer could be obtained by evaluating the amount of hepatic lipase protein by Western blot analysis. This has not been done in the present study. Other data in our study showed that the kinetics of the hepatic lipase in CRF rats are altered in that its V_{max} is reduced and its K_m is increased. It is, therefore plausible to propose that both the production as well as the activity of hepatic lipase are adversely affected in CRF.

Certain data support the proposition that an increase in $[Ca^{2+}]i$ of hepatocytes, induced by other agonists such as epinephrine (24), vasopressin (15, 25), or calcium ionophore, reduces the activity of hepatic lipase (26, 27). Also, PTH (15), epinephrine (24, 28), and vasopressin (29) increase the generation of cAMP by the liver, and cAMP inhibits the activity of hepatic lipase as shown by others (3) and by the present study. cAMP also raises $[Ca^{2+}]i$ of hepatocytes (15). Thus, it is reasonable to suggest that the inhibitory effect of PTH on hepatic lipase activity is due to hormone-mediated rise in $[Ca^{2+}]i$ and increase in cAMP production.

Our studies of liver perfusion clearly demonstrated that the heparin-induced hepatic lipase release is impaired in CRF, and this derangement is again due to the state of chronic excess of PTH in CRF. These in vitro studies are similar to those reported in vivo in CRF rats where it was shown that the postheparin hepatic lipase activity in plasma is reduced in these animals (6) but normal in CRF-PTX and CRF-V animals (5). Further support for the effect of excess PTH on heparin-induced hepatic lipase release is found in our observation that perfusion of livers from normal animals with PTH inhibited this process. It is of interest that heparin did not increase hepatic lipase activity in both the hepatocytes or their culture media while it increased the activity of the enzyme in the effluent of the liver perfusate. This finding could be interpreted that heparin does not stimulate hepatic lipase production but causes the release of the enzyme attached to the vascular elements of the liver (31, 32). Others (30), however, reported that during prolonged (24–72 h) culture of rat hepatocytes, heparin does stimulate hepatic lipase production.

The impairment in the heparin-induced hepatic lipase release in CRF could be due to reduced availability of the enzyme, if the production of enzyme is reduced and/or due to impairment of the release process. The blocking of heparin-induced hepatic lipase release by PTH by livers from normal animals is consistent with an inhibitory effect of hormone on the release process since the normal livers should have adequate availability of the enzyme as demonstrated by the brisk hepatic-lipase release induced by heparin alone. Taken together, the data are consistent with the notion that the impaired hepatic release in CRF is most likely due to both reduced availability of the enzyme and to interference with the release process.

The results of the present study shed light on the molecular and cellular mechanisms responsible for the impaired hepatic lipase activity in CRF. It is of interest that Mordasini et al. (33) reported a selective deficiency of hepatic lipase in uremic patients; our observations provide an explanation for their finding.

Our data also provide insight into potential approaches for the prevention of the derangements in the metabolism of hepatic lipase. Indeed, both the prevention of secondary hyperparathyroidism of CRF or the use of calcium channel blocker which interferes with the action of PTH on the liver may prevent the derangement of hepatic lipase activity. These potential therapeutic approaches were found useful both in dogs (5) and rats (6) in the amelioration of the hyperlipidemia of CRF; indeed, we have found that the fasting hypertriglyceridemia of CRF was prevented by PTX of dogs or rats with similar degree and duration of CRF (5, 6) or by the treatment of CRF rats with verapamil (6). Similar studies are needed in CRF patients before the use of calcium channel blockers or the surgical or medical treatment of secondary hyperparathyroidism are recommended for the treatment of hyperlipidemia of CRF in humans.

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