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Decreased O_2 consumption by PMNL from humans and rats with CRF: Role of secondary hyperparathyroidism

MACIEJ KIERSZTEJN, MIROSLAW SMOGORZEWSKI, PRASERT THANAKITCHARU, GEORGE Z. FADDA, and SHAUL G. MASSRY

Division of Nephrology, the Department of Medicine, University of Southern California School of Medicine, Los Angeles, California, USA

Decreased O_2 consumption by PMNL from humans and rats with CRF: Role of secondary hyperparathyroidism. Bactericidal ability of polymorphonuclear leukocytes (PMNL) is impaired in chronic renal failure (CRF). This function of PMNL is mediated by the generation of oxidizing radicals and the latter event requires O_2 consumption by these cells. The present study examined both basal and FMLP-stimulated rise in cytosolic calcium ($[Ca^{2+}]_i$) and O_2 consumption of PMNL from normal subjects and hemodialysis patients and from CRF rats, and evaluated the potential role of secondary hyperparathyroidism of CRF on these properties of PMNL. Basal levels of $[Ca^{2+}]_i$ were significantly higher, and FMLP-induced increments in $[Ca^{2+}]_i$ were significantly lower in PMNL of both humans and rats with CRF than in normals. Basal and FMLP-stimulated O_2 consumption were significantly lower in CRF subjects and rats than in normals. These derangements were prevented by prior parathyroidectomy of CRF rats or by their treatment with verapamil from day one of CRF. Also, therapy of rats with pre-existing CRF with this drug reversed the abnormalities in $[Ca^{2+}]_i$ and in O_2 consumption of PMNL. The data indicate that: (1) CRF is associated with derangements in the homeostasis of $[Ca^{2+}]_i$ of PMNL and their oxygen consumption, (2) these abnormalities are, most likely, mediated by the state of secondary hyperparathyroidism of CRF, and (3) verapamil, which blocks the PTH-induced entry of calcium into cells, and prevents as well as reverses these PMNL dysfunctions. These results implicate the excess PTH of CRF in the genesis of the defective bactericidal function of PMNL, and assign a new dimension to PTH toxicity in CRF.

Certain data indicate that the bactericidal ability of polymorphonuclear leukocytes (PMNL) is impaired in chronic renal failure (CRF) [1, 2]. In order for the PMNL to kill bacteria, these cells must first ingest the bacteria and subsequently kill them by oxidizing agents generated during the respiratory burst [3–5]. We, along with others, have shown that ingestion of particles by PMNL from uremic patients [6–8] or rats with CRF [9] is impaired. This derangement is mediated, at least in part, by the state of secondary hyperparathyroidism of CRF which causes a significant rise in the basal levels of cytosolic calcium ($[Ca^+]_i$) and a significant reduction in ATP content of the PMNL [8, 9]. Indeed, prevention of the changes in $[Ca^+]_i$ and ATP content of PMNL by parathyroidectomy of CRF rats or by their treatment with the calcium channel blocker, verapamil, resulted in the normalization of phagocytosis [9].

The impaired ingestion of particles by PMNL in CRF could explain the reduced bactericidal activity of these cells. However, it is also possible that CRF is associated with impaired respiratory burst and hence reduced production of oxidizing agents. Such an additional derangement provides another mechanism underlying the impaired bactericidal activity of PMNL in CRF. Available data on effect of CRF on respiratory burst are controversial. Eckardt et al [10], utilizing chemiluminesce technique and whole blood samples, reported enhanced phorbol myristate acetate (PMA)-induced respiratory burst activity in CRF and dialysis patients. In contrast, Hirabayashi et al [11] found that PMA-induced production of H_2O_2 by PMNL of hemodialysis patients is reduced, indicating impaired respiratory burst. It is possible that, as in the case of particle ingestion by PMNL, the secondary hyperparathyroidism of CRF and the consequent metabolic derangements in PMNL [8, 9] interfere with their respiratory burst.

During the activation of the respiratory burst the PMNL consumes oxygen, and therefore, oxygen consumption is an indicator of the magnitude of the respiratory burst. We, therefore, examined basal and N-formyl-L-methionyl-L-leucyl-L-phenylalamine (FMLP) stimulated oxygen consumption by PMNL from CRF patients and from CRF rats with and without excess PTH, and evaluated the usefulness of the calcium channel blocker, verapamil, in preventing or reversing the potential derangements in oxygen consumption by PMNL.

Methods

Experiments were done on both humans and animals. The human studies were done in 18 hemodialysis patients (HD) and 15 normal subjects (NS). The age of HD patients ranged between 20 to 55 (37.2 ± 2.4) years and there were eight females and 10 males. The duration of the HD treatment was 4 to 62 (27.4 ± 4.1) months. The etiology of the renal failure was essential hypertension in 10 patients, chronic glomerulonephritis in five and unknown in three. In all patients, blood pressure was maintained normal by appropriate medications which did not include verpamil. Among the normal subjects there were seven females and eight males, and their ages ranged between 24 and 45 (34.6 ± 1.7) years. Peripheral venous blood was drawn under sterile conditions into vacutainers containing 20 U preservative-free heparin (Gibco Laboratories, Grand Island,

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New York, USA) per every 1.0 ml of blood. The blood samples from dialysis patients were collected before the dialysis procedure. These blood samples were used for the separation of PMNL.

The animal studies were done in Sprague-Dawley rats weighing 320 to 480 g. They were fed rat chow diet (Wayne Research Animal Diets, Chicago, Illinois, USA) throughout the study and allowed to drink *ad libitum*. The diet contained 1.4% calcium and 0.97% phosphorus. Two sets of studies were done. In the first set, there were five groups of animals including: (a) normal rats, (b) rats with chronic renal failure (CRF) of 42 days duration, (c) normocalcemic parathyroidectomized (PTX) rats with CRF of 42 days duration (CRF-PTX rats), (d) rats with CRF of 42 days duration treated daily with verapamil from day one of CRF (CRF-VER rats), and (e) normal rats treated with verapamil for 42 days (normal-VER rats).

In the second set of animal studies, CRF was maintained for 84 days. On day 42 of CRF, the rats were randomized to two groups which were matched in regard to their creatinine clearance and blood levels of PTH. One group began to receive verapamil from day 42 through day 84 of CRF, while the second group received no verapamil.

Parathyroidectomy was done by electrocautery, and the success of the procedure was ascertained by a decrease in plasma levels of calcium of at least 2 mg/dl. The PTX rats were freely allowed to drink water containing 50 g/liter of calcium gluconate. This procedure is adequate to normalize plasma calcium in PTX rats. Animals that did not display a fall in plasma calcium of 2 mg/dl or more after PTX, and the PTX rats that did not attain normocalcemia with drinking calcium containing water were discarded and not used in the study. Seven days after PTX, the animals underwent right partial nephrectomy through a flank incision; a week later, a left nephrectomy was performed. This nephrectomy procedure was also performed in rats with intact parathyroid glands. Verapamil (Isoptin, Knoll, AG, Ludwigshafen, Germany) was injected subcutaneously in a dose of 0.1 μg/g body weight twice daily. Two days before the experiments, the rats were housed in metabolic cages for the collection of two 24-hour urine outputs which were used for the measurement of creatinine clearance. The latter was also measured at days 40, 41, 82 and 83 of CRF in rats that were maintained for 84 days. On the day of the experiment, the rats were put under light ether anesthesia and blood was drawn directly from the heart under sterile conditions and placed into sterile test tubes containing 100 U of preservative-free heparin (Gibco) per 1.0 ml of blood.

The separation of PMNL from peripheral blood was made according to the method described by Ferrante and Thong [12] with certain modifications. The details of the method of separation of PMNL from blood of HD patients and NS have already been reported from our laboratory [8]. In brief, 3.0 ml of fresh heparinized blood was placed over two layers Ficoll-hypaque solution in 15 ml conical polypropylene tubes. The bottom layer was made of 3 ml of Mono-Poly Resolving Medium with density of 1.114 g/ml (Flow Laboratories, Inc., McLean, Virginia, USA) and second layer of 1.0 ml Isolymph with density of 1.077 g/ml (Gallard-Schlesinger Industries Inc., Carle Place, New York, USA). The tubes were centrifuged at 1270 × g for 45 minutes at room temperature. This procedure resulted in the separation of mononuclear and polymorphonuclear cells into two distinct bands with the red blood cell pellet at the bottom of the tube. The PMNL were aspirated with Pasteur pipette and washed twice in Hank's balanced salt solution (HBSS). Cells from two to three animals were pooled from each study. Cell purity determined by Wright's staining was greater than 97% and cell viability assayed by the trypan blue exclusion method exceeded 98%. Difficulty in the separation of PMNL was encountered in few blood specimens and this was overcome by extending the time of centrifugation. To remove red blood cells that might have remained with PMNL samples obtained from humans or rats, hypotonic lysis with 3 ml of 0.2% NaCl was done for 30 seconds followed by the addition of 3 ml of 1.6% NaCl. The samples were centrifuged for 15 minutes at 250 × g. This procedure was done twice. The PMNL pellet was resuspended in adequate volume of calcium-free Hank's solution containing 20 mM HEPES and 5.6 mM glucose to give 2×10^8 PMNL/ml. The samples were kept on ice and used for the measurements of basal and FMLP-stimulated oxygen consumption and cytosolic calcium ($[Ca^{2+}]_i$).

Oxygen consumption was determined polarographically by means of a Clark oxygen electrode (Gilson Medical Electronics, Middelton, Wisconsin, USA), fitted to a plexiglass chamber of 2.0 ml capacity as described originally by Chance and Williams [13]. The method used for this measurement in PMNL was described by Rossi et al [14]. First 2.0 ml of Hank's solution containing 1.25 mM of $CaCl_2$ was placed in the plexiglass chamber for 5 to 10 minutes at 37°C; thereafter 600 μl of this solution was removed and 500 μl of the PMNL suspension (1×10^7 cells) and 50 μl of KCN was added to give a final concentration of 1 mM. The latter was added to inhibit mitochondrial oxygen consumption. Basal oxygen consumption by PMNL was monitored for five minutes followed by the addition of 50 μl of FMLP to give a final concentration of 2 μM inside the chamber. Oxygen consumption was then monitored for 10 minutes and calculated using a solubility coefficient of 0.024 μmol/ml of medium and expressed as nmol $O_2/2 \times 10^7$ PMNL/min.

Resting levels of $[Ca^{2+}]_i$ of the PMNL and FMLP-induced rise in $[Ca^{2+}]_i$ were estimated with Fura2-AM (Sigma Chemical Co., St. Louis, Missouri, USA). A sample of 5×10^6 PMNL was washed with solution I containing in mM: NaCl, 132; KCl, 3; $MgSO_4$, 1; NaH_2PO_4, 1.2; D-glucose, 10; HEPES, 10; $CaCl_2$ 0.02 (pH was adjusted to 7.4 with Tris buffer) and spun at at 300 × g for 15 minutes. The pellet was resuspended in 490 μl of the above solution and 10 μl of Fura2-AM dissolved in DMSO giving a final concentration of 4 μM of Fura2. The mixture was then incubated in a water bath of 37°C for 30 minutes. After this incubation, the cells were washed and resuspended in solution I. Measurements of $[Ca^{2+}]_i$ were done with Perkin Elmer fluorescence spectrophotometer model LS 5B (Perkin-Elmer Corp., Norwalk, Connecticut, USA) at excitation wavelength of 340 nm and 380 nm and emission wavelength of 510 nm with a slit of 10 and 20 mm, respectively. An aliquot of 100 μl of PMNL suspension was added to a spectrophotometer cuvette containing 1.9 ml of solution II, which was the same as solution I except for its $CaCl_2$ concentration being 1 mM. Autofluorescence from cells and/or added reagents was monitored during each experiment and was not found to be a significant factor. Maximal fluorescence (F_{max}) and minimal fluorescence (F_{min}) were measured with 0.05% Triton and 5 mM EGTA in Tris Base

Table 1. Basal levels and FMLP-induced changes in $[Ca^{2+}]_i$ and oxygen consumption of PMNL of humans and rats with chronic renal failure

	N	$[Ca^{2+}]_i$ nM		$\Delta[Ca^{2+}]_i$ After FMLP nM	$\frac{\Delta[Ca^{2+}]_i}{\text{basal } [Ca^{2+}]_i}$ ratio	Oxygen consumption nmol $O_2/2 \times 10^7$ PMN/min	
		Basal	After FMLP			Basal	After FMLP
I. Human studies							
Normal subjects	15	57 ± 1.1	198 ± 7.6	170 ± 7.4	2.45 ± 0.13	5.8 ± 0.42	61 ± 0.3
Hemodialysis patients	18	68 ± 1.1[a]	182 ± 3.3	114 ± 2.9[a]	1.70 ± 0.04[a]	3.5 ± 0.43[a]	32 ± 1.8[a]
II. Rat studies							
A. 6 Week rat studies							
Normal	20	104 ± 1.3	197 ± 1.9	93 ± 2.7	0.87 ± 0.03	3.2 ± 0.10	29 ± 0.5
CRF	20	133 ± 3.1[a]	189 ± 2.9	56 ± 4.1[a]	0.43 ± 0.03[a]	2.1 ± 0.16[a]	14 ± 0.7[a]
CRF-PTX	12	103 ± 1.6	196 ± 2.3	93 ± 3.1	0.90 ± 0.04	3.0 ± 0.16	27 ± 0.8
CRF-VER	19	103 ± 1.0	188 ± 3.0	85 ± 3.0	0.82 ± 0.02	2.9 ± 0.18	26 ± 0.7
Normal-VER	20	105 ± 2.2	190 ± 2.0	85 ± 2.3	0.83 ± 0.03	2.9 ± 0.13	30 ± 1.0
B. 12 Week rat studies							
CRF without VER	17	141 ± 3.1[a]	190 ± 1.9	49 ± 0.3[a]	0.34 ± 0.03[a]	2.2 ± 0.08[a]	14 ± 0.7[a]
CRF with 6 weeks VER	16	106 ± 2.0	190 ± 4.9	84 ± 3.0	0.78 ± 0.03	3.0 ± 0.20	23 ± 1.1

Data are presented as mean ± SE.

[a] $P < 0.01$ vs. normal subjects (Human studies), vs. normal, CRF-PTX, CRF-VER, and Normal-VER (6 week rat studies) and vs. CRF-VER (12 week rat studies)

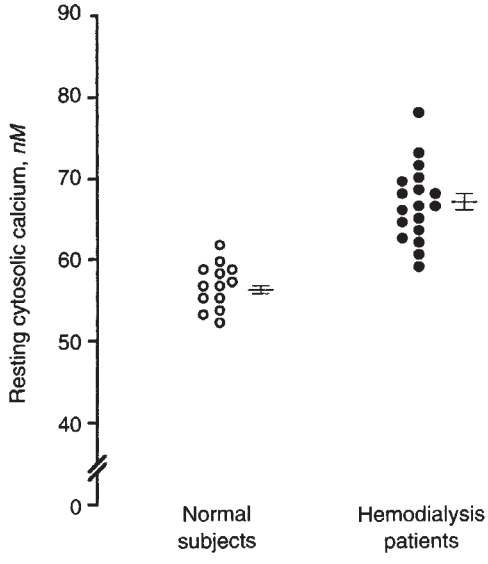

Fig. 1. *Basal levels of $[Ca^{2+}]_i$ of PMNL of normal subjects and hemodialysis patients.* Each datum point represents one individual and brackets denote mean ± 1 SE. The values between the two groups are significantly ($P < 0.01$) different.

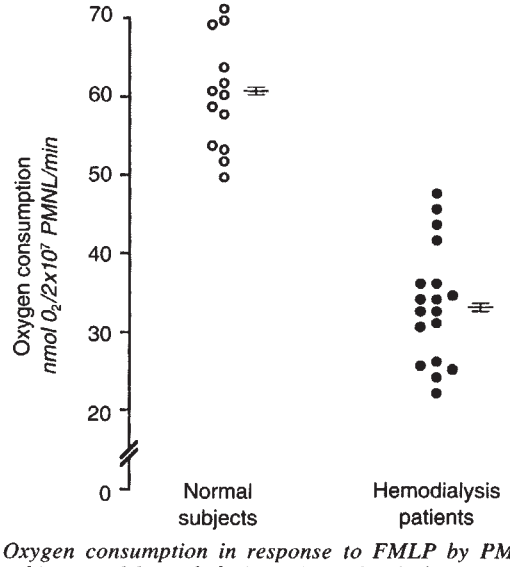

Fig. 2. *Oxygen consumption in response to FMLP by PMNL from normal subjects and hemodialysis patients.* Each datum point represents one patient and brackets denote mean ± 1 SE. The values between the two groups are significantly different.

buffer (pH 8.0), respectively. Calculation of $[Ca^{2+}]_i$ was done using the Grynkiewicz equation and the dissociation constant for Ca^{2+}-Fura2 was assumed to be 225 nM [15].

The measurement of calcium in plasma was made with Perkin Elmer atomic absorption spectrophotometer model 503 (Perkin Elmer Corp.) and those of creatinine and phosphorus by Technicon autoanalyzer (Technicon Instrument, Inc., Tarrytown, New York, USA). PTH levels in serum from rats were determined by INS-PTH immunoassay kit (Nichols Institute Dignostics, San Juan Capistrano, California, USA). This assay recognizes the aminoterminal fragment of PTH. The lowest detectable level is 3 pg/ml. The interassay variation is 7.3% and the intrassay variation is 4%.

PTH in humans was measured with radioimmunoassay utilizing sheep anti-sera 478 (supplied by Dr. Claude Arnaud), ^{125}I-labeled bovine PTH and pooled sera from patients with renal failure as standard. This antibody reacts predominantly with an immunologic determinant in the carboxyl region of human PTH, and it detects both the intact hormone and its carboxyterminal fragment. The values for this assay in 63 normal subjects ranged from undetectable to 15 (5.7 ± 0.7) μlEq/ml. The blood levels of PTH were detectable in 33 of the 63 (52%) normal subjects. Elevated blood levels of PTH were found in all 60 patients with CRF. The lower unit of detectability is 1 μlEq/ml.

Statistical evaluation was made with one way analysis of variance and Tukey's HSD (Honest Statistical Difference) for

Table 2. Biochemical data in rats with six weeks of chronic renal failure

	N	Body weight g	Plasma mg/dL			Creatinine clearance μL/min/100 g	Serum PTH pg/mL
			Calcium	Phosphorus	Creatinine		
Normal	20	347 ± 4.8	9.4 ± 0.1	8.1 ± 0.06	0.40 ± 0.01	453 ± 10	24 ± 2.5
CRF	20	346 ± 3.9	9.3 ± 0.1	7.5 ± 0.11[b]	1.15 ± 0.02[a]	161 ± 10[a]	61 ± 3.4[a]
CRF-PTX	12	354 ± 7.8	9.3 ± 0.1	8.0 ± 0.10	1.13 ± 0.03[a]	167 ± 10[a]	13 ± 0.5[c]
CRF-VER	19	340 ± 4.8	9.4 ± 0.1	7.1 ± 0.07[b]	1.12 ± 0.02[a]	150 ± 10[a]	54 ± 3.6[a]
Normal-VER	20	341 ± 4.9	9.5 ± 0.1	8.1 ± 0.04	0.40 ± 0.01	443 ± 10	21 ± 1.1

Data are presented as mean ± 1 SE.
[a] $P < 0.01$ vs. normal and normal-VER
[b] $P < 0.01$ vs. normal CRF-PTX and normal-VER
[c] $P < 0.01$ vs. other groups

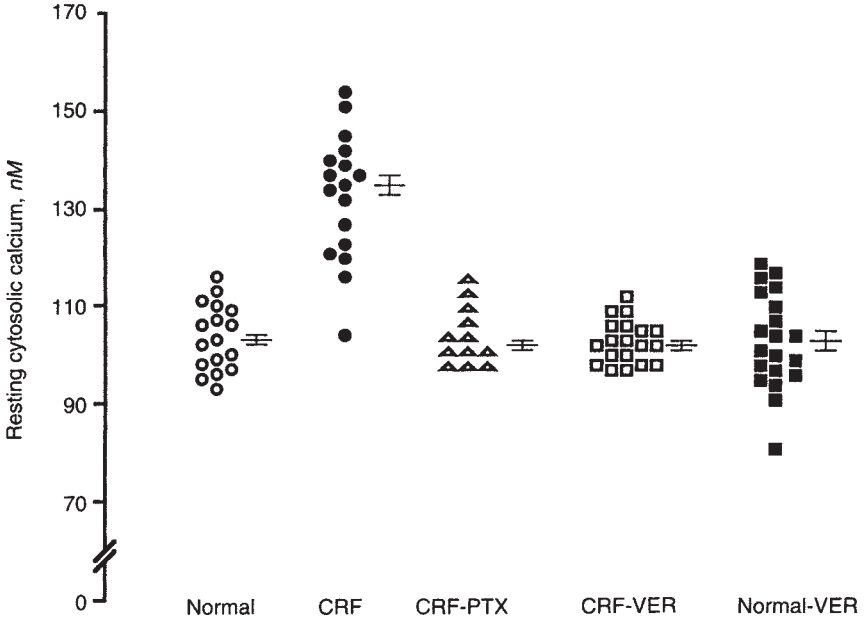

Fig. 3. *Basal levels of $[Ca^{2+}]_i$ of PMNL from the five groups of rats studied for six weeks.* Each datum point represents one study of PMNL pooled from 2 to 3 rats. Brackets denote mean ± 1 SE. The values in CRF rats are significantly ($P < 0.01$) higher than those in the other 4 groups of animals.

multiple comparison between groups, and unpaired *t*-test for comparison of parameters within each group. Data are expressed as mean ± SE.

Results

Studies in humans

The HD patients have markedly elevated serum levels of PTH (486 ± 104 μlEq/ml). There were no significant differences in plasma levels of calcium between the NS and HD patients (9.4 ± 0.23 vs. 8.9 ± 0.5 mg/dl). The plasma levels of phosphorus in HD patients (4.7 ± 0.20 mg/dl) was significantly ($P < 0.01$) higher than in NS (3.5 ± 0.11 mg/dl). The resting levels of $[Ca^{2+}]_i$ in PMNL of HD patients were significantly ($P < 0.01$) higher than those in NS (Table 1, Fig. 1). The rise (Δ) in $[Ca^{2+}]_i$ as well as the ratio of Δ $[Ca^{2+}]_i$/basal$[Ca^{2+}]_i$ after FMLP were significantly ($P < 0.01$) smaller in HD patients than in NS (Table 1). Both basal and FMLP stimulated oxygen consumption in PMNL of HD patients were significantly ($P < 0.01$) lower than in NS (Table 1, Fig. 2).

Studies in rats with six weeks of CRF

Table 2 provides the biochemical data in these rats. There were no significant differences in body weight among the rats of the five groups studied. The 5/6 nephrectomy produced significant ($P < 0.01$) increments in plasma levels of creatinine and significant ($P < 0.01$) decrements in creatinine clearance. Indeed, the serum levels of creatinine were almost three times higher and the values of creatinine clearance three times lower in CRF, CRF-PTX and CRF-VER than in normal and normal-VER rats. There were no significant differences between these parameters among the CRF, CRF-PTX and CRF-VER animals. Similarly, the plasma levels of calcium were not different among the five groups of animals. The plasma levels of phosphorus were significantly ($P < 0.01$) lower in CRF and CRF-VER rats than in normal, CRF-PTX and normal-VER animals. The serum levels of PTH were significantly ($P < 0.01$) elevated in CRF and CRF-VER animals than in normal, CRF-PTX and normal-VER.

Resting levels of $[Ca^{2+}]_i$ in PMNL of rats with six weeks of CRF were significantly ($P < 0.01$) higher than those in normal,

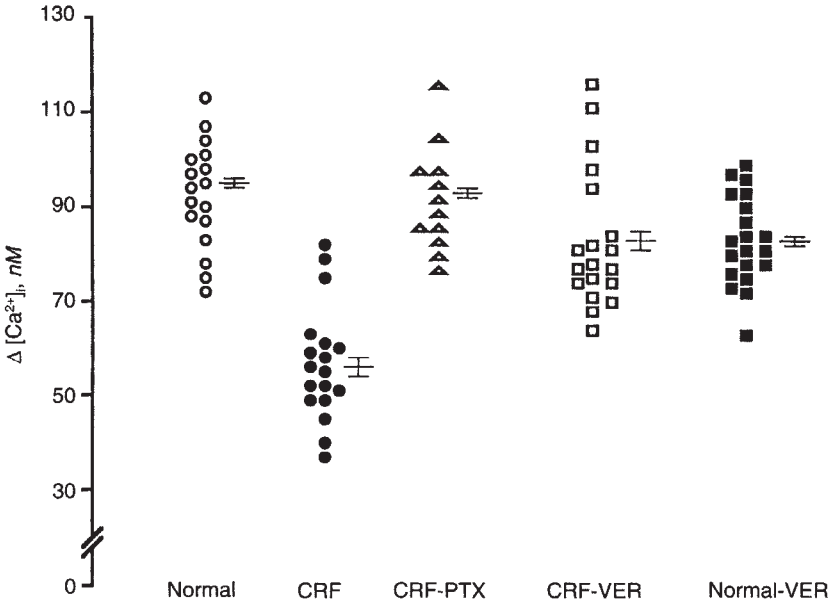

Fig. 4. *The change in $[Ca^{2+}]_i$ in response to FMLP in PMNL from the five groups studied for six weeks.* Each datum point represents one study of PMNL pooled from 2 to 3 rats. Brackets denote mean ± 1 SE. The values in CRF rats are significantly ($P < 0.01$) lower than those in the other 4 groups of animals.

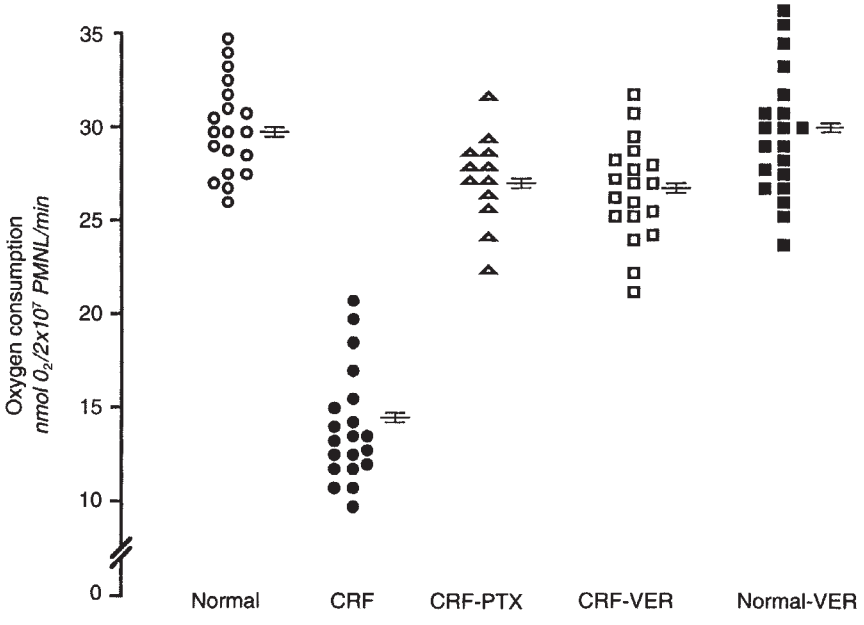

Fig. 5. *Oxygen consumption in response to FMLP by PMNL from the five groups of rats studied for six weeks.* Each datum point represents one study of PMNL pooled from 2 to 3 rats. Brackets denote mean ± 1 SE. The values in CRF rats are significantly ($P < 0.01$) lower than those in the other 4 groups of animals.

CRF-PTX, CRF-VER and normal-VER rats and there were no significant differences between the values of PMNL $[Ca^{2+}]_i$ in the latter four groups of animals (Table 1, Fig. 3). FMLP produced significantly ($P < 0.01$) smaller rise in $[Ca^{2+}]_i$ (Table 1, Fig. 4) and in oxygen consumption (Table 1, Fig. 5) in PMNL of rats with CRF than in those of the other four groups, and there were no differences in these parameters among normal, CRF-PTX, CRF-VER and normal-VER. Basal levels of oxygen consumption in PMNL of CRF rats was significantly lower than in PMNL of the other four groups (Table 1).

Twelve week rat studies

The biochemical data in these rats are provided in Table 3. At the time of randomization (6 weeks of CRF), there were no significant differences in the body weight, plasma levels of calcium, phosphorus and creatinine, serum levels of PTH and creatinine clearance in the animals which were to be maintained for 12 weeks of CRF with or without treatment with verapamil. Similarly, these parameters were not different between the two subgroups at the time of study (12 weeks of CRF).

The resting levels of $[Ca^{2+}]_i$ of PMNL in 12 weeks CRF rats were significantly ($P < 0.01$) higher than in those treated with verapamil for the last six weeks of CRF (Table 1, Fig. 6). Basal levels of oxygen consumption by PMNL from 12 weeks CRF rats was significantly ($P < 0.01$) lower than from CRF-V rats. FMLP caused a significantly ($P < 0.01$) smaller rise in $[Ca^{2+}]_i$ (Table 1, Fig. 7) and oxygen consumption (Table 1, Fig. 8) in PMNL of 12 weeks CRF rats than in those treated with verapamil for the last six weeks of CRF.

Table 3. Biochemical data of twelve week rat studies

	N	Body weight g	Plasma mg/dL Calcium	Phosphorus	Creatinine	Creatinine clearance μL/min/100 g	Serum PTH pg/mL
I. At time of randomization (6 weeks of CRF)							
Rats to be maintained without verapamil	17	363 ± 5.3	9.4 ± 0.1	7.2 ± 0.06	1.10 ± 0.04	150 ± 4	71 ± 6.1
Rats to be treated with verapamil	16	370 ± 2.1	9.5 ± 0.1	7.1 ± 0.05	1.09 ± 0.04	155 ± 8	65 ± 6.4
II. After 12 weeks of CRF							
CRF only	17	443 ± 4.7	9.2 ± 0.2	6.8 ± 0.15	1.12 ± 0.18	138 ± 2	68 ± 5.9
CRF with verapamil treatment	16	432 ± 1.9	9.0 ± 0.2	6.8 ± 0.10	1.00 ± 0.08	174 ± 8	69 ± 5.5

Data are presented at mean ± 1 SE.

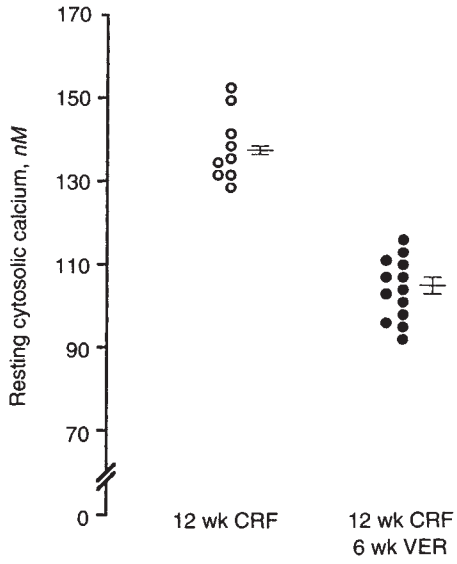

Fig. 6. *Basal levels of $[Ca^{2+}]_i$ of PMNL of CRF rats studied for 12 weeks.* Each datum point represents the study of PMNL pooled from 2 to 3 rats. Brackets denote mean ± 1 SE. The values of 12 weeks (wk) rats are significantly ($P < 0.01$) higher than those in 12 weeks CRF rats treated with verapamil for the last 6 weeks.

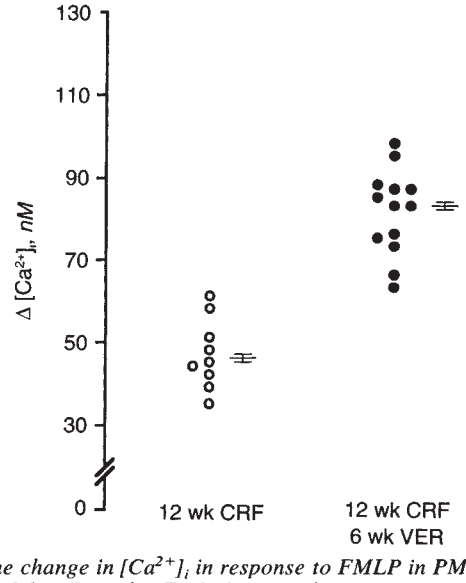

Fig. 7. *The change in $[Ca^{2+}]_i$ in response to FMLP in PMNL of CRF rats studied for 12 weeks.* Each datum point represents one study of PMNL pooled from 2 to 3 rats. Brackets denote mean ± 1 SE. The values in 12 weeks (wk) CRF rats are significantly ($P < 0.01$) lower than those in 12 weeks CRF rats treated with verapamil for the last 6 weeks.

Discussion

The results of the present study demonstrate that PMNL from both HD patients and rats with CRF have lower basal and FMLP-stimulated oxygen consumption than corresponding normal values. This defect is most likely mediated by the secondary hyperparathyroidism associated with CRF, since parathyroidectomy of CRF-rats and the prevention of the rise in serum PTH levels by this procedure normalized both basal and the FMLP-stimulated oxygen consumption.

The mechanism(s) through which excess PTH exerts its deleterious effects on oxygen consumption could be multiple. However, one consequence of chronic excess PTH, such as an elevation in basal $[Ca^{2+}]_i$, could play a major role in this defect. It has been shown that chronic excess of PTH in the presence [8, 9, 16–20] or absence of CRF [17, 21] is associated with a rise in basal levels of $[Ca^{2+}]_i$ in many cells, including pancreatic islets [16, 21], brain synaptosomes [17], T cells [18], B cells [19], PMNL [8, 9] and platelets [20]. This elevation in basal $[Ca^{2+}]_i$ of these cells has been implicated in the disturbances of their function in CRF [8, 9, 16, 21, 22], since the prevention of this rise in basal $[Ca^{2+}]_i$ was followed by normalization of function despite CRF [9, 22–24]. The present study again demonstrates that PMNL of the HD patients and rats with CRF have elevated basal levels of $[Ca^{2+}]_i$, and this derangement could be responsible for their impaired oxygen consumption. Indeed, the treatment of CRF rats with verapamil from day one of CRF prevented the elevation in basal $[Ca^{2+}]_i$ as well as the abnormality in oxygen consumption.

It is of interest that verapamil treatment of CRF rats not only prevented the abnormalities in $[Ca^{2+}]_i$ and oxygen consumption in PMNL when the drug was given from day one of CRF, but also reversed these derangements in PMNL when the drug was given to rats with pre-existing CRF. This observation has important clinical implications and points toward the usefulness of this calcium channel blocker in the management of already developed manifestations of the uremic syndrome. Indeed, Thanakitcharu et al [25] have also shown that treatment of rats with pre-existing renal failure with verapamil reversed the CRF-induced abnormalities in the metabolism and function of pancreatic islets.

It is of interest that verapamil treatment of normal rats did not affect the $[Ca^{2+}]_i$ of their PMNL. Similar observations were found in pancreatic islets [24] and brain synaptosomes [17]. A decrease in calcium entry into cells of normal animals by

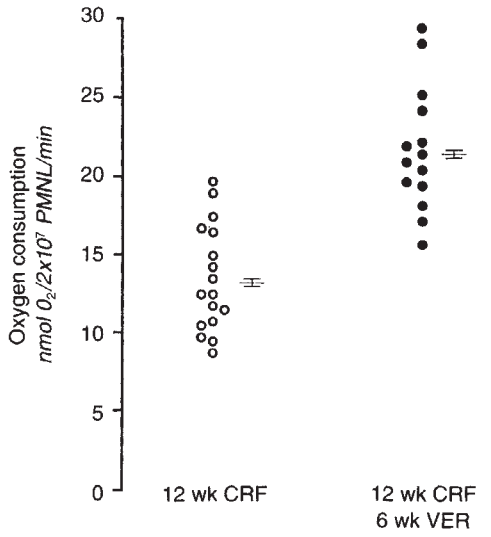

Fig. 8. *Oxygen consumption in response to FMLP in PMNL of CRF rats studied for 12 weeks.* Each datum point represents one study of PMNL pooled from 2 to 3 rats. Brackets denote mean ± 1 SE. The values in 12 weeks (wk) CRF rats are significantly ($P < 0.01$) lower than those in 12 weeks CRF rats treated with verapamil for the last 6 weeks.

verapamil is counterbalanced by a decrease in calcium extrusion out of the cells due to a modest adaptive decrease in the V_{max} of Ca^{2+} ATPase [24, 26]. It is possible that a similar phenomenon occurs in PMNL of normal rats treated with verapamil.

Certain data suggest that PMNL may not have L-type voltage-dependent calcium channels [27] which are blocked by verapamil [28]. On the other hand, Schimchowitz and Spilberg [29] showed that ^{45}Ca influx in PMNL is inhibited by verapamil. Therefore, our finding that verapamil prevented the rise in $[Ca^{2+}]_i$ in PMNL of CRF rats suggests that either L-type calcium channels do exist in PMNL, or other types of calcium channels may be present in PMNL [30] and are affected by verapamil, or the drug may affect $[Ca^{2+}]_i$ by other mechanisms, the nature of which has not been elucidated by our studies.

It has been proposed that for an agonist to elicit an appropriate physiologic response, it should induce an adequate calcium signal and an appropriate $\Delta[Ca^{2+}]_i/\text{basal}[Ca^{2+}]_i$ ratio [31]. A rise in basal $[Ca^{2+}]_i$ of cells may interfere with the magnitude of the calcium signal and/or the $\Delta[Ca^{2+}]_i/\text{basal}[Ca^{2+}]_i$ induced by an agonist and hence an impairment of the cell response to the agonist. Indeed, an elevated basal $[Ca^{2+}]_i$ of pancreatic islets of CRF rats displayed a smaller rise in $[Ca^{2+}]_i$ and in $\Delta[Ca^{2+}]_i/\text{basal}[Ca^{2+}]_i$, and impaired insulin secretion in response to potassium [32] or glucose [33] as compared to islets from normal rats and with normal basal $[Ca^{2+}]_i$. Normalization of the basal $[Ca^{2+}]_i$ in the CRF islets resulted in restoration of the calcium signal, $\Delta[Ca^{2+}]_i/\text{basal}[Ca^{2+}]_i$ and insulin secretion in response to those insulin secretogogues [32, 33]. Also, PMNL from CRF patients had elevated basal levels of $[Ca^{2+}]_i$ and smaller calcium signal and $\Delta[Ca^{2+}]_i/\text{basal}[Ca^{2+}]_i$ in response to monoclonal antibody 3G8 with specificity anti-CD16 as compared to PMNL from normal subjects [8].

FMLP, after binding to its receptor on the PMNL [34], induces a rise in $[Ca^{2+}]_i$ [35, 36] which in turn activates the respiratory burst [36, 37] and hence an increase in oxygen consumption [38]. It is possible, therefore, that the elevated basal levels of $[Ca^{2+}]_i$ in PMNL from humans or rats with CRF interferes with the calcium signal and/or $\Delta[Ca^{2+}]_i/\text{basal}[Ca^{2+}]_i$ induced by FMLP and hence in impaired oxygen consumption. Indeed, our data show that both the magnitude of the calcium signal and the $\Delta[Ca^{2+}]_i/\text{basal}[Ca^{2+}]_i$ as well as the rise in oxygen consumption in PMNL of patients and animals with CRF in response to FMLP are significantly ($P < 0.01$) smaller than in PMNL from normal subjects and normal rats. Further, the normalization of basal $[Ca^{2+}]_i$ in PMNL of CRF rats by parathyroidectomy or by treatment with verapamil resulted in restoration of the responses in $[Ca^{2+}]_i$ as well as in oxygen consumption to FMLP. Additional support for this notion is found by the data of Korchak et al [37], demonstrating that the buffering of the FMLP-induced rise in $[Ca^{2+}]_i$ of PMNL is associated with a significant reduction in generation of oxygen radicals and thus in oxygen consumption. Further, calcium ionophore that increase $[Ca^{2+}]_i$ trigger activation of respiratory burst [39, 40].

Although the above-cited arguments suggest a cause and effect relationship between the rise in $[Ca^{2+}]_i$ and oxygen consumption, other data challenge this proposition. Grinstein and Furuya [41], using electropermeabilized PMNL, found that the FMLP-induced oxygen consumption persisted even in cell depleted intracellular $[Ca^{2+}]_i$. They concluded that changes in $[Ca^{2+}]_i$ are not required for FMLP-stimulated respiratory burst. Studies by O'Flaherty et al [42] also suggested that calcium transient may not be required but may potentiate the FMLP receptor-mediated respiratory burst. They also suggested that some calcium pool may affect receptor expression in PMNL. It is, therefore, theoretically possible that a rise in basal levels of $[Ca^{2+}]_i$ in CRF may be associated with reduced expression of FMLP receptors in PMNL, resulting in a reduced rise in $[Ca^{2+}]_i$ and in reduced oxygen consumption without necessarily a cause and effect between these two parameters. Also, one can speculate that the elevated basal levels of $[Ca^{2+}]_i$ of PMNL in CRF may induce damage to these cells through activation of proteases and lipases, and therefore impede their FMLP-induced oxygen consumption.

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Reprint requests to Shaul G. Massry, M.D., Department of Medicine, Division of Nephrology, University of Southern California, School of Medicine, 2025 Zonal Avenue, Los Angeles, California 90033 USA.

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