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Research Article

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Both the amino-terminal (1-34) PTH and the intact (1-84) PTH, but not the carboxy-terminal (53-84) PTH, produced significant increases in osmotic fragility. This effect was abolished by prior inactivation of the hormone. There was a dose-response relationship between both moieties of PTH and the increase in osmotic fragility. This action of PTH required calcium, was mimicked by calcium ionophore, and was partially blocked by verapamil. PTH caused significant influx of ^{45}Ca into RBC, which was not associated with potassium leak. The hormone did not affect water content of RBC. Scanning electron microscopy revealed that the incubation of RBC with PTH was associated with the appearance of membrane filamentous extensions, which anchor RBC together.

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ABSTRACT The survival of erythrocytes (RBC) is shortened in uremia, and it has been shown that calcium influx into RBC evoked crenation and increased their rigidity. The high blood levels of parathyroid hormone (PTH) may augment entry of calcium into RBC and hence affect their integrity. We examined the effect of PTH on osmotic fragility of human RBC and investigated the mechanisms through which PTH interacts with RBC.

Both the amino-terminal (1-34) PTH and the intact (1-84) PTH, but not the carboxy-terminal (53-84) PTH, produced significant increases in osmotic fragility. This effect was abolished by prior inactivation of the hormone. There was a dose-response relationship between both moieties of PTH and the increase in osmotic fragility. This action of PTH required calcium, was mimicked by calcium ionophore, and was partially blocked by verapamil. PTH caused significant influx of ^{45}Ca into RBC, which was not associated with potassium leak. The hormone did not affect water content of RBC. Scanning electron microscopy revealed that the incubation of RBC with PTH was associated with the appearance of membrane filamentous extensions, which anchor RBC together.

Inhibition of glycolytic activity of RBC with NaF or inhibition of Na-K-activated ATPase with ouabain did not abolish the effect of PTH on osmotic fragility. PTH did not stimulate RBC Na-K-activated ATPase or Mg-dependent ATPase but caused marked and significant stimulation of Ca-activated ATPase. The basal activity of the RBC adenylate cyclase was low and PTH produced only a modest stimulation of this enzyme. Both cyclic AMP and dibutyryl cyclic AMP had no effect on osmotic fragility.

The data indicate that: (a) the RBC is a target organ for PTH, (b) the hormone increases osmotic fragility

of RBC, and (c) this effect of PTH is due to enhanced calcium entry into RBC. We suggest that the increased calcium influx may affect the spectrin-actin of the cytoskeletal network of the RBC and may alter the stability and integrity of the cell membrane. This action of PTH on the RBC could be, at least in part, responsible for the shortened survival of RBC in uremia, and assign a new role for PTH in the pathogenesis of the anemia of uremia.

INTRODUCTION

Erythrocyte (RBC)¹ survival is reduced in patients with advanced renal failure (1, 2). Since RBC from uremic patients have normal survival when infused into normal subjects, whereas RBC from normal individuals display shortened survival when infused into uremic patients, one or more extracorporeal factors have been considered responsible for this phenomenon (2, 4).

Evaluation of RBC survival of patients treated with maintenance hemodialysis using ^{51}Cr and Di (isopropofluoro) ^{32}P techniques revealed that the half-life of the RBC is markedly reduced (5) and that increasing the frequency of dialysis did not normalize the half-life of the cells (6). These observations indicate that the serum factor (or factors) responsible for the reduced survival of the RBC was not effectively removed by dialysis. The nature of the hemolytic factor(s) in uremia has not, as yet, been elucidated.

Patients with chronic renal failure have secondary hyperparathyroidism (7) and markedly elevated blood levels of parathyroid hormone (PTH) (8, 9). This hormone is known to augment entry of calcium into a variety of mammalian cells (10-12), and it has been

¹ Abbreviations used in this paper: MOF, median osmotic fragility; PBS, phosphate-buffered saline; PTH, parathyroid hormone; RBC, erythrocyte.

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shown that excess blood levels of PTH in uremia are responsible for an increase in the calcium content of many tissues such as skin (13), cornea (14), blood vessels (15), muscle (16), brain (17), peripheral nerves (18), and heart (19).

Theoretically, PTH may also promote calcium entry into RBC, and it has been shown that small increases in intracellular calcium of RBC evoked crenation and increased rigidity (20–24). Thus, excess blood levels of PTH in patients with uremia could be, at least in part, responsible for the reduced survival of RBC in these patients. The present study examined the effects of PTH on RBC.

METHODS

The osmotic fragility of human RBC was evaluated by the method of Parpart et al. (25). Blood samples were withdrawn into heparinized test tubes from normal volunteers. The samples were centrifuged for 5 min at 600 *g* and both the plasma and the buffy coat were discarded. The packed RBC were washed three times. The first two washes were made with 6 vol of a solution containing 0.9% NaCl and 10 μ M EDTA, and the final wash with isotonic phosphate-buffered saline (PBS), pH 7.4, containing 100 mg/100 ml bovine serum albumin. Each time, the RBC were mixed with the solution by gentle inversion and centrifuged for 3 min at 600 *g*. The washed packed RBC were then suspended in PBS to provide a hematocrit of 10%. We first measured the median osmotic fragility (MOF) for RBC of each individual by incubating 50 μ l of the RBC in solutions containing various concentrations of NaCl. The absorbance of the supernate vs. water was determined with Beckman spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 575 μ m, and the percentage of RBC lysed in each solution was calculated. The concentration at which 50% of RBC were lysed was considered the MOF. These studies also allowed the construction of osmotic fragility curves.

The effects of the various experimental conditions on the osmotic fragility of the RBC were examined by the same procedure described above. Two sets of test tubes were prepared to study hemolysis in the absence (control) and presence of the experimental condition. The test materials were added to the PBS without changing the volume of the solution or the concentration of NaCl. All studies were performed in duplicate.

Preliminary studies showed that the intact molecule of PTH causes hemolysis of RBC. Therefore, we evaluated the effects of various concentrations (0.1, 1.0, and 10.0 U/ml) of the intact hormone 1-84 bPTH (Sigma Chemical Co., St. Louis, MO), of its amino-terminal fragment 1-34 bPTH (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA), of 1.2 μ g/ml of the commercially available carboxy-terminal fragment 53-84 hPTH (Bachem Inc., Marina Del Rey, CA), and of inactivated PTH on the osmotic fragility of human RBC. All studies were performed with PBS solution containing 1.0 mM calcium. 1.2 μ g of 53-84 hPTH is equivalent to 10 U of 1-84 bPTH. Inactivation of the hormone was done as follows: 10–15 μ g of PTH was dissolved in 60 μ l of 0.15 N acetic acid, and to this solution 40 μ l of 30% vol/vol H₂O₂ was added. The solution was incubated at 37°C for 45 min and the reaction was terminated by freezing and lyophilizing. The effects of various concentrations of calcium (0, 0.01, 0.10, and 1.0 mM) in PBS on the action of PTH on osmotic

fragility of RBC were also examined. To ensure that the PBS was free of calcium, studies were also performed with calcium-free PBS to which 1 mM EDTA was added. The MOF in the presence of PTH with and without EDTA in the calcium-free PBS was not different. Studies were also carried out to examine the effect of preincubation of RBC with PTH in isotonic PBS containing 1 mM calcium for 30 min. The cells were then washed three times with isotonic PBS without PTH and their osmotic fragility was then determined.

The effects of 10 μ M cyclic AMP (Sigma Chemical Co.) and 1 μ M diluetylryl cyclic AMP (Sigma Chemical Co.) in the absence and presence of 1.0 mM calcium on osmotic fragility of RBC were also evaluated.

Studies were also done to investigate whether the effect of PTH on RBC is mimicked by the free acid form of calcium ionophore A23187, which contains no calcium (kindly supplied by Eli Lilly & Co., Indianapolis, IN) or inhibited by verapamil (Isoptene, Knoll AG, Ludwingshagen, West Germany). We evaluated the effects of 1.0 and 10 μ M calcium ionophore A23187 on the osmotic fragility of the RBC in the absence of calcium and PTH and in the presence of 1.0 mM calcium. We also examined the effect of 27.5 μ M verapamil alone and in the presence of PTH and compared the results with the effect of PTH alone.

The possibility that PTH may exert its effect on the osmotic fragility of the RBC through an action on glycolytic activity of the cells or on the Na-K-activated ATPase of the RBC membrane was also examined. This was done by evaluating the effect of PTH on the osmotic fragility of RBC in the presence and absence of 25 mM NaF (an inhibitor of glycolytic activity) and in the presence and absence of 2.5 mM ouabain (an inhibitor of Na-K-activated ATPase).

The effects of PTH on the activity of Na-K-activated ATPase, Ca-activated ATPase, and Mg-dependent ATPase of the RBC membrane were also evaluated. Processing of the RBC for the measurements of the ATPase activity was made according to the method of Sen and Post (26) with either ouabain, EGTA, or calcium, or a combination thereof was added, depending on the enzyme assay. The activity of each enzyme was measured by hydrolysis of ATP to inorganic phosphorus, which was measured according to the method of Fiske and Subbarow (27) and expressed per milligram of protein, as determined by the method of Lowry et al. (28). Assays of the enzyme activity were done in duplicates.

Assay of adenylate cyclase activity of RBC ghosts were made in the absence and presence of 10 U/ml of PTH, according to the methods described by Bilezikian and Aurbach (29) and by Rodan et al. (30), with modifications. The ghosts were prepared according to the method of Dodge et al. (31) and their protein content was determined by the method of Lowry et al. (28). Cyclic AMP was determined by the method of Steiner et al. (32) by means of radioimmunoassay and a commercially available reagent kit from New England Nuclear (Boston, MA). Assays of the enzyme activity were done in duplicate.

The effects of PTH in the presence or absence of calcium, verapamil, or calcium ionophore A23187, and of the latter in the presence or absence of calcium or verapamil on the leakage of magnesium and potassium from RBC, were also investigated. The RBC were prepared as described above. They were then suspended in PBS containing 0.1% bovine serum, pH 7.4, to give a hematocrit of 12.5%. 100 μ l of the suspension (1.80×10^8 RBC) was added to 900 μ l of isotonic PBS solution containing various combinations of calcium (1 mM), 1-84 bPTH (20 U), calcium ionophore A23187 (1 μ M), or verapamil (10 μ M). The solutions were preincubated in

a water bath at 37°C for 10 min until the temperature reached equilibrium. The reaction was started by adding the cell suspension to the media, followed by incubation for 60 min. The reaction was stopped after 15 min of centrifugation at 1,000 *g*. The supernate was removed for the determinations of hemoglobin, magnesium, and potassium. Hemoglobin was measured spectrophotometrically at 575 μm , potassium with Instrumentation Laboratory flame photometer (Instrumentation Laboratory, Inc., Lexington, MA), and magnesium with Perkin-Elmer atomic absorption spectrophotometer model 503 (Perkin-Elmer Corp., Instrument Div., Norwalk, CT).

The effect of PTH on water content of RBC was examined in five studies. RBC were prepared as described above and the same cell population was incubated with isotonic PBS containing 1 mM calcium in the presence and absence of PTH for 60 min. The solutions were centrifuged for 1 h at 15,000 rpm. Trapped PBS was estimated by tagging the solution with ^{125}I HSA (0.15 $\mu\text{Ci/ml}$). The water content was estimated by drying the sample at 105°C to a constant weight (generally within 24–36 h). This value was corrected for the trapped PBS solution.

We also examined the effects of PTH, calcium ionophore A23187, and verapamil on ^{45}Ca uptake by the RBC. The procedures for the preparation of the RBC were similar to those described above, but in these studies 100 μl of RBC suspension (12.5% hematocrit and equal to 1.8×10^8 RBC) were added to 900 μl of isotonic PBS solution containing 1 mM of calcium and 0.1 μCi of ^{45}Ca . After 60 min of incubation, the solution was centrifuged at 15,000 rpm for 30 min and the supernate removed. The pellet was washed and centrifuged three times with PBS containing no radioactive

calcium. The radioactivity was measured in the packed cells. They were decolorized with hydrogen peroxide and counted in scintillation fluid (619 ml toluene, 293 ml Triton X-100, 88 ml H_2O containing 3.53 g purified protein derivative and 44 mg POPOP).

In preliminary studies we found that PTH exerts similar effects on RBC of rabbits. Evaluation of the effects of PTH, in the presence of calcium on the morphology of the rabbit RBC was performed. The cells were prepared and the experiments were done as described above. The packed cells were fixed in 1% glutaraldehyde for evaluation with scanning electron microscope.

RESULTS

The effects of the intact molecule of PTH (1-84 bPTH), its amino-terminal fragment (1-34 bPTH), its carboxy-terminal fragment (53-84 hPTH) and of inactivated PTH on the osmotic fragility of RBC are given in Table I and Figs. 1 and 2. Both 1-84 bPTH and 1-34 bPTH produced significant increases in osmotic fragility of human RBC. The MOF increased from 0.414 ± 0.006 (SE) to 0.452 ± 0.007 ($P < 0.01$) and to 0.454 ± 0.007 ($P < 0.01$) in the presence of 1 and 10 U/ml of 1-84 bPTH. This change in MOF rendered the RBC more susceptible to hypotonic lysis. From Fig. 1, it could be noted that at concentrations of NaCl that were associated with 10% hemolysis in the absence of PTH, 50% of the cells were lysed in the presence

TABLE I
Effect of PTH on MOF of Human RBC

Experiment	Concentration of PTH, U/ml			
	0	0.1	1	10
Studies with 1-84 bPTH (<i>n</i> = 6)				
MOF	0.411±0.006	0.419±0.007	0.450±0.007	0.454±0.007
<i>P</i> vs. control		NS	<0.01	<0.01
ΔMOF		0.008±0.003	0.039±0.002	0.043±0.002
<i>P</i> vs. control			<0.01	<0.01
Studies with 1-34 bPTH (<i>n</i> = 4)				
MOF	0.410±0.01	0.411±0.008	0.441±0.01	0.452±0.01
<i>P</i> vs. control		NS	<0.05	<0.01
ΔMOF		0.001±0.002	0.031±0.002	0.042±0.001
<i>P</i> vs. control		NS	<0.01	<0.01
Studies with inactivated PTH				
Inactivated 1-84 bPTH (<i>n</i> = 5)				
MOF	0.408±0.01			0.410±0.01
<i>P</i> vs. control				NS
Inactivated 1-34 bPTH (<i>n</i> = 5)				
MOF	0.412±0.01			0.411±0.02
<i>P</i> vs. control				NS
Studies with 53-84 hPTH (<i>n</i> = 5)				
Dose, $\mu\text{g/ml}$	0	1.2	2.4	3.6
MOF	0.410±0.01	0.409±0.01	0.410±0.009	0.410±0.01
<i>P</i> vs. control		NS	NS	NS

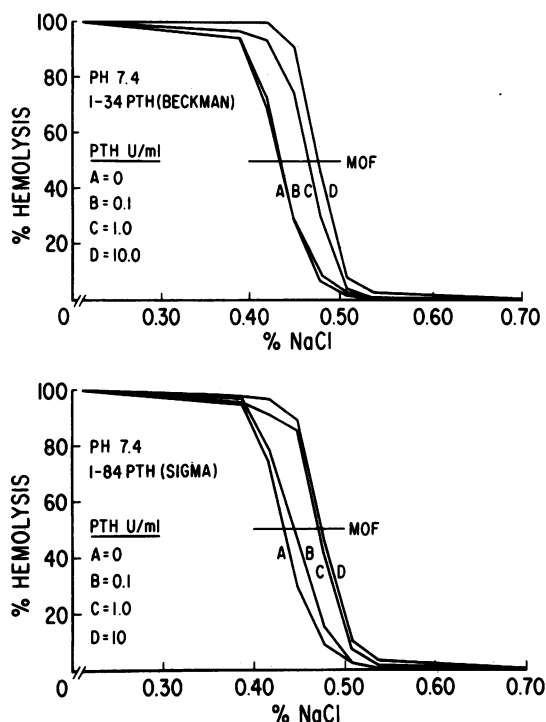


FIGURE 1 Effects of 1-34 PTH (upper panel) and of 1-84 PTH (lower panel) on percentage of hemolysis and MOF of human RBC.

of 1.0 or 10 U/ml. These effects of 1-34 bPTH and 1-84 bPTH on MOF displayed a dose-response relationship (Fig. 2). Inactivation of both these moieties of PTH abolished the hemolytic action of the hormone. In contrast to 1-34 and 1-84 bPTH, the carboxy-terminal fragment 53-84 hPTH had no effect on the osmotic fragility of the RBC. Preincubation of RBC with PTH did not affect their MOF. The latter was

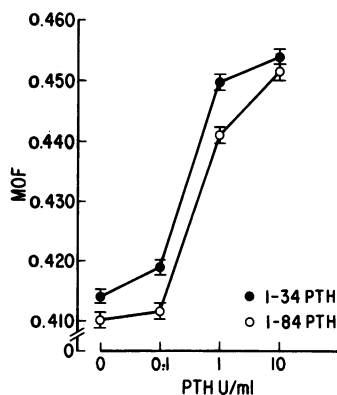


FIGURE 2 A dose-response curve between 1-34 PTH and 1-84 PTH and MOF of human RBC. Each data point is the mean \pm SE of five to seven studies.

0.416 ± 0.01 ($n = 6$) in the control studies and 0.414 ± 0.01 in studies with cells preincubated with PTH. These data indicate that the PTH must be present in the media to exert its effect on MOF.

Fig. 3 depicts the effects of various concentrations of calcium in PBS on the effect of 1 U/ml of 1-84 bPTH on MOF. It is evident that in the absence of calcium, PTH did not produce a change in MOF, and there was a dose-response relationship between the calcium concentration and the change in MOF induced by PTH.

Neither NaF (an inhibitor of glycolysis) nor ouabain (an inhibitor of Na-K activated ATPase) affected the changes in MOF induced by PTH. The increase in MOF was 0.038 ± 0.002 with PTH alone, 0.039 ± 0.002 with PTH and NaF, and 0.039 ± 0.001 with PTH and ouabain.

The results of the studies on the effects of the calcium ionophore A23187 on osmotic fragility of RBC are shown in Table II. The calcium ionophore ($10 \mu\text{M}$) in the absence of calcium reduced MOF, and the change (-0.034 ± 0.002) was significant ($P < 0.01$). In the presence of 1 mM calcium in PBS, this effect of calcium ionophore was not apparent and MOF increased by 0.010 ± 0.002 . Thus, in the presence of calcium, the change in MOF equals the sum of the change induced by ionophore without calcium and that noted in the presence of calcium. The sum total is an increase in MOF of 0.044 ± 0.002 , a change similar to that induced by PTH in the presence of calcium.

Verapamil alone did not affect the osmotic fragility of the RBC, and when used with PTH it blocked almost 50% of the effect of PTH on MOF. The change in MOF with PTH alone was 0.052 ± 0.003 and with PTH and verapamil it was 0.024 ± 0.002 ($P < 0.01$).

The basal activity of adenylate cyclase of RBC obtained from seven studies was 3.54 ± 0.26 pmol cyclic AMP/2 h per mg protein and 4.51 ± 0.37 pmol cyclic AMP/2 h per mg protein in the presence of 10 U/ml bPTH. This represents a modest ($27 \pm 2\%$), but significant ($P < 0.01$ by paired t test) increment. Neither cyclic AMP nor dibutyryl cyclic AMP in the absence or presence of 1 mM Ca had an effect on the osmotic fragility of human RBC.

The effects of 1-84 bPTH on the activity of Mg-dependent, Ca-activated, and Na-K-activated ATPase of the RBC are depicted in Table III. The hormone produced significant ($P < 0.01$) stimulation of the Ca-activated ATPase only. The activity of the enzyme doubled in the presence of the hormone.

Calcium ionophore produced marked and significant leakage of potassium from RBC; the presence of calcium or PTH in PBS did not augment this effect and verapamil did not abolish it. Calcium ionophore also caused marked leakage of magnesium and this

TABLE II
Effect of Calcium Ionophore A23187 on MOF of Human RBC

	Ionophore concentration, μM		
	0	1.0	10
No calcium			
MOF	0.428 \pm 0.03	0.416 \pm 0.004	0.394 \pm 0.004
Δ MOF		-0.012 \pm 0.002	-0.034 \pm 0.002
1 mM calcium			
MOF	0.410 \pm 0.003	0.413 \pm 0.003	0.420 \pm 0.003
Δ MOF		0.003 \pm 0.002	0.010 \pm 0.002

Each data point represents mean \pm SE of five experiments.

effect was potentiated in the presence of calcium in PBS and again was not prevented by verapamil. Significant leakage of hemoglobin occurred in the simultaneous presence of calcium and calcium ionophore in the PBS. PTH alone and in the presence of calcium and/or verapamil did not induce potassium leak, but caused modest leakage of magnesium when calcium was present in PBS; the latter effect was blunted in the presence of verapamil. There was no effect of PTH on the water content of RBC. The water content was 67 \pm 2% in the absence of PTH and 68 \pm 3% with PTH.

Summary of the data on the effect of 1-84 bPTH, calcium ionophore, verapamil, or various combinations of these agents on calcium uptake by the RBC is shown in Table IV. PTH produced significant ($P < 0.01$) calcium uptake by the RBC (from 0.09 \pm 0.01 to 1.50 \pm 0.19 μ mol/h per 1.8×10^8 RBC). Verapamil only partially but significantly ($P < 0.01$) blocked calcium uptake induced by PTH. The uptake of calcium was most marked in the presence of calcium ionophore and was 14 times greater than that induced by PTH alone; verapamil did not block the effect of calcium ionophore or calcium uptake.

Fig. 4 depicts a scanning electron micrograph of RBC after the incubation in PBS containing 1-84

bPTH and calcium. Many RBC showed membranous processes extending toward other RBC and linking them together.

DISCUSSION

The results of the present study demonstrate that both the intact PTH and its amino-terminal fragment produced significant increases in the osmotic fragility of human RBC. This effect was dose dependent, required the presence of calcium, and was due neither to inhibition of the glycolytic activity of the cells nor to the stimulation of their Na-K-activated ATPase. The demonstration that inactivation of PTH abolished its effect on RBC is consistent with the action's being related to the biologic activity of the hormone and not to a contaminant of the hormone preparation. Furthermore, our data show that the MOF of RBC preincubation with PTH is not altered and that the hormone must be present in the media to exert its effect.

PTH is known to augment entry of calcium into a variety of mammalian cells (10-12). Two observations in our study indicate that PTH enhances calcium movement into the RBC as well. An increase in the calcium concentration in the *cis*-side of the RBC membrane activates the transport system responsible for the

TABLE III
Effects of 10 U/ml of 1-84 bPTH on the Activity of Mg-dependent, Ca-activated, and Na-K-activated ATPase of RBC

	Mg-dependent ATPase	Ca-activated ATPase	Na-K-activated ATPase
	<i>nmol Pi/mg protein/min</i>		
-PTH	62 \pm 4.5	70 \pm 5.2	46 \pm 3.1
+PTH	63 \pm 5.1 (102 \pm 4.0)	153 \pm 25.9 (213 \pm 23.8)	48 \pm 5.2 (103 \pm 5.9)
<i>P</i>	NS	0.01	NS

Data are presented as mean \pm SE of six studies. Numbers in brackets indicate the percentage increase from control value.

TABLE IV
Effect of PTH, Verapamil, and Calcium Ionophore on Calcium Entry into the RBC

PTH	Verapamil	A23187	Calcium entered	Change
			$\mu\text{mol/h}/1.8 \times 10^8 \text{ RBC}$	%
-	-	-	0.09±0.01	0
+	-	-	1.50±0.19*	+1,667
-	+	-	0.07±0.01	-22
-	-	+	21.00±3.90*	+23,333
+	+	-	1.00±0.10†	+1,256
-	+	+	21.00±2.36*	+23,333

Data are presented as the mean±SE of 32 studies.

* $P < 0.01$ from control.

† $P < 0.01$ from PTH alone.

extrusion of this ion from the cells (33), and this system depends on the activity of Ca-activated ATPase (33). The finding that PTH stimulated the Ca-activated ATPase of the RBC is in agreement with PTH augmenting calcium entry into the cells. In addition, the results of the studies with ^{45}Ca provide direct evidence that PTH increases calcium uptake by the RBC. The mechanisms through which PTH augments calcium influx into the RBC are not fully elucidated by the present study. This action of the hormone could be mediated by increased production of cyclic AMP via the stimulation of an adenylate cyclase. But the finding that PTH caused only a modest stimulation of the activity of the RBC adenylate cyclase and the observation that both cyclic AMP and dibutyryl cyclic AMP did not have an effect on the osmotic fragility of the cells make this possibility unlikely.

The hormone may, however, directly enhance calcium influx into the RBC. Such an action of PTH could be due to an effect of the hormone on the phospholipids of the cell membrane. Lo et al. (34) reported

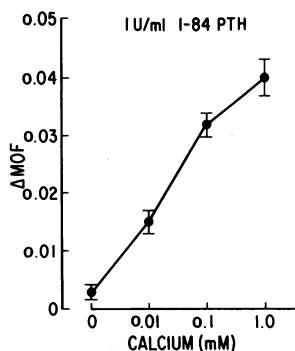


FIGURE 3 Effect of calcium on the change in MOF induced by 1 U/ml of 1-84 PTH.

that PTH increased the incorporation of ^{32}Pi into phosphatidic acids and phosphatidylinositol in renal cortical slices. Mitchel (35) found that the incorporation of ^{32}Pi into these components precedes the movement of calcium across cell membrane. Finally, Green et al. (36) showed that phosphatidic acid and phosphatidylinositol are potent calcium ionophores.

Several findings in our study provide support for the notion that the action of PTH on the osmotic fragility of the RBC is related to the enhanced calcium entry into the cells. First, PTH did not exert its effect in the absence of calcium in the media. Second, calcium ionophore in the presence of calcium produced an effect on osmotic fragility similar to that of PTH. Third, verapamil, which blocks movement of calcium into cells, blocked partially but significantly the effect of the hormone on the osmotic fragility.

The cellular events leading to an increase in osmotic fragility of the RBC by the PTH-mediated increment in calcium influx are not evident. Several possibilities should be considered. First, it has been reported that calcium loading of RBC by ionophore is associated with marked potassium loss and shrinkage of the cells (37). The latter changes do not appear to play a role in the effect of PTH on osmotic fragility, since the enhanced calcium influx produced by PTH was not associated with potassium loss from the cells.

Second, augmented calcium influx into the RBC (38, 39) and stimulation of calcium-activated ATPase (33, 39) utilize ATP, which results in ATP depletion. Recent studies suggest that ATP may have a direct effect on membrane flexibility by affecting membrane-associated structural proteins (40, 41). Thus, ATP depletion may affect membrane stability and lead to cell fragmentation.

Third, the RBC have a membrane skeleton located at the membrane-cytosol surface with its major component made of spectrin-actin (42, 43) and this cytoskeletal network of the RBC participates in several membrane functions, including control of shape, viscoelastic properties, surface topology, and membrane stability (42-44). An increase in calcium concentration of RBC causes cross linking of membrane proteins of intact cells and it has been suggested that the intrinsic cross linking of membrane proteins is a key signal for the removal of the RBC from the circulation (45). It is possible that PTH, by enhancing calcium influx into RBC, causes cross linking of membrane proteins and as a result affects membrane structure and stability. That PTH may have an effect on the RBC membrane is further supported by the finding of filamentous extensions with scanning electron microscopy. PTH may also reduce RBC surface area by liberation of membrane vesicles and/or by lipid or protein loss; further studies to examine these possibilities are needed.

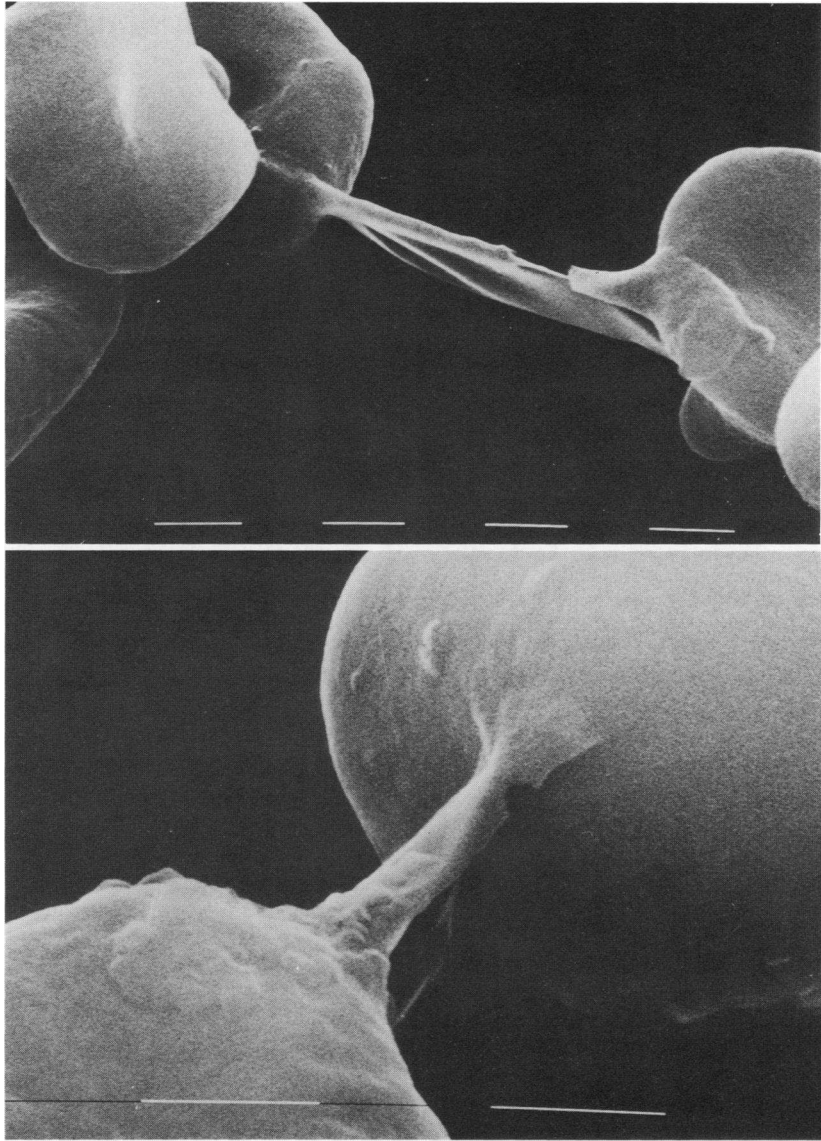


FIGURE 4 Scanning electron micrograph depicting the filamentous extension from the RBC membranes and adhesions between cells (upper panel) and (lower panel) close-up photograph. Marker is 1.0 μm .

Since the osmotic fragility of a given RBC represents a measure of its surface area/volume ratio and since PTH did not affect water content of RBC, it is reasonable to suggest that the effect of the hormone on MOF is mediated through a reduction in surface area secondary to changes in cell membrane shape and/or integrity.

The results of the present study and those previously reported by us (46) and others (47-49) provide information on the multiple and complex pathways through which excess blood levels of PTH in patients with

uremia may participate in the genesis of their anemia. There are at least three pathogenetic mechanisms. PTH inhibits erythropoiesis (46, 49), affects RBC survival, and produces bone marrow fibrosis (48, 49), which reduces the availability of the red marrow to produce erythrocytes.

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