Parathyroid Hormone Increases Sodium/Calcium Exchange Activity in Renal Cells and the Blunting of the Response in Aging*

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Hiroyuki Hanai, Makoto Ishida, C. Tony Liang, and Bertram Sacktor‡

From the Laboratory of Biological Chemistry, National Institute on Aging, National Institutes of Health, Gerontology Research Center, Baltimore, Maryland 21224

Na+-dependent Ca2+ efflux was demonstrated in cells isolated from the rat renal cortex, suggestive of the presence of a Na+/Ca2+ exchange carrier in the cells. Parathyroid hormone, when incubated with the cells in vitro, increased Na+-dependent Ca2+ efflux about 60%. The effect of the hormone was specific for biologically active parathyroid hormone analogs and could be mimicked by cyclic nucleotides and forskolin. The effects of parathyroid hormone concentration on Ca2+ efflux and cyclic AMP formation were similar. These findings would be consistent with the view that the cyclic nucleotide might act as the intracellular messenger to increase Na+/Ca2+ exchange activity. Cells isolated from parathyroidectomized rats had decreased Na+-dependent Ca2+ efflux. When these cells were treated in vitro with parathyroid hormone, Na+-dependent Ca2+ efflux was enhanced to the same rate as found with cells from sham-operated animals. Parathyroid hormone-sensitive Na+/Ca2+ exchange activity was markedly blunted in cells from senescent (24 months) rats. Basal Na+-dependent Ca2+ efflux and Na+-independent Ca2+ efflux were not altered in the aged animal. Parathyroid-stimulated adenylate cyclase was also decreased in aging. In contrast, forskolin-stimulated Na+-dependent Ca2+ efflux and adenylate cyclase did not change with senescence. These findings would be compatible with a mechanism of desensitization that occurred at the level of the receptor or hormone-receptor coupling to adenylate cyclase. These results may be of physiological significance in understanding calcium homeostasis and the imbalances in mineral metabolism associated with old age.

The Na+/Ca2+ exchange system in plasma membranes plays a key role in the extrusion of cellular Ca2+ and the control of the cytosolic Ca2+ concentration (1). Although the carrier system has been studied most extensively in excitable tissues, such as nerve, heart, and skeletal muscle (2), evidence has accumulated indicating that the exchange mechanism also functions in the regulation of cellular Ca2+ in nonexcitable tissues. In the kidney, initial support for the occurrence of Na+/Ca2+ exchange was from findings that in microperfused tubules removal of Na+ from, or addition of ouabain to, the peritubular fluid inhibits active Ca2+ transport (3), and alterations in the magnitude of the Na+ electrochemical gradient across the peritubular cell membrane modulate the intracellular level of Ca2+ (4). Confirmation of the presence of the exchange system has come from direct examinations of carrier activity in basolateral membrane vesicles prepared from the plasma membrane fraction of the renal cortex (5-8).

Because the renal cytosolic free Ca2+ concentration is in the submicromolar range (9), plasma and filtrate Ca2+ concentrations are about 2.5 mM and the membrane potential is cell interior negative, it is generally assumed that Ca2+ enters the tubular cell crossing the luminal segment of the plasma membrane by a diffusional mechanism, but that the cation has to be transported actively out of the cell crossing the basolateral segment of the plasma membrane. Two different Ca2+ transport systems, localized in the basolateral membrane, have been proposed to translocate the divalent cation: one is a high affinity Ca2+ ATPase which serves as a Ca2+ pump (7, 10-13), the other is the secondary active process mediated by the Na+/Ca2+ exchanger. It is not clear whether the two systems operate in parallel nor how they are regulated by intrinsic and extrinsic effectors.

Parathyroid hormone (PTH) has long been known to stimulate renal Ca2+ reabsorption (14, 15). The biochemical mechanism by which the hormone enhances Ca2+ transport has remained largely unknown. PTH receptors have been localized to the basolateral membrane of the tubular cell, and reception is coupled to increases in cAMP (15-17). Recently, we reported that Na+/Ca2+ exchange activity in basolateral membrane vesicles prepared from rats thyroparathyroidectomized 48 h previously is decreased about 40%, and this activity is restored when synthetic PTH(1-34) is infused in the thyroparathyroidectomized animal (6). These results have been confirmed in the dog (8). Moreover, it has been found that the alteration in activity is an apparent Vmax effect with no change in the apparent Ks for Ca2+ (8). However, these earlier findings do not establish that when PTH is administered in vivo the hormone acts directly on renal tubular cells to modulate Na+/Ca2+ exchange activity. In the present paper, we report experiments describing the effects of PTH and other agonists when incubated with isolated renal cells in vitro. We also report that the action of PTH is blunted in the senescent animal. A portion of this work has appeared in abstract form (18).

EXPERIMENTAL PROCEDURES

Isolation of Renal Cells—Wistar-derived male rats were obtained from the Animal Facility, Gerontology Research Center, National Institute on Aging. The animals were 2 months old, except as given in the experiments examining the effect of age. The kidneys were perfused in situ with Hanks’ balanced salt solution containing 11 mM

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† To whom correspondence may be addressed.

‡ The abbreviations used are: PTH, parathyroid hormone; EGTA, (ethylenebis(oxyethylenenitrilo)tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; cAMP, adenosine 3',5' monophosphate.
glucose (isolation medium). The renal cortices from the 2 kidneys from each rat were finely minced and the tissue suspended in 30 ml of the isolation medium containing collagenase (1-1.67 mg/ml) and hyaluronidase (1 mg/ml). The suspension was incubated with constant agitation in a metabolic shaker for 30-40 min at 37 °C. The suspension was then filtered at 130 s for 1 s, and the supernatant was discarded. The pellet was resuspended in 10-15 ml of isolation medium and the mixture recentrifuged. The pellet was resuspended and the suspension was centrifuged at 45 × g for 10 s. The resultant supernatant, containing the cells, was centrifuged again at the higher gravitational force, and the sedimented cells were washed twice by alternating suspension and centrifugation. Then, the cells were suspended in an incubation medium containing 140 mM KCl, 10 mM Hepes-Tris buffer, pH 7.4, 10 mM mannose, 0.5 mM β-hydroxybutyrate, and 2.5 mM glutamine and washed twice. Finally, the cell preparation was suspended in the same incubation medium at a concentration of 10-15 mg of cell protein/ml.

Microscopic examinations revealed that the preparations consisted of single cells with clusters of from 2 to 6 cells. At least 85% of the cells excluded trypan blue. The general metabolism of the cells was evaluated by determining ATP content of cells when freshly isolated and after the cells were incubated for 1 h under conditions (described below) for preloading the cells with calcium and measuring calcium efflux. The ATP contents of freshly prepared cells of 6- and 24-month-old rats were 7.7 ± 0.7 and 7.4 ± 0.5 nmol·mg⁻¹ of cell protein, respectively. After 1 h, the ATP contents were 7.1 ± 0.1 and 7.6 ± 1.0 nmol·mg⁻¹ of cell protein for the respective two ages. Intracellular space determinations, based on the uptake of α-methylglucoside at equilibrium (19), indicated a relationship of 3.3 μl·mg⁻¹ of cell protein.

The general metabolism of cells was also examined by measuring the rate of CO₂ formation from [6-¹⁴C]glucose. Cells from rats 2, 6, 12, and 24 months of age produced 1.0 ± 0.22, 1.39 ± 0.28, and 1.51 ± 0.53 nmol of CO₂·h⁻¹·mg⁻¹ of protein, respectively, which were not significantly different. Cellular integrity was assessed by measuring the leakage of lactic dehydrogenase activity. Cells were incubated in incubation medium for 1 h at 37 °C. They were reincubated for 20 min at 37 °C in the absence and presence of 0.3% Triton X-100. After the reincubation, the cells were centrifuged and dehydrogenase activity measured in the supernatant. Lactic dehydrogenase activity in the extracellular medium after detergent treatment increased more than 6 times, indicating that the plasma membrane of the cells not exposed to Triton X-100 was highly preserved. There were no significant differences between cells from various aged animals nor between cells tested prior to or after the 1-h incubation period.

Measurement of Calcium Efflux—A 50-μl aliquot of the freshly prepared cell suspension was preincubated at 37 °C for 20 min. The cells were preincubated with calcium by adding to the suspension 50 μl of the incubation medium containing 2 mM CaCl₂ labeled with 0.25 μCi of "Ca and incubating the mixture with constant shaking for 30 min (Preliminary study showed that the "Ca content of the cells reached a steady state in 20-25 min.) After the preloading period, efflux of efflux was initiated by the addition of 900 μl of efflux medium containing either 140 mM NaCl or choline chloride, plus 2 mM EGTA and 10 mM Hepes-Tris buffer, pH 7.4. Efflux at 5 s, unless noted otherwise, was terminated by the addition of 3 ml of ice-cold efflux medium and the mixture rapidly filtered on 5-µm Millipore filters (SMWP02500). The reaction tube and filter were washed 3 times, each with 3 ml of ice-cold stopping solution. The cells and filter were digested with 1 ml of 0.1 N NaOH for several hours, 10 ml of scintillation fluid (Beckman Ready Solv. MP) was then added, and radioactivity counted. Zero time values (0% efflux) were estimated from reactions in which the ice-cold stopping solution was added prior to the efflux medium and the contents of the reaction tube immediately filtered. All incubations were carried out at least in triplicate. Each experiment was repeated a minimum of 5 times, each with different cell preparations. Values are reported as the mean ± S.E. of 5 experiments, each with different cell preparations.

Other Assays—The synthesis of cAMP by hormone-stimulated renal cells was determined as previously described (21). Briefly, cAMP in neutralized perchloric acid extracts of the incubation reactions was eluted from AG 1-X2 columns (recovery of [3H]cAMP was 80-90%) and then quantitated with a commercial radioimmunoassay kit (Immuno Nuclear). Adenylate cyclase activity in renal cell membranes was measured as reported (22). Lactic dehydrogenase activity (23), cell ATP content (24), and protein (25) were estimated by standard procedures.

Parathyroidectomy—Rats were anesthetized with ether and sodium pentobarbital (5 mg/100 g body wt) and parathyroid glands surgically removed by electrocautery or sham-operated. Parathyroidectomized rats were used 48 h after surgery. Completeness of gland removal was verified by the decrease in serum calcium concentration, from 2.14 ± 0.04 mM before to 1.55 ± 0.04 mM 48 h after surgery, whereas the concentrations in the sham-operated animals did not change, being 2.30 ± 0.08 mM and 2.28 ± 0.07 mM before and after the sham procedure, respectively. Each datum represents the mean ± S.E. of 10-12 rats.

Materials—"Ca (50 mCi/ml) and [3H]cAMP (30 Ci/mmol) were obtained from New England Nuclear. PTH(1-84) (lot 6009 A, 1227 units/mg) was purchased from Inolox. PTH(1-34), lot 006946, 6,800 units/mg and PTH(3-34) were from Peninsula. Hyaluronidase, Type I-S, dibutyryl cAMP, and 8-bromo-cAMP were purchased from Sigma. Forskolin was obtained from Calbiochem-Behring. Collagenase CLS II was from Cooper Biomedical.

RESULTS

Na⁺-dependent Efflux of Calcium—Fig. 1 shows the time course of "Ca⁺ efflux from renal cells preloaded with "Ca⁺ and then diluted 1:10 with a medium containing EGTA and either Na⁺ or choline chloride. When the extracellular medium contained choline⁺, efflux was relatively slow, only 14% of the "Ca⁺ was released after 20 s. Similar results were obtained when KCl was substituted for choline chloride (data not illustrated). In the presence of extracellular Na⁺, efflux of "Ca⁺ was rapid, being greater at all measured time points than those in the presence of choline⁺. At 5 s, the time interval used to roughly approximate initial rates, 16% of the "Ca⁺ effused, compared to 5% in the presence of extracellular choline⁺. When the calcium ionophore A23187 (1 μM) was added to the medium, efflux was nearly complete, 91% and 95% in the presence of EGTA and, respectively, choline⁺ and Na⁺. This finding indicated that the total exchangeable Ca⁺ was not altered by the extracellular cation. Assuming isotopic equilibrium, the cell calcium content immediately prior to the initiation of efflux was 2.66 nmol·mg⁻¹ of cell protein and this decreased to about 0.2 nmol·mg⁻¹ of cell protein after the exposure to ionophore. Previous studies on Na⁺/Ca⁺ exchange in renal basolateral membrane vesicles demonstrated that the Na⁺ effect on Ca⁺ flux was specific since Na⁺ could not be replaced by Li⁺, K⁺, or Rb⁺ (6, 8). Addition of a Na⁺ gradient by monensin inhibited Ca⁺ transport (6). Also, the possibility that Na⁺ enhanced the debinding of Ca⁺ from noncarrier binding sites on the membrane was largely excluded (6). Hence, the finding that efflux of Na⁺ enhanced the efflux of Ca⁺ from renal cells preloaded with Na⁺/Ca⁺ exchange activity...
cellular Na⁺ increased the efflux of Ca²⁺ from the renal cell provided evidence for the presence of Na⁺/Ca²⁺ exchange in these isolated renal cells. The effect of the concentration of extracellular Na⁺ on the Na⁺-dependent rate of Ca²⁺ efflux is shown in Fig. 2. The Ca²⁺ efflux system was saturated with respect to extracellular Na⁺ at a concentration of about 100 mM. The relationship between Na⁺ concentration and rate (5 s of efflux was sigmoidal, suggesting the interaction of more than one class of Na⁺ sites. A Hill transformation of the data (Fig. 2, inset), yielded a straight line \( r = 0.99 \), with a calculated \([Na⁺]_0\) of 10 mM. This value was similar to that obtained for the half-maximal concentration of Na⁺ effecting Ca²⁺ efflux from basolateral membrane vesicles (6, 8).

**Effect of PTH on Na⁺-dependent Calcium Efflux**—Highly purified bovine PTH(1–84) or vehicle control was incubated with the renal cells for 1.5 min prior to initiation of Ca²⁺ efflux, and then efflux was measured in the Na⁺- or choline⁺-containing medium (Fig. 3). Earlier experiments revealed that the maximal effect of PTH on efflux was obtained with a 1.5-1.5-min exposure of the cells to the hormone (data not shown). As illustrated, 10 units/ml PTH stimulated the Na⁺-dependent efflux (5 s) of ⁴⁵Ca²⁺ 55%, from 17.6 ± 4.6% in the control to 27.3 ± 3.8% in hormone-treated cells (\( p < 0.01 \)). Na⁺-independent ⁴⁵Ca²⁺ efflux was not affected by the hormone, 4.8 ± 3.1% and 4.5 ± 3.8% in control and treated cells, respectively.

The specificity of the action of PTH is indicated by the experiments shown in Fig. 4. Equivalent units (10 units/ml) of the synthetic tetradecapeptide PTH(1–34) resulted in an increase in Na⁺-dependent ⁴⁵Ca²⁺ efflux comparable to that found with PTH(1–84). In contrast, the equivalent weight of the inactive form of the hormone, PTH(3–34), did not significantly affect Na⁺-stimulated Ca²⁺ efflux. The biological activity of the different PTH analogs was confirmed in the renal cell system used in the present study by measuring the cAMP formed. After the 1.5-min preincubation of the cells with the analogs, the cAMP found in the cells and medium was 8 ± 2 pmol·mg⁻¹ of cell protein in control cells and 12 ± 3 (\( p < 0.05 \)) and 13 ± 3 (\( p < 0.02 \)) for cells exposed to PTH(1–84) and PTH(1–34), respectively. The cAMP found when the cells were treated with PTH(3–34) was 9 ± 2 pmol·mg⁻¹ of cell protein, a value not significantly different from the value measured with control cells. The data in Fig. 4 show additionally that forskolin, an activator of renal cell adenylate cyclase (26), and the cAMP analogs dibutyryl cAMP and 8-bromo-cAMP, also stimulated Na⁺-dependent ⁴⁵Ca²⁺ efflux.

The effects of the concentration of PTH(1–84) on ⁴⁵Ca²⁺ efflux and on the production of cAMP are shown in Fig. 5. Efflux was enhanced with increased concentration of hormone with a maximal effect obtained with about 10 units/ml. Elevating the hormone concentration to 50 units/ml did not stimulate efflux additionally. Na⁺-independent Ca²⁺ efflux was not affected at any of the tested concentrations of hormone (data not shown). The formation of cAMP also increased with increasing concentrations of PTH (Fig. 5).

**Effect of Parathyroidectomy on Na⁺-dependent Calcium Efflux**—Parathyroid glands were removed from rats, and 48 h later renal cells were prepared. At the time of death, the endogenous levels of PTH in the parathyroidectomized rats would be negligible in view of the short (24-min) half-life of the hormone in the parathyroidectomized rat (27), and this would be supported by the decrease in serum calcium concentration, noted under “Experimental Procedures.” As shown...

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**Fig. 2.** The relationship between extracellular Na⁺ concentration and Na⁺-dependent ⁴⁵Ca²⁺ efflux. The efflux was 5 s. Na⁺-dependent efflux represents the difference between the efflux in the presence of Na⁺ and the efflux in the presence of choline. Osmolarity was maintained constant by the appropriate combinations of NaCl and choline chloride. Each datum represents the mean ± S.E. of 4 separate experiments, each carried out in triplicate. The inset describes a Hill plot of the data.

**Fig. 3.** The effect of parathyroid hormone (PTH 1–84) on ⁴⁵Ca²⁺ efflux from cells in the presence of extracellular Na⁺ or extracellular choline⁺. PTH (10 units/ml) was added to the cell suspension 1.5 min prior to the initiation of efflux. The time of efflux was 5 s. Each datum represents the mean ± S.E. of 6–8 experiments, each replicated.

**Fig. 4.** The specificity of the action of parathyroid hormone. The effect of the hormone and agonists was determined in the presence of NaCl. Forskolin and the cAMP analogs did not change ⁴⁵Ca²⁺ efflux when choline⁺ was substituted for Na⁺ in the extracellular medium (not illustrated). The concentrations were forskolin, 10 µM; dibutyryl cAMP (dibut. cAMP), 1 mM; and 8-bromo-cAMP (8-Br-cAMP), 0.2 mM. The PTH analogs and forskolin were added to the cell suspension 1.5 min prior to initiation of efflux. The cAMP analogs were added 10 min prior to initiation of efflux. Each datum represents the mean ± S.E. of 5 experiments, each replicated at least in triplicate.
Fig. 5. The effects of the concentration of parathyroid hormone (PTH 1–84) on Na⁺-dependent ⁴⁵Ca²⁺ efflux and generation of cAMP. Na⁺-dependent efflux was measured as the difference (△) between the efflux in the presence of extracellular Na⁺, without added PTH, shown as zero△ efflux, and the efflux in the presence of Na⁺, with the indicated concentrations of PTH. The△ cAMP formed was calculated in a similar manner. Cells were exposed to PTH for 1.5 min prior to measurements of efflux and cAMP. The cAMP in cells as well as in the medium was determined. Each datum represents the mean of 4–6 experiments, each replicated.

Fig. 6. The effect of parathyroidectomy on Na⁺-dependent ⁴⁵Ca²⁺ efflux. Na⁺-dependent efflux was measured as the difference between the efflux in the presence of extracellular Na⁺ and extracellular choline⁺. Other details of the experiment are described in the text. Each datum represents the mean ± S.E. of 8–10 experiments, each replicated.

in Fig. 6, Na⁺-dependent ⁴⁵Ca²⁺ efflux with cells from parathyroidectomized animals was decreased 25% from the value found with cells from sham-operated rats, 10.6 ± 0.6% to 8.0 ± 0.6% (p < 0.01). ⁴⁵Ca²⁺ efflux measured in the presence of extracellular choline⁺ instead of Na⁺ was not altered by the removal of the parathyroids (data not illustrated). These findings suggested that Na⁺-dependent ⁴⁵Ca²⁺ flux in renal cells was modulated by endogenous levels of PTH.

Cells from control and parathyroidectomized rats were incubated in vitro for 1.5 min with 10 units/ml PTH(1–84). With cells from sham-operated rats, Na⁺-dependent ⁴⁵Ca²⁺ efflux was enhanced 61%, from 10.6 ± 0.6 to 17.1 ± 0.9% (p < 0.01). With cells from parathyroidectomized animals, PTH increased efflux 106%, from 8.0 ± 0.6 to 16.5 ± 1.5% (p < 0.01). The difference between 17.1 ± 0.9%, the efflux found when control cells were incubated with PTH in vitro, and 16.5 ± 1.5%, the efflux found when cells from parathyroidectomized animals were incubated with PTH in vitro, was not significantly different. These results indicated that ⁴⁵Ca²⁺ efflux from control and parathyroidectomized rats treated with PTH in vitro could be increased to the same level.

Effect of Age of the Rat on the Sensitivity of Na⁺-dependent Calcium Efflux to PTH.—Because calcium homeostasis is a critical problem in the aging animal and the senescent rat was reported to have increased levels of immunoreactive PTH (28, 29) and the accumulation of cAMP in response to PTH in renal cortical slices from 12-month-old rats was found to be decreased in comparison to cells from 2-month-old animals (30), we examined the effect of age of the rat on renal cell ⁴⁵Ca²⁺ efflux and the responsiveness of the system to PTH. ⁴⁵Ca²⁺ efflux into a choline⁺-containing medium was not altered by the age of the animal, from 2 to 24 months (data not shown). Fig. 7 shows that Na⁺-dependent ⁴⁵Ca²⁺ efflux also did not significantly differ in the cells from the different aged rats, varying from a low of 8.3 ± 1.4% in cells from 12-month-old rats to a high of 10.3 ± 1.0% in the cells from 24-month-old animals. In contrast, the PTH responsiveness of the transport system did depend on age. When the cells were preincubated for 1.5 min with 10 units/ml PTH(1–34), Na⁺-dependent ⁴⁵Ca²⁺ efflux from cells from 2-month-old animals increased from 10.0 ± 1.0% to 17 ± 2.2%, a stimulation of 69% (p < 0.01). The PTH stimulation was 62% (p < 0.01) and 59% (p < 0.01) with cells from 6- and 12-month-old rats, respectively. However, stimulation with cells from senescent animals (24 months) was markedly decreased, being 10.3 ± 1.0% without PTH and 12.8 ± 1.3% with PTH, a change of 24% which was not statistically significant. These findings indicated that the PTH sensitivity of the renal cell Na⁺-dependent ⁴⁵Ca²⁺ efflux system was blunted in the senescent rat. The decrease in PTH responsiveness with aging could not be attributed to an age-dependent difference in the cellular uptake of labeled Ca²⁺ during preloading of the cells. The calcium contents of cells from the different aged rats at steady state immediately prior to initiation of efflux varied from 2.45 to 2.66 nmoi·mg⁻¹ of protein, values which were not significantly different.

This loss in the responsiveness of the transport system to PTH with age was in accord with the finding of a decrease in PTH-sensitive adenylate cyclase in membranes prepared from the renal cells from senescent rats. Table I shows that basal adenylate cyclase activity was not altered with age, being 49 ± 10 pmol·15 min⁻¹·mg⁻¹ of protein and 47 ± 9 in membranes from 2- and 24-month-old animals, respectively. In the presence of 10 units/ml PTH(1–34), cyclase activity increased 3.46-fold with preparations from the young animals, but only 2.11-fold with membranes from the aged rats. In the presence of a concentration of GTP (1 μM), which by itself had little affect on basal activity, PTH increased adenylate cyclase activity 4.69- and 1.96-fold in membranes from 2- and 24-month-old rats, respectively. In contrast, forskolin-stimulated
TABLE I

Effect of PTH and age on adenylate cyclase activity in renal cell membranes

| Hormone       | Basal (2 months) | Basal (24 months) |
|---------------|------------------|-------------------|
| PTH           | 3.46 ± 0.76      | 2.11 ± 0.32       |
| GTP           | 1.18 ± 0.19      | 1.19 ± 0.14       |
| PTH + GTP     | 4.69 ± 0.94      | 1.95 ± 0.26       |
| Forskolin     | 14.2 ± 3.9       | 10.5 ± 1.0        |

FIG. 8. Effect of age of the rat on the sensitivity Na+-dependent 45Ca2+ to forskolin. Cells were treated with 10 μM forskolin for 1.5 min prior to initiation of efflux. Na+-dependent 45Ca2+ efflux was calculated as described in Fig. 6. Each datum represents the mean ± S.E. for 5–8 experiments, each replicated.

The present findings provide evidence supporting the postulate of a direct effect of the hormone administered in vivo on isolated renal tubular cells to regulate Na+/Ca2+ exchange activity or whether the modulation was effected indirectly, such as by changing renal hemodynamics, altering serum calcium levels, or influencing the titers of other hormones. The present study now demonstrated that PTH incubated in vitro with isolated renal cortical cells increased the cellular efflux of Ca2+. The response to the hormone was dependent on the presence of Na+ in the extracellular medium. Thus, these findings provided evidence supporting the postulate of a direct effect of PTH on renal cells which resulted in regulation of Na+/Ca2+ exchange activity. The present observations that PTH enhanced Ca2+ efflux would be consistent with the recent report that PTH lowered the intracellular free Ca2+ concentration in tubular cells (36).

The specificity of the action of PTH was indicated by the findings that the biological active bovine PTH(1–84) and the synthetic tetraacacetate peptide PTH(1–34) increased Na+-dependent 45Ca2+ efflux, whereas the biological inactive analog PTH(3–34) did not. In addition, the effect on PTH(1–84) was concentration-dependent. Although the concentration of hormone needed for maximal response in our in vitro system was relatively high, approximately 10 units/ml, the dose was in accord with that used in previous investigations on the in vitro effect of bovine PTH on adenylate cyclase activity in renal tubular segments (37) and cultured renal cells (38). One possible explanation for the required high dosage was the discordance between the animal species from which the cell was obtained and the animal from which the PTH molecule was derived. Recently, it was reported that rat PTH(1–34) was significantly more potent than bovine or human PTH(1–34) when tested by in vitro activation of rat renal adenylate cyclase (39). Another possible explanation for the relatively high concentration of PTH needed to demonstrate response in these isolated cells could be an effect of collagenase-hyaluronidase, used in obtaining the preparations, on the hormone receptor-adenylate cyclase complex.

The present findings that 1) PTH when incubated with renal cells for 1.5 min elicited both Ca2+ transport and cAMP formation responses in a comparable concentration-dependent manner; 2) PTH analogs which increased Na+-dependent Ca2+ efflux also increased cellular cAMP, whereas the analog that did not affect transport did not generate cAMP; and 3) dibutyryl cAMP and forskolin, the activator of adenylate cyclase, mimicked the action of PTH on Ca2+ efflux supported the view that cAMP might act as an intracellular messenger to increase Na+/Ca2+ exchange activity.

The precise region of the nephron from which the isolated cortical cells were derived has not been established. This question gains importance because of the functional heterogeneity of the nephron with respect of Ca2+ transport and of the multiple loci along the nephron of PTH-stimulated adenylate cyclase. More than half of the Ca2+ reabsorbed by the nephron was found to take place in the proximal tubule; the remainder occurred distally, in the cortical thick ascending limb of Henle and the cortical distal convoluted and collecting tubule (31–40). Substantial PTH-stimulated adenylate cyclase was demonstrated in the rat nephron in the proximal tubule, cortical thick ascending limb, and distal convoluted tubule, with only marginal activity in the cortical connecting tubule and no measurable PTH-stimulated cyclase in medullary regions (37, 43). Furthermore, it had been long argued...
that Ca\(^{2+}\) flux in the proximal tubule was mostly passive or occurred paracellularly, whereas the remaining component of the filtered Ca\(^{2+}\), which was reabsorbed distally, was regulated (14, 15). However, recent evidence would suggest that a significant fraction of the filtered Ca\(^{2+}\) conserved by the proximal tubule was reabsorbed actively (3, 44). Moreover, the presence of the Na\(^{+}/Ca\(^{2+}\) exchange system in the proximal tubule was inferred from physiological experiments (3, 45), and Na\(^{+}/Ca\(^{2+}\) exchange activity was demonstrated in basolateral membranes derived largely, but not exclusively, from the proximal tubule (5–8). Because the cells used in this study were prepared from the rat renal cortex, it is unreasonable to expect elements from all cortical nephron segments to be present in the preparation. The pattern of hormone-stimulated cyclase in the cell preparation, i.e. responsive to PTH but only moderately (2-fold over basal) responsive to vasopressin and not at all responsive to calcitonin,\(^2\) might suggest that the cells were derived predominantly from the proximal tubule. This finding would be consistent with the fact that the bulk of the nephrogenous elements in the cortex would be proximal tubules. In addition, the proximal tubule would be the locus of PTH-sensitive phosphate transport (46) and 25-hydroxyvitamin D\(_3\) hydroxylation (47). On the other hand, the cortical thick ascending limb of Henle in the rat should contain calcitonin- and PTH-stimulated adenylate cyclase as well as some enzyme responsive to vasopressin. Thus, the failure to find activation of the cyclase by calcitonin in the isolated cells would not be concordant with their derivation from the thick ascending limb. The possibility that during the preparation of the cells calcitonin-stimulated adenylate cyclase was lost is not precluded, however. Therefore, based on the present evidence, we would propose the hypothesis that PTH modulated Na\(^{+}\)-dependent Ca\(^{2+}\) efflux in proximal tubular cells. This view would be consistent with a preliminary communication that PTH increased the active transport rate for Ca\(^{2+}\) in the microperfused proximal tubule (48) and with the recent report that the hormone and dibutryl CAMP decreased the concentration of intracellular Ca\(^{2+}\) in the proximal tubule (36). Since the effects of PTH and cyclic nucleotides in increasing Ca\(^{2+}\) transport in distal regions of the nephron were well documented (49), it could be postulated that the hormone also acted on Na\(^{+}/Ca\(^{2+}\) exchange in these nephron segments. Thus, although it is clear that PTH enhanced Na\(^{+}/Ca\(^{2+}\) exchange in renal cells, the precise loci of the carrier in the heterogenous nephron and its sensitivity to the hormone remain to be determined as well.

The present study demonstrated that PTH-sensitive Na\(^{+}/Ca\(^{2+}\) exchange activity was markedly decreased in the senescent rat. Basal Na\(^{+}/Ca\(^{2+}\) exchange and Na\(^{+}\)-independent efflux of Ca\(^{2+}\) were not altered in the aging animal. In contrast, forskolin-stimulated Na\(^{+}/Ca\(^{2+}\) exchange activity did not decrease in the aged rat. In accord with these results, it was found that PTH-stimulated adenylate cyclase activity was decreased in cells from senescent rats, whereas forskolin-stimulated adenylate cyclase activity did not change with age. These decrements with age appeared not to be due to an age-dependent decrease in the general metabolism and integrity of the cells, for there were no differences with age in the ATP content of the cells, the rate of glucose catabolism, the leakage of the cytosolic enzyme lactate dehydrogenase, and the exclusion of trypan blue. However, a possible age-dependent increase in the susceptibility of the PTH receptor or hormone-receptor coupling to adenylate cyclase to damage by the cell isolation procedure has not been definitively excluded.

\(^2\) C. T. Liang, H. Hanai, and B. Sacktor, unpublished results.

These results would be consistent with reports that the aged rat had increased levels of immunoreactive PTH (28, 29), and cultured renal cells developed refractoriness in their cAMP response to PTH (38, 50, 51). Our findings would be compatible with a mechanism of desensitization that occurred at the level of the receptor or hormone-receptor coupling to adenylate cyclase. Additional studies on PTH receptor number and sensitivity as well as the activities of the stimulatory and inhibitory GTP-binding proteins with respect to age are indicated. In summary, the present biochemical results demonstrating PTH-sensitive Na\(^{+}\)-dependent Ca\(^{2+}\) efflux in renal cells and the blunting of the response with age may be of physiological significance in understanding calcium homeostasis and the imbalances in mineral metabolism associated with old age.

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