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Parathyroid Hormone-Induced Changes of the Brush Border Topography and Cytoskeleton in Cultured Renal Proximal Tubular Cells

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Summary. In order to examine the possibility of parathyroid hormone-mediated ultrastructural rearrangements in target epithelium, isolated canine renal proximal tubular cells were grown on a collagen-coated semipermeable membrane in a defined medium. Scanning and transmission electron microscopy of these monolayers revealed abundant microvilli. Exposure of the proximal tubular cells to parathyroid hormone resulted in a biphasic changes involving: (1) dramatic shortening and rarefaction of microvilli within 1 min; and (2) recovery of microvillar topography after 5 min. A similar shortening of microvilli was observed following exposure to ionomycin, whereas incubation with cyclic AMP resulted in an elongation of microvilli. Parathyroid hormone stimulated cyclic AMP production and increased cytoplasmic free calcium concentration in cultured proximal tubular cells. Pretreatment of cells with a calmodulin inhibitor abolished the effect of parathyroid hormone on brush border topography. Shortening of microvilli was associated with a disappearance of microvillar core filaments. Staining of F-actin with fluorescein-phalloidin showed that parathyroid hormone resulted in fragmentation of stress fibers. It is concluded that parathyroid hormone-induced cell activation involves cytoplasmic-free calcium, calmodulin, and the cytoskeleton.

Key Words kidney · parathyroid hormone · angiotensin II · calcium · brush border · cytoskeleton

Introduction

The microvillus cytoskeleton represents a complex network of multiple structural and Ca^{2+}-binding proteins [3, 33, 43], which stabilize the peculiar shape of the brush border membrane. In certain conditions, such as fasting [32] or increased in vitro concentration of either ATP [4, 26] or calcium [3, 4, 14, 26], the rigidity of the microvilli is lost and they dramatically change their shape. The location of the brush border membrane, its expanded surface area and characteristic membrane fluidity [30] are specializations that favor transport processes and are felt to be regulated.

In the renal proximal tubular cell, a number of transport processes have been shown to be regulated at the level of the brush border membrane [for review *see* 8, 10, 42]. The recent demonstration of structural changes in the apical membrane of renal collecting tubule cells and toad urinary bladder epithelium in response to ADH [12, 17, 19, 29] evokes an intriguing question of structural rearrangements serving as a prerequisite for functional adaptation of transport processes. These hormone-induced morphologic changes are consistent with, and may represent a particular case of, the currently developing concept of transepithelial "cross-talk" between basolateral and luminal membranes [6, 11].

Parathyroid hormone elicits multiple effects on the transport processes in the renal proximal tubular cell, such as inhibition of phosphate uptake, inhibition of Na^+/H^+ exchange and activation of contraluminal Na^+-Ca^{2+} exchange [8, 10, 13, 22, 23, 25, 27, 42]. We attempted to examine the possibility of parathyroid hormone-induced structural changes in the brush border topography. We report biphasic changes in the brush border topography which occur upon exposure of cultured proximal tubular cells to parathyroid hormone.

Materials and Methods

Preparation of Proximal Tubular Segments and Cell Culture Technique

Mongrel dogs of either sex were anesthetized with pentobarbital and nephrectomy was performed via flank incision. The renal artery was immediately canulated and the kidney perfused with ice-cold saline. Renal cortical tissue was sliced with a Stadie-Riggs microtome and incubated for 45 min in a shaking water bath in a Krebs-Henseleit buffer, pH 7.4, containing 0.1 mg/dl collagenase (Type 1, Sigma Chemical, St. Louis, MO) and 0.3 g/dl bovine serum albumin, in an atmosphere of 95% O_2 + 5% CO_2

at 37°C in a shaking water bath. The homogenate was centrifuged in a Percoll gradient, as described by Vinay et al. [41].

Tubular segments of proximal tubular origin, obtained from band IV of the Percoll gradient, were seeded at hyperconfluent densities on collagen-coated polycarbonate membranes (0.8 μm pore size, 13 mm; Nucleopore Corp., Pleasanton, CA) glued to rubber rings (Millipore, Bedford, MA) with Millipore cement formulation #1. The cells were grown in Dulbecco's modified MEM and F-12 medium (1 : 1) containing the following additions: transferrin 5 μg/ml; insulin 3 μg/ml; PGE_1 25 ng/ml; hydrocortisone 5×10^{-8} M and triiodothyronine 5×10^{-12} M. During the first three days, 5% fetal calf serum and 5% horse serum were used to promote anchoring of the cells. The cells were used 3 weeks after the initiation of the cultures. At this time the cells formed a confluent monolayer, as determined by phase contrast microscopy in vivo, and by both transmission and scanning electron microscopy of fixed specimens. By this time the monolayers expressed polarity with the apical-negative transepithelial potential difference of 0.28 ± 0.09 mV, short-circuit current 1.3 ± 0.23 $\mu A\ cm^{-2}$, and a resistance of $95.0 \pm 9.3\ \Omega\ cm^2$ ($n = 23$).

The cell monolayers were exposed to bovine 1-84 parathyroid hormone (gift from Dr. J. Morrisey), (Nle^8, Nle^{18}, Tyr^{34}) bovine parathyroid hormone (3-34) amide (Sigma Chemical, St. Louis, MO), ionomycin (Calbiochem, San Diego, CA), cyclic 3',5' adenosine monophosphate or dibutyryl cyclic 3',5'-adenosine monophosphate, angiotensin II, apamin and compound 48/80 (all from Sigma Chemical, St. Louis, MO) in one ml of culture medium for different time intervals and at concentrations as specified in the results.

Transmission and Scanning Electron Microscopy

Cell monolayers on polycarbonate membranes were fixed in cold cacodylate-buffered 2% glutaraldehyde (48 hr), and rinsed in cacodylate buffer. Specimens for TEM[1] were post-fixed for one hour with 1% osmium tetroxide in the same buffer, dehydrated through a graded ethanol series, and embedded in Epon. In a parallel series of experiments, phosphate-buffered saline was used in place of the cacodylate buffer for the preparation of TEM specimens. Ultrathin sections were cut normal to the membrane surface with a Porter Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and observed under a Philips 200 TEM.

Specimens for study in the SEM were fixed in 2% glutaraldehyde (24 hr), dehydrated in a graded ethanol series, and dried out of liquid CO_2 by the critical point method in a Denton DCP-1 apparatus. After drying, the polycarbonate membranes were glued to SEM specimen studs with Millipore filter cement formulation #1 and coated with approximately 15 nm of gold in a Denton Desk-1 cold sputter unit. All of these specimens were examined and photographed with a Philips 501 SEM.

Cyclic AMP Formation

Proximal tubular cells grown on a solid support were suspended in Krebs-Henseleit buffer, pH 7.4, by scraping with a rubber policeman, and incubated in a shaking water bath at 37°C in an atmosphere of $95\%O_2/5\%CO_2$ for 30 min. The cells were divided into 1-ml samples, and 1 mM methylisobutylxantine was added to each sample. Parathyroid hormone (10^{-9}–10^{-6} M) was added, and after 5 min, the reaction was terminated by 0.3 N perchloric acid. The reaction mixture was neutralized with KOH, and the precipitate was removed by centrifugation. The cyclic AMP concentration in the supernatant was measured by the previously described radioimmunoassay technique [18, 38], in Dr. K. Martin's laboratory. Measurements were performed in quadruplicate in four separate experiments. Cyclic AMP production was expressed as fmol of cAMP per 10^6 cells.

Changes in Cytoplasmic Free Calcium Concentration

Proximal tubular cells in suspension were loaded with a cell-permeant fluorescent calcium indicator quin 2/AM as described by Tsien et al. [40]. The final intracellular concentration of quin 2 was estimated to be 0.5–0.6 mM. In the preliminary experiments, lower concentrations of quin 2/AM (5 μM) were tested. This protocol resulted in an intracellular quin 2 concentration of 0.1–0.2 mM, with no significant changes in fluorescence patterns evoked by different stimuli. Leakage of the dye out of the cells averaged 5–7% during 30 min of incubation. Measurements of individual cell fluorescence were performed by means of fluorescence flow cytometry (Coulter Electronics, Hialeah, FL) with multiparameter data acquisition and display system. Cells were excited at 347 nm wave length from an argon laser, and fluorescence was monitored at 480–515 nm. A blocking filter at 480 nm allowed us to cut off the fluorescence of pyridine nucleotides. For each measurement, the fluorescence of 10,000–25,000 cells was collected and fluorescence intensity was plotted against the number of counted cells. After the basal fluorescence of the cell suspension was determined, 10^{-7}–10^{-8} M parathyroid hormone was added and recording was immediately repeated. These repeated measurements were completed within 40–120 sec, thus an impact of quin 2 leakage on fluorescence measurements was negligible. Under the experimental conditions parathyroid hormone did not affect the autofluorescence of the cells. The detailed account of monitoring cytoplasmic calcium transients in response to PTH has been done elsewhere [21].

F-Actin Staining with Fluorescein Phalloidin

Renal proximal tubular cells were grown in petri dishes. At confluence, the cells were treated with either 10^{-5} M cytochalasin B for 10–20 min or 10^{-8}–10^{-7} M parathyroid hormone for 1-min. After repeated washing with phosphate-buffered saline, the cell monolayers were fixed in 3.7% formaldehyde solution in the same buffer for 10 min at room temperature. The samples were again washed in phosphate-buffered saline and exposed to absolute acetone for 4 min at −20°C, and then air-dried, according to the previously described technique [44]. Fluorescein phalloidin (Molecular Probes, Junction City, OR) was applied to the fixed and permeabilized monolayers at a concentration 0.05 mg/ml in phosphate-buffered saline with an addition of 1% dimethyl sulfoxide for 40 min in an incubator. After thorough washing, cell monolayers in petri dishes were immersed in 30% glycerol in the same buffer and covered with coverslips. The preparations were examined with a Nikon fluorescence microscope and photographs were taken with Kodak Tri-X pan film 400 ASA.

[1] *Abbreviations used:* TEM—transmission electron microscopy; SEM—scanning electron microscopy; PTH—parathyroid hormone.

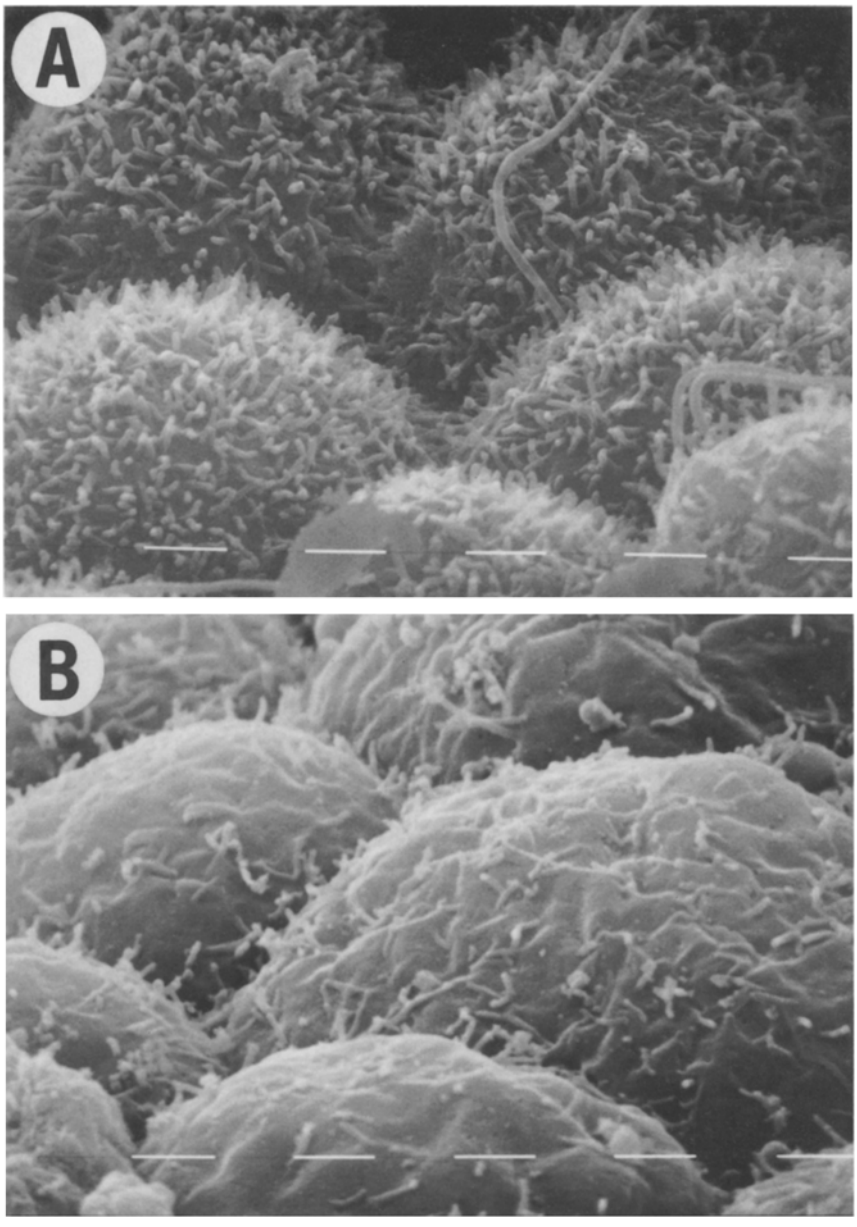


Fig. 1. (*A*) Scanning electron micrograph of renal proximal tubular cells grown on a polycarbonate membrane. Numerous microvilli measuring up to 1 μm in length cover the apical surface of the cells. Magnification ×10,000; bar = 1 μm. (*B*) Scanning electron micrograph of proximal tubular cells exposed for 1 min to bovine 1-84 parathyroid hormone (10^{-8} M). Brush border is markedly reduced in development. ×10,000; bar = 1 μm

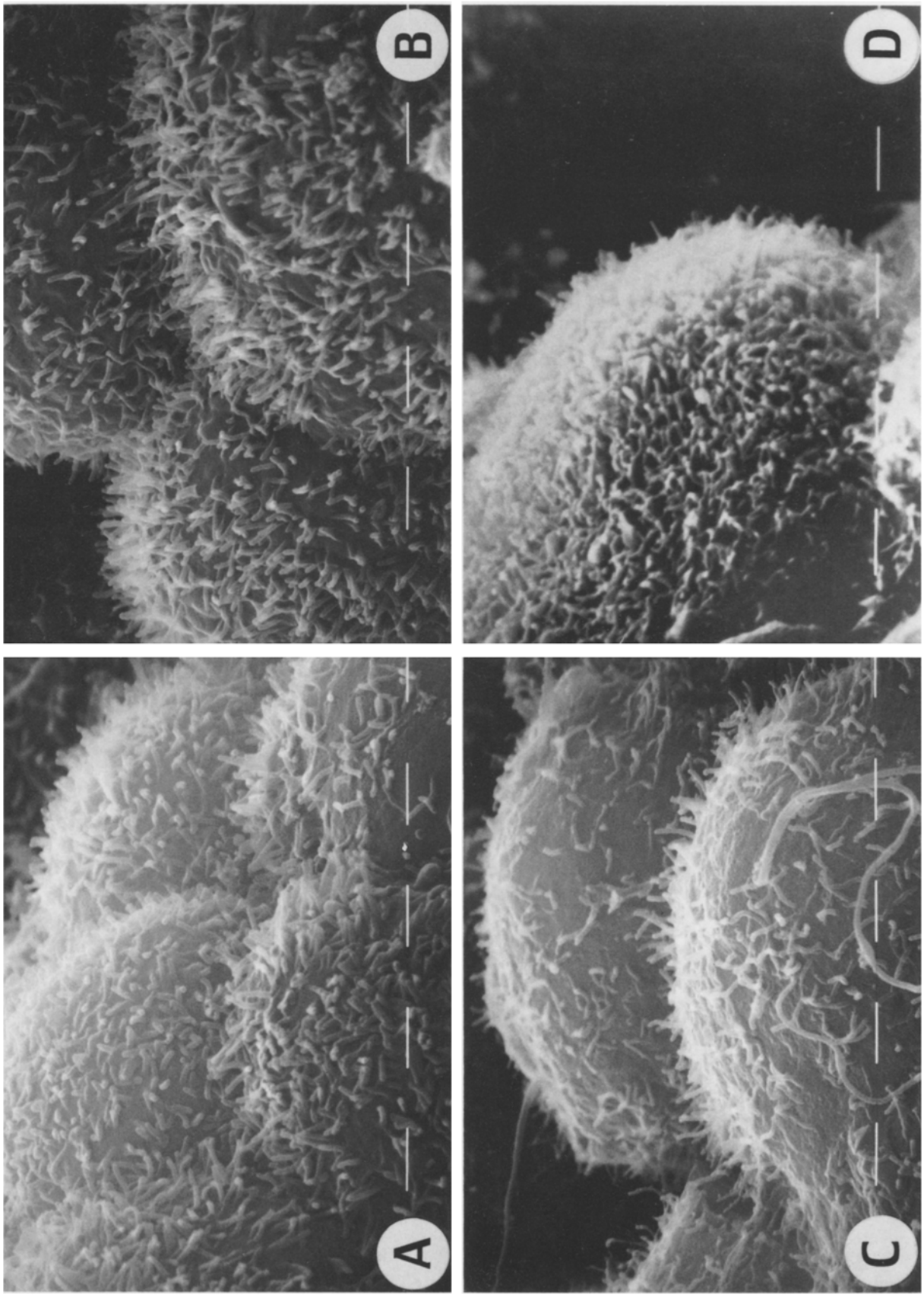
Results

Biphasic Effect of Parathyroid Hormone on the Brush Border Topography

Resting proximal tubular cells form a monolayer on a polycarbonate collagen-coated membrane. Transmission and scanning electron microscopy revealed abundant microvilli reaching approximately 1 μm in length (Figs. 1*A* and 3*A*).

Exposure to 10^{-7}–10^{-8} M bovine 1-84 parathyroid hormone for 1 min resulted in a striking shortening and rarefaction of microvilli with an almost complete disappearance of the brush border (Figs. 1*B* and 3*B*). After 5 min of incubation with the hormone, however, there was a marked recovery of the brush border topography (Fig. 2*A*). Transmission electron microscopy of the hormone-treated cells revealed, complemental to SEM data, an initial shortening of microvilli and an apparent loss of their characteristic core filaments (*compare* Fig. 3*B* with the control 3*A*). The inactive analog of parathyroid hormone (Nle^8,Nle^{18},Tyr^{34}) bPTH-(3-34) amide, did not affect the shape of the microvilli (Fig. 2*D*).

To assess quantitatively a possible parathyroid hormone-induced change in microvillar density, the total number of microvilli was counted in four ran-


B
D
A
C

domly selected 10-μm^2 areas in each experimental series (data was collected from 32 photographs). The results are summarized in the Table. By the first minute following exposure to parathyroid hormone, there was a twofold decrease in the microvillar density ($P < 0.01$). After 5 min of exposure to the hormone the microvillar density returned to the control level. Thus the first phase of the parathyroid hormone action on proximal tubular cells was characterized by a reduction in both microvillar length and density. There was almost complete recovery of both of these parameters during the second phase of the parathyroid hormone effect.

Intracellular Messengers of Parathyroid Hormone

It has been postulated that the effects of parathyroid hormone on proximal tubular cells are mediated by a dual messenger system, involving the generation of cyclic AMP and an increase in cytoplasmic free calcium concentration [36]. While there is no doubt that parathyroid hormone stimulates adenylate cyclase in target cells, the effect on cytoplasmic calcium remains a matter of controversy [2, 13, 22, 45]. We thus attempted to examine cyclic AMP formation and changes in cytoplasmic free calcium concentration in proximal tubular cells following exposure to parathyroid hormone. Figure 4*A* shows the dose-response curve of cyclic AMP production in suspensions of proximal tubular cells during a 5-min exposure to parathyroid hormone. These results are similar to those previously obtained in cultured renal tubular cells [5, 20, 39]. They demonstrate that in the concentration range 10^{-8}–10^{-7} M, parathyroid hormone significantly stimulated cyclic AMP production. From this data, and those obtained by others in cultured cells [5, 20, 39], it is apparent that the sensitivity to the hormone is reduced compared to proximal tubular cells *in situ*.

To study changes in cytoplasmic-free calcium concentration, cells were loaded with quin 2/AM, and flow cytometric recording of quin 2 fluorescence was performed. Figure 4*B* represents a typical (four separate experiments utilizing different batches of proximal tubular cells) response of quin 2 fluorescence upon exposure to parathyroid hormone. There was an immediate increase in fluorescence intensity (appearance of the second "hump" on the recording), reflecting qualitatively an increase in cytoplasmic-free calcium concentration. Thus both cyclic AMP and cytoplasmic free calcium participate in the cellular response to parathyroid hormone in cultured renal proximal tubular cells. Hence, both could contribute to the observed changes in brush border topography. This was the reason we studied their effects on the brush border topography separately.

Effects of Cyclic AMP and Ca^{2+}-Ionophore on the Brush Border Topography

Cyclic AMP (10^{-4}–10^{-3} M) or dibutyryl cyclic-AMP (10^{-7}–10^{-4} M) elicited no shortening of the microvilli at 1, 5 and 30 min of observations, but rather a lengthening of the brush border microvilli occurred after 5 min of exposure to these compounds (Fig. 2*B*). Proximal tubular cells exposed to 250 nM ionomycin showed a significant shortening and rarefaction of microvilli by 1 min (Fig. 2*C*), similar to the first phase of the parathyroid hormone effect. This concentration of ionomycin caused an increase in cytoplasmic calcium concentration comparable to that produced by parathyroid hormone (*data not shown*). Unlike parathyroid hormone, however, there was no subsequent restoration of microvillar shape within the 30-min time frame of these studies.

Comparative Effects of Other Hormones on the Brush Border Topography

To ascertain further the specificity of the PTH-induced topographic changes, the effects of different hormonal stimuli were tested. Angiotensin II increases cytoplasmic calcium concentration in cultured proximal tubular cells (*personal observation*). Figure 5 demonstrates the time course of the effect of angiotensin II on the brush border topography. The immediate phase is remarkably similar to that observed following exposure to parathyroid hormone (for comparison *see* Fig. 1*B*). The recovery process, however, was significantly delayed (up to

Fig. 2 (*facing page*). (*A*) Reversibility of the effect of parathyroid hormone. Five minutes following exposure to the hormone there is an almost complete recovery of microvillar shape and length. ×10,000; bar = 1 μm. (*B*) Scanning electron micrograph of proximal tubular cells exposed to 10^{-7} M dibutyryl cyclic AMP. Note the uniform elongation of microvilli and occasional aggregation of their free ends. ×10,000; bar = 1 μm. (*C*) Scanning electron micrographs of proximal tubular cells exposed to 250 nM ionomycin. Note the uniform shortening and rarefaction of microvilli. There is a striking similarity with the initial effect of parathyroid hormone (Fig. 1*B*). ×10,000; bar = 1 μm. (*D*) Scanning electron micrograph of proximal tubular cells following treatment with 10^{-7} M 3-34 fragment of parathyroid hormone. This PTH analog does not reproduce the observed effect of 1-84 parathyroid hormone (*compare* with Fig. 1*B*). ×10,000; bar = 1 μm

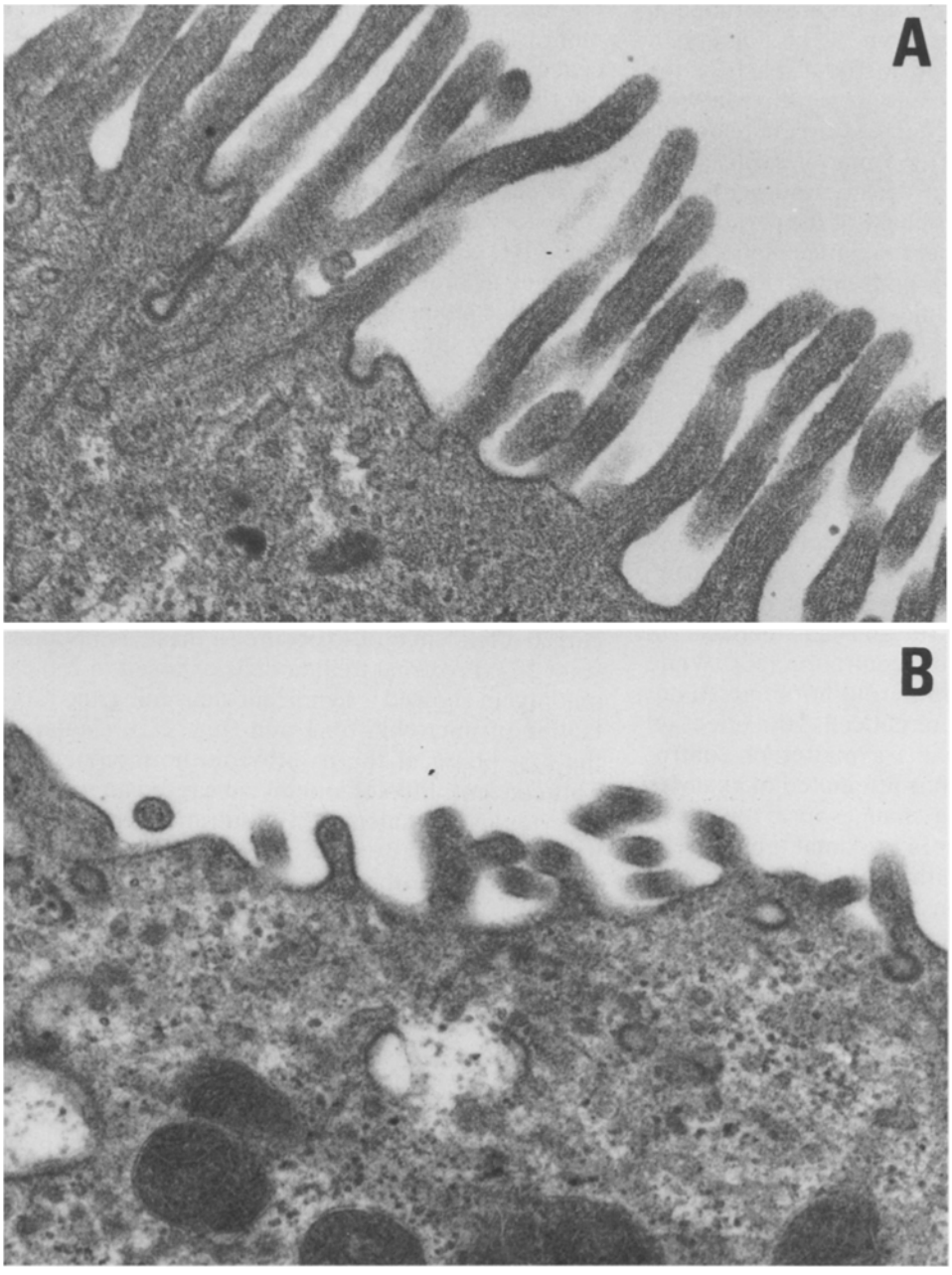


Fig. 3. (*A & B*) Transmission electron micrographs of microvillar ultrastructure in the intact (*A*) and parathyroid hormone-treated (*B*) proximal tubular cells. Note the presence of microvillar core filaments and rootlets in the control (*A*) and their disappearance following parathyroid hormone (*B*). ×30,000

30 min of observation). Since angiotensin II does not affect cyclic AMP production, as parathyroid hormone does, it is conceivable that the rate of the recovery of the brush border topography is related to the production of this adenine nucleotide.

In addition, we tested the effects of insulin, which affects neither cytoplasmic calcium concentration nor cyclic AMP production, on the brush border topography. No changes in microvillar shape and density occurred up to 30 min following administration of insulin (*data not shown*).

Thus comparative data on the effects of biologically active compounds, which differ in the intracellular messengers involved, demonstrated a possible role of cytoplasmic calcium and cyclic AMP in changes of the brush border topography. Specifi-

Table. Density of microvilli on the apical surface of cultured renal proximal tubular cells (mean ± SD)

	Microvilli/10 μm^2	*P* value
1. Control	315.5 ± 18.6	$P_{1-2} < 0.01$
2. 1 min after PTH	166.0 ± 18.3	$P_{2-3} < 0.05$
3. 5 min after PTH	268.0 ± 27.9	P_{1-3} NS[a]

[a] NS—not significant

cally, the immediate phase of the PTH effect exhibited a close similarity to that produced by another calcium-mobilizing hormone, angiotensin II, suggesting a possible involvement of cytoplasmic calcium in this process. The recovery phase following angiotensin II was protracted as compared to PTH, compatible with a possible role of cyclic AMP generation in prompt restoration of the brush border topography following parathyroid hormone administration.

Cell Swelling vs. Primary Involvement of the Cytoskeleton

Shortening and rarefaction of microvilli upon exposure to parathyroid hormone could, theoretically, result from hormone-induced cell swelling. Ca^{2+}-activated K^+ efflux is implicated in subsequent regulatory volume decrease [15, 28], and in fact occurs in renal proximal tubular cells following administration of parathyroid hormone [7]. By blocking potassium efflux it could be possible to amplify and visualize the effect of a hormone-induced cell swelling on the brush border topography. In the next series of experiments, the influence of apamin, a specific blocker of Ca^{2+}-activated K^+ channels [9, 37], was examined. Proximal tubular cells were pretreated with 10 nM apamin for 5 min and then exposed to parathyroid hormone for 1–5 min. This protocol resulted in striking cellular swelling accompanied by vesiculation of microvilli (Fig. 6*A*); however, it did not affect their length and density. This phenomenon was not due to a toxic effect of apamin, since alone it did not exert any topographic changes. These data indicate that cell swelling, per se, is not likely to be a significant contributor to the perturbations in the brush border topography induced by parathyroid hormone. Direct observations using differential-interference optics of proximal tubular cells in vivo exposed to parathyroid hormone confirmed this conclusion (*data not shown*).

It has been demonstrated that calmodulin mediates calcium-induced contraction of the terminal web [26]. Alternatively, calmodulin may be required for activation of actin-binding proteins [35].

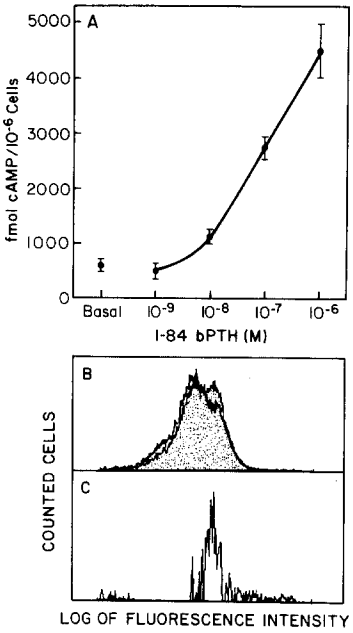


Fig. 4. (*A*) Parathyroid hormone-induced production of cyclic AMP. (*B*) Qualitative assessment of cytoplasmic free calcium transients following administration of parathyroid hormone. 10,000 cells were counted by means of flow cytometry, and their integrated fluorescence profile was generated. Parathyroid hormone was added, and fluorescence profile measurements of 10,000 cells were repeated. This resulted in an increase of the integrated fluorescence (right shift of the shaded curve), which reflects qualtitatively an increase in cytoplasmic calcium concentration in the representative population of proximal tubular cells. After 5 min of incubation with parathyroid hormone, fluorescence of proximal tubular cells was still elevated (*data not shown*). Control samples without parathyroid hormone did not exhibit any shift of fluorescence intensity during 5 min of observation. (*C*) Subtraction of the basal fluorescence curve from the curve generated after parathyroid hormone treatment

We further examined the effect of a specific calmodulin inhibitor, compound 48/80, on the microvillar topography. Renal proximal tubular cells were pretreated with 50 μg/ml of compound 48/80 for 10 min (control), and parathyroid hormone was then administered into the incubation medium for 1, 5 and 30 min. Compound 48/80 by itself had no discernible effect on the brush border topography (*data not shown*), but it clearly abolished the characteristic effect of parathyroid hormone (Fig. 6*B*). Shortening and rarefaction of microvilli did not occur within the 30-min time frame of observation.

Direct evidence of the involvement of the cytoskeleton during the early phase of the response to parathyroid hormone was obtained in the experiments with fluorescein-phalloidin staining of F-actin. Figure 7 summarizes these results. Proximal tubular cells grown in petri dishes displayed an abundant network of actin filaments (Fig. 7*A*),

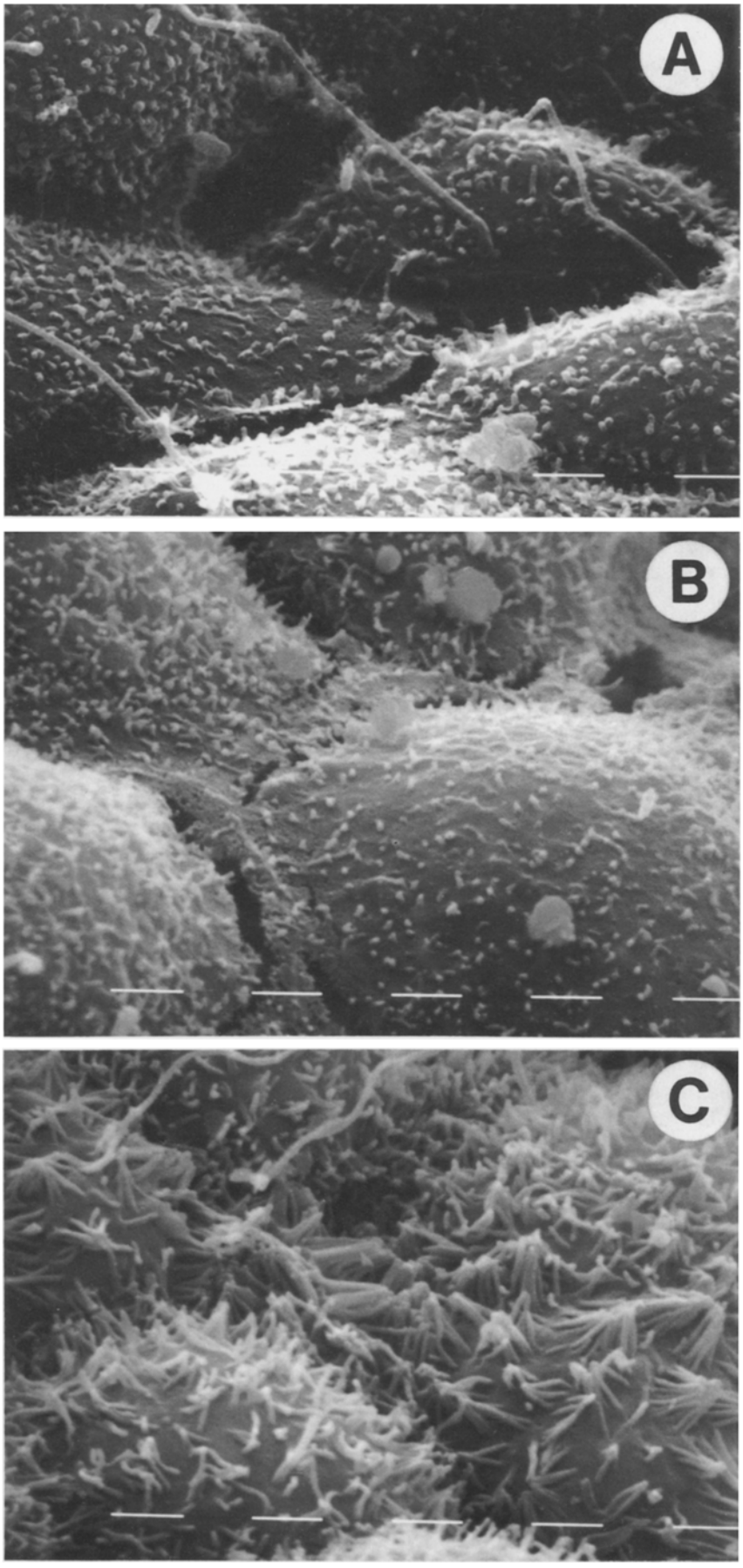


Fig. 5. (*A–C*) The effect of angiotensin on the brush border topography. (*A*) 1 min following angiotensin II. (*B*) 5 min following angiotensin II. (*C*) 30 min following angiotensin II. Note a remarkable similarity of the initial phase of the effect of angiotensin II on microvillar topography to that produced by parathyroid hormone, and in contrast to the latter, a protracted recovery of the brush border topography (up to 30 min). ×10,000; bar = 1 μm

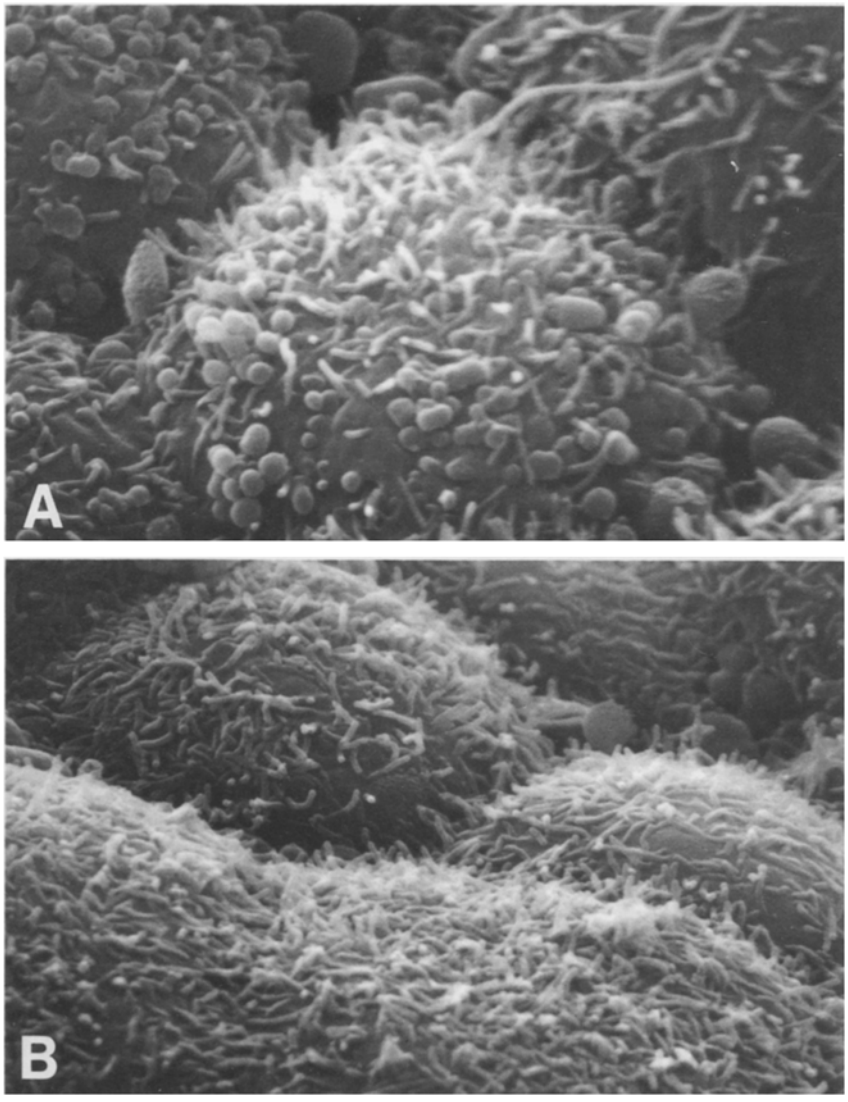


Fig. 6. Scanning electron micrographs of proximal tubular cells pretreated with apamin (*A*) and compound 48/80 (*B*) before exposure to parathyroid hormone. (*A*) Apamin interference with the effect of parathyroid hormone. Cells were pretreated with 10 nM apamin for 5 min, following by parathyroid hormone. Apparent cell swelling with a bulging of luminal membrane and vesiculation of microvilli occurred, not accompanied by discernible shortening or rarefaction of microvilli. ×10,000; bar = 1 μm. (*B*) Calmodulin inhibitor, compound 48/80 abolishes the effect of parathyroid hormone on brush border topography. ×10,000; bar = 1 μm

which was significantly affected by cytochalasin B (Fig. 7*B*). Exposure to parathyroid hormone for 1 min resulted in a uniform fragmentation of microfilaments (Fig. 7*C*). If a similar process takes place in microvilli, as suggested by the TEM data (Fig.3*B*), it could underlie the observed changes in their shape and density.

Discussion

The above observations demonstrated that parathyroid hormone causes a dramatic rearrangement of the brush border topography in cultured proximal tubular cells. One minute exposure to the hormone resulted in an almost complete disappearance of microvilli. This was accompanied by a significant but temporary decrease in their density. This effect was followed by a remarkably rapid recovery phase. It is noteworthy that neither of these effects was reproduced by the inactive 3-34 fragment of parathyroid hormone.

The striking similarity of the initial changes in the brush border topography induced by parathyroid hormone and Ca^{2+}-ionophore may indicate that an increase in cytoplasmic free calcium concentration precipitates microvillar shortening and rarefaction. In a series of studies employing the equilibrium ^{45}Ca dilution technique, it was demonstrated that parathyroid hormone increases the cytoplasmic calcium pool [2]. Our data on quin 2 fluorescence monitored with a flow cytometer, spectrofluorimeter [21], as well as observations made with fluorescence microscopy [45] confirmed this finding. It is

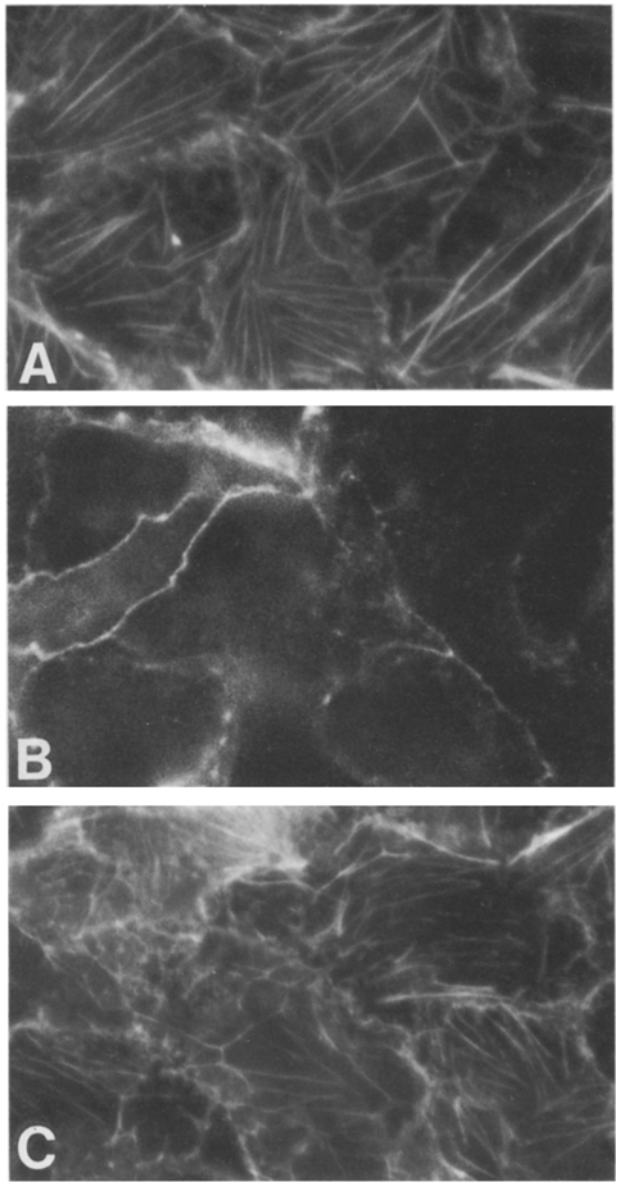


Fig. 7. Fluorescein-phalloidin staining of F-actin. (*A*) Intact monolayer. (*B*) After pretreatment with cytochalasin B for 15 min. (*C*) One minute following exposure to parathyroid hormone. ×350

tempting to speculate that an early effect of parathyroid hormone on microvillar shape and density is mediated via cytoplasmic free calcium-induced modulation of the cytoskeleton. Such a possibility has been explored previously in vitro [4, 14, 26]. It has been shown that an increase in calcium concentration promotes villin-induced severing of actin microfilaments [4, 14]. Our TEM observation of the disappearance of microvillar core filaments upon exposure to parathyroid hormone, together with the finding of fragmentation of fluorescein-phalloidin stained actin filaments are consistent with the possibility of cytoskeletal involvement in the hormone action.

It is theoretically possible that changes in the brush border topography following exposure to parathyroid hormone are due to cell swelling. To accentuate such an effect, if it exists, we used a specific blocker of Ca^{2+}-activated K^+ channels, apamin [9, 37]. When chased by parathyroid hormone, it resulted in a completely different phenomenon, which included bulging and vesiculation of the microvilli, but their length and density remained unaffected. This observation makes it unlikely that significant cell swelling is a major contributor to parathyroid hormone-induced shortening and rarefaction of microvilli.

The finding that a specific calmodulin inhibitor, compound 48/80, prevents the effect of parathyroid hormone on microvillar topography, could further support the primary role of cytoplasmic calcium in these structural metamorphoses [1]. In light of the multiple cellular functions of calmodulin, the interpretation of these results is highly complicated. Nevertheless, it is not excluded that calmodulin is required for activation of actin-binding protein(s), thus modulating the severing-polymerization cycle of actin microfilaments [35]. It is of interest in this connection that trifluoperazin inhibits the effect of parathyroid hormone on phosphate transport in isolated renal brush border membrane vesicles [24], and parathyroid hormone has been shown to induce a calmodulin-dependent phosphodiesterase activity in the rat kidney [31]. The data obtained thus far, however, do not allow us to identify the specific mechanism of parathyroid hormone-induced shortening and rarefaction of microvilli. These topographic changes may be due to the calcium-mediated solation of microvillar core filaments [4]; alternatively, they could be caused by calcium-calmodulin induced contraction of the terminal web [26], though both of these mechanisms are clearly calcium related.

The delayed phase of the parathyroid hormone effect is characterized by a recovery of the brush border topography. Since the single effect of cyclic AMP consists in elongation of microvilli, and an increased formation of cyclic AMP in response to parathyroid hormone has been well documented [22] and further confirmed in this study, it is possible that this second messenger participates in the recovery phase. It is interesting that essentially the same effect of cyclic AMP on the microvillar morphology of toad urinary bladder epithelium has recently been reported [34]. Another Ca^{2+}-mobilizing hormone (angiotensin II), which does not affect cyclic AMP formation, similarly resulted in immediate

changes in microvillar shape and density, but the recovery phase was protracted. Although not conclusive, the observed changes in the cell topography might reflect the parathyroid hormone-induced dual messenger response in the proximal tubular cell [36]. It is not excluded that these rapid structural metamorphoses are a necessary prerequisite for a prompt phosphorylation of specific membrane proteins [16, 22], or that they represent a particular case of an expression of transcellular "cross-talk" between basolateral and luminal membranes [6, 11].

In summary, parathyroid hormone mediates a striking shortening and rarefaction of microvilli in cultured renal proximal tubular cells in the span of 1 min, with an almost complete restoration of the brush border topography by 5 min. The first phase of this response can be mimicked by the Ca^{2+} ionophore ionomycin. Since calmodulin and the cytoskeleton are involved in these structural metamorphoses, it is attractive to propose that parathyroid hormone triggers the cell activation cycle involving the calcium-calmodulin-cytoskeleton axis. This phenomenon could furnish the calcium-mediated coupling of basolateral and apical membranes, and underlie parathyroid hormone-induced changes in ion transport.

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