Regulation of renal epithelial cell affinity for calcium oxalate monohydrate crystals

JOHN C. LIESKE, ERICK HUANG, AND F. GARY TOBACK
Department of Medicine, University of Chicago, Chicago, Illinois 60637

Lieske, John C., Erick Huang, and F. Gary Toback. Regulation of renal epithelial cell affinity for calcium oxalate monohydrate crystals. Am. J. Physiol. Renal Physiol. 278: F130–F137, 2000.—The binding and internalization of calcium oxalate monohydrate (COM) crystals by tubular epithelial cells may be a critical step leading to kidney stone formation. Exposure of MDCK cells to arachidonic acid (AA) for 3 days, but not oleic or linoleic acid, decreased COM crystal adhesion by 55%. Exogenous prostaglandin PGE, or PGE_{2} decreased crystal binding 96% within 8 h, as did other agents that raise intracellular cAMP. Actinomycin D, cycloheximide, or tunicamycin each blocked the action of PGE_{2}, suggesting that gene transcription, protein synthesis, and N-glycosylation were required. Blockade of crystal binding by AA was not prevented by the cyclooxygenase inhibitor flurbiprofen, and was mimicked by the nonmetabolizable AA analog eicosatetryanoic acid (ETYA), suggesting that generation of PGE from AA is not the pathway by which AA exerts its effect. These studies provide new evidence that binding of COM crystals to renal cells is regulated by physiological signals that could modify exposure of cell surface molecules to which the crystals bind. Intrarenal AA, PGs, and/or other agents that raise the intracellular concentration of cAMP may serve a protective function by preventing crystal adhesion along the nephron, thereby defending the kidney against crystal retention and stone formation.

arachidonic acid; adenosine 3',5'-cyclic monophosphate; cytotoxic protection; MDCK cells; prostaglandins

Urine is usually supersaturated with calcium and oxalate ions that nucleate to form calcium oxalate crystals (10). Unless these small crystals grow large enough to occlude a tubule lumen, aggregate with other crystals to form a mass large enough to do so, or adhere to the tubular epithelium, they will be swept out of the nephron in the flowing fluid within a few minutes, and kidney stones will not form. Indeed, calculations based on the rate of fluid flow and time required for crystals to nucleate from ions in tubular fluid suggest that an individual crystal will pass into the urine before it grows large enough to occlude the lumen and be retained in the nephron (14, 20).

We and others have hypothesized that attachment of nascent crystals to the tubular cell surface (23, 24, 32, 33, 38, 39) and the cellular responses that follow (26–28) could result in crystal retention and thereby set in motion a series of events that lead to pathological renal calcification. Adhesion of calcium oxalate monohydrate (COM) crystals, the most common crystal in renal stones, to anionic, sialic acid-containing molecules on the surface of renal epithelial cells is crystal-type specific and can be blocked by competing soluble anions in tubular fluid such as glycosaminoglycans, citrate, or glycoproteins (23, 24). Recent evidence suggests that the plasma membrane phospholipid composition may influence adhesion of COM crystals to renal cells, because enriching the phosphatidylserine (PS) content of rat inner medullary collecting duct (IMCD) cells with PS-loaded liposomes increased COM crystal binding (5). Plasma membrane fluidity is also strongly influenced by the lipid content; greater fluidity is associated with enhanced crystal binding (6). Therefore, the lipid composition of plasma membranes could alter crystal binding via several different mechanisms.

Arachidonic acid (AA) is a major component of plasma membrane phospholipids and a determinant of membrane fluidity. Supplementation of MDCK cell culture medium with AA is known to alter plasma membrane composition (22). In addition, metabolism of AA produces second messengers such as prostaglandins (PGs) and cAMP. Exposure of gastric mucosal cells to PGs is cytoprotective, and deletion of endogenous PGs has been implicated in nonsteroidal anti-inflammatory drug (NSAID)-induced gastric mucosal impairment (45). We reasoned that AA and PGs might regulate the renal cell response to COM crystals via diverse pathways. This hypothesis was tested by supplementing the culture medium of renal epithelial cells with AA, PGs, or related metabolites/second messengers to determine their effect on the kidney cell-COM crystal interaction.

MATERIALS AND METHODS

Cell culture. Renal epithelial cells of the Madin-Darby canine kidney (MDCK) line were grown in Dulbecco-Vogt modified Eagle’s medium containing 25 mM glucose (DMEM) at 38°C in a CO_{2} incubator as previously described (28). To prepare high-density, quiescent cultures, 8 \times 10^{6} cells/35-mm plastic plate (9.62 cm{sup 2}); Nunc, Naperville, IL) were plated in DMEM containing 2% calf serum and 1.6 \mu M biotin. Two days later, when they were confluent at a density of 10^{6} cells/dish, the medium was aspirated and replaced with fresh medium containing 0.5% calf serum and 1.6 \mu M biotin. Cells were used for study the next day.

BALB/c 3T3 fibroblasts were grown in DMEM containing 10% calf serum as before (28). To prepare high-density, quiescent cultures, 2 \times 10^{6} cells were plated in a 35-mm dish. Two days later the medium was aspirated and replaced with fresh medium containing 1% calf serum and 1.6 \mu M biotin.

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The cells were used for study the next day, when they were confluent at a density of $6 \times 10^6$ cells/dish.

To evaluate the effect of prior exposure of cells to AA or related metabolites on adhesion of crystals, the agent under study was added directly to the culture medium and replenished each time the medium was changed. For agents that required ethanol [PGE$_2$, flurbiprofen, dibutylryl cAMP (DB-cAMP), 17-octadecynoic acid (ODYA), cytoheximide, actinomycin D, tunicamycin] or dimethyl sulfoxide [AA, eicosatetraynoic acid (ETYA)] as a solvent, an equal volume of the vehicle was added to control cultures. When added to the culture medium to block new RNA or protein synthesis, actinomycin D (2.5 µg/ml) inhibited incorporation of [3H]uridine into RNA by 99% and cytoheximide (1 µg/ml) blocked incorporation of [3H]leucine into protein by 85% over the course of the next 6 h.

Adhesion of crystals to cells. To measure adhesion of crystals to cells, culture medium was aspirated and replaced with 2 ml of PBS (10 mM Na$_3$PO$_4$, 155 mM NaCl, and 5.4 mM KCl, pH 7.4) at 38°C. [14C]COM crystals were added to the buffer to achieve a final concentration of 200 µg/ml (41.6 µg/cm$^2$ of cells) from a sterile slurry in distilled water that was constantly stirred at 1,500 rpm to prevent aggregation. The culture dishes were gently agitated for 5 s to uniformly distribute the crystals that then settled to the surface of the cell monolayer under the force of gravity. After 2 min, buffer was aspirated, and the cells were washed three times with PBS (2 ml). The cells were then scraped directly into a scintillation vial containing 6 H Cl (0.5 ml) to which 4.5 ml of Ecoscint (National Diagnostics, E. Palmetto, FL) was added, and the amount of radioactivity was measured (32).

Our previous studies have demonstrated that the amount of cell-associated radioactivity is an index of the number of adherent crystals (26).

Prostaglandin assay. Tissue culture medium PGE$_3$ concentration was measured in a 96-well plate using a competitive immunoassay kit according to the manufacturer’s instructions (Correlate-EIA Prostaglandin E$_3$ Enzyme Immunoassay Kit; Assay Designs, Ann Arbor, MI). The antibody used in this kit has a 70% cross-reactivity with PGE$_1$, and a 16% cross-reactivity with PGE$_3$.

Materials. Crystals of COM were prepared from supersaturated solutions by Y. Nakagawa (University of Chicago) as reported previously (30). To prepare radioactive COM crystals, [14C]oxalic acid (30–60 mCi/mmol: ICN Biomedicals, Irvine, CA) was added to a sodium oxalate solution to produce a specific activity of $10^5$ cpm/mm$^2$, and sufficient calcium chloride was then added to form a supersaturated solution. The COM crystals that precipitated when different batches were prepared had a specific activity that varied from 375,000 to 800,000 cpm/mg.

Crystal size and shape were assessed by light and scanning electron microscopy (26). COM crystals were cuboidal to spindle shaped and uniformly small at 1–2 µm in largest diameter. Crystals were sterilized by heating to 180°C overnight. X-ray crystallography performed by S. Deganello (University of Palermo, Italy) demonstrated that heating did not alter the structure of COM crystals. Other reagents were purchased from Sigma Chemical, St. Louis, MO, unless otherwise indicated.

Statistics. Data were compared by Student’s t-test; $P < 0.05$ was accepted as significant. Values are means ± SE. When no measure of variance appears on a graph, it is because the variance is smaller than the symbol used for the mean.

RESULTS

Effect of AA on COM crystal adhesion to MDCK cells. As the content of AA as well as other saturated and unsaturated fatty acids in the plasma membrane is altered by addition of fatty acids to the culture medium (22), we first explored the effect of exogenous AA on the capacity of MDCK cells to bind COM crystals. Cultures were prepared as described in MATERIALS AND METHODS, and the medium was supplemented with fatty acid or vehicle when cells were plated (day 0) and each time the medium was changed (days 1 and 2). The affinity of cells for crystals was assessed by replacing the medium with PBS and measuring the amount of [14C]COM crystals in PBS that bound to cells during a 2-min assay (24). Supplementation of the medium for 3 days with 50 or 100 µM AA decreased COM crystal adhesion by 17% and 55%, respectively ($P < 0.001$, Fig. 1A). Oleic acid, which does not significantly alter the plasma membrane lipid composition of these cells (22), had little effect on crystal binding (Fig. 1A). Surprisingly, linoleic acid, which increases MDCK cell plasma membrane AA content to a similar extent as does exogenous AA (22), had no effect on crystal binding (Fig. 1A). The effect of AA on crystal adhesion to MDCK cells required exposure for more than 24 h (Fig. 1B), perhaps to allow efficient incorporation of fatty acid into the plasma membrane and/or for generation of second messengers.

Cell counts of cultures exposed to AA (100 µM) for 1 or 3 days did not differ significantly from control, indicating that the decline in crystal binding was not mediated by a reduced cell number.

Blockade of AA metabolism. AA can be modified via cyclooxygenase to produce PGs or metabolized by the lipoxygenase or cytochrome P-450 systems (11, 13). Addition of previously established effective doses (11, 12, 19) of the cyclooxygenase inhibitor flurbiprofen, the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA), the cytochrome P-450 inhibitor OYA, or quinacrine, which blocks all pathways of AA metabolism, each did not reverse the inhibitory effect of AA on crystal adhesion to MDCK cells (Fig. 2). Addition of NDGA, OYA, or quinacrine alone did not alter COM crystal adhesion, whereas flurbiprofen alone increased adhesion (see below), although it did not reverse the effect of AA. Administration of the free radical scavenger butylated hydroxyanisole (BHA, 1 mM) (19) together with AA, also did not alter its anti-adhesion action (data not shown). Even when a concentration range of each inhibitor was used, including values in excess of the previously established effective dose for each (11, 12, 19), the inhibitory effect of AA on crystal adhesion was never reversed. However, we cannot exclude the possibility that the targeted metabolic pathway(s) were not completely blocked.

Supplementation of MDCK culture medium with ETYA, a nonmetabolizable AA analog that is incorporated into plasma membrane phospholipids (2), also decreased COM crystal adhesion to MDCK cells (Fig. 3). As with AA, the effect of ETYA required exposure for
longer than 1 day. To remove PGs present in calf serum, MDCK cells were plated and grown for 2 days in 2.5% calf serum, followed by 2 days in 1% low-lipid formula serum replacement (controlled process serum replacement type 1, Sigma). The PGE concentration on day 4, measured by radioimmunoassay, was 380 pg/ml (1.3 x 10^{-2} µM) in the medium of control cultures, increased to 4,750 pg/ml (1.3 x 10^{-2} µM) with exposure to AA (100 µM), but fell to 40 pg/ml with flurbiprofen (10 µM) present, although flurbiprofen did not block the effect of AA on crystal adhesion. These results suggest that the capacity of AA to inhibit binding of COM crystals is not a consequence of its metabolism.

Effect of PGs and cAMP on COM crystal adhesion to MDCK cells. Although AA-mediated inhibition of crystal adhesion appeared independent of its metabolism (Fig. 2), we tested the hypothesis that PGE could regulate crystal adhesion, because PGE2 is the most abundant PG produced by MDCK cells (22), and renal distal tubular cells posses PG receptors (8). MDCK culture medium was supplemented with PGE2, PGE1, or vehicle at the time of a final medium change (day 2), and affinity of cells for crystals was assessed. Supplementation of the medium for 1 day with 1 µM PGE2 or 10 µM PGE1 significantly reduced COM crystal adhesion, with maximal inhibition (70%) at 1 mM (Fig. 4A). Exposure of cells to PGE2 for 6 h was sufficient to decrease COM crystal adhesion (Fig. 4B), whereas AA required >24 h to exert its effect (Fig. 1B). Modulation of COM crystal adhesion appeared relatively specific for the renal epithelial cell type, because exposure of 3T3 fibroblasts to PGE2 (0.1 µM for 1 day) or AA (100 µM for 3 days) did not change crystal binding (data not shown).

Fig. 1. Effect of arachidonic acid (AA) on calcium oxalate monohydrate (COM) crystal adhesion to canine kidney  
epithelial cells of the MDCK line. Cultures were prepared as described in MATERIALS AND METHODS. Medium was  
supplemented with a different fatty acid or vehicle on the day cells were plated (day 0) and at the time the medium  
was changed (days 1 and 2). Affinity of cells for crystals was assessed by replacing the medium with PBS and  
measuring the amount of exogenous [14C]COM crystals in PBS that bound to cells during a 2-min period. A:  
supplementation of medium for 3 days with 50 or 100 µM AA decreased COM crystal adhesion by 17% or 55%,  
respectively, whereas oleic acid (OA) or linoleic acid (LA) had no effect. B: supplementation with AA (100 µM) for 72  
h, but not 24 h, was sufficient to decrease COM crystal adhesion. Specific activity of COM crystals adherent to cells  
was 375 cpm/µg. *P < 0.001 vs. control (Cont).

Fig. 2. Effect of inhibitors of AA metabolism on COM crystal adhesion to MDCK cells. Culture medium was supplemented with AA or  
AA plus an inhibitor on the day of plating (day 0) and at the time of each medium change (days 1 and 2). Affinity of cells for crystals was  
assessed after replacing the medium with PBS and measuring the amount of added [14C]COM crystals that bound to the cells during a  
2-min assay. Supplementation of culture medium for 3 days with AA (75 µM) decreased COM crystal adhesion by 56% (P < 0.001).  
Addition of flurbiprofen (+F, 10^{-5} M), nordihydroguaiaretic acid (NDGA; +N, 1 µM), 17-octadecynoic acid (ODYA; +O, 45 µM), or  
quinacrine (+Q, 3.0 µM) did not reverse AA-mediated inhibition of crystal adhesion. Addition of NDGA, ODYA, or quinacrine alone did  
not alter adhesion compared with control, whereas flurbiprofen alone increased adhesion (see Fig. 6) but did not reverse the effect of AA.  
Specific activity of COM crystals bound to cells was 650 cpm/µg. *P < 0.001 vs. control.
PGE binds to renal cell surface receptors and can increase intracellular cAMP (8). Therefore, we next explored the effect of DBcAMP, a cAMP analog that can permeate plasma membranes and increase the concentration of cAMP in the cytosol (21). Exposure of MDCK cells to DBcAMP (1 mM) decreased COM crystal adhesion and required only 4 h to do so (Fig. 5A). Exposure to the phosphodiesterase inhibitor isobutylmethylxanthine (IMX), another strategy to raise cAMP levels inside cells (7), also decreased COM crystal adhesion (Fig. 5B). Therefore intracellular cAMP appears to set into motion a cascade of events that results in decreased adhesion of crystals to MDCK cells.

PGE₂ regulation of COM crystal adhesion to MDCK cells. We then explored other mechanisms by which PGE₂ might act on cells to regulate COM crystal adhesion. As MDCK cells constitutively produce PGE₂ in culture (22), we asked whether reducing PG generation by cyclooxygenase blockade would enhance crystal adhesion. Addition of flurbiprofen at concentrations ≥0.1 µM significantly increased COM crystal adhesion, with maximal stimulation at >10 µM (62% increase, Fig. 6A). In the presence of flurbiprofen, PGE levels in conditioned medium of MDCK cells fell from >5,000 to 477 ± 26 pg/ml (1.3 × 10⁻³ µM) on day 2 and to 71 ± 36 pg/ml (0.2 × 10⁻³ µM) on day 3, confirming that flurbiprofen decreased PGE concentration sufficiently to influence crystal binding (Fig. 4A). Addition of ≥0.1 mM PGE₂ to the culture medium for 6 h reversed the effect of 3 days exposure to flurbiprofen (10 µM), with a maximal decrease of 58% (compared with flurbiprofen alone) when 1.0 mM PGE₂ was employed (Fig. 6B). Therefore, MDCK cells constitutively produce PGEs, blockade of which increases COM crystal adhesion.

Next, we looked for a mechanism by which PGE₂ might decrease crystal binding to MDCK cells. As COM crystals can adhere to sialic acid-containing glycoproteins on the surface of renal cells (23), we asked whether new DNA synthesis, protein synthesis, and/or N-glycosylation were required for PGE₂-mediated inhibition of crystal binding. Addition of actinomycin D (2.5 µg/ml) (1), cycloheximide (1 µg/ml) (37), or tunicamycin (500 ng/ml) (34) with PGE₂ each reversed its inhibitory effect, so that crystals bound to the same extent as to control cells in which endogenous PGE production was blocked by flurbiprofen (Fig. 6C). These data suggest...
that the mechanism(s) by which PGE2 inhibits crystal adhesion is mediated, at least in part, by gene transcription, protein synthesis, and N-glycosylation. Parallel experiments were not technically feasible with AA, because 3 days are required for its inhibitory effect on crystal binding (Fig. 1B), and MDCK cells could not tolerate these metabolic inhibitors for that long.

Crystal binding to MDCK cells decreases progressively during the first 4 days after plating (Fig. 7) (40), although the mechanisms that mediate this change are unknown. Addition of the N-glycosylation inhibitor tunicamycin prevented the decline in crystal binding (Fig. 7), suggesting that progressive glycosylation of cell surface proteins might block adhesion of crystals. When MDCK cells were exposed to PGE2 for 3 days after plating, crystal binding also decreased (Fig. 4), suggesting that inhibition of crystal binding by PGE2 might also be mediated by glycosylation of cell surface proteins.

Fig. 5. Effect of intracellular cAMP on COM crystal adhesion to MDCK cells. Culture medium was supplemented with dibutylryl cAMP (DBcAMP) or isobutylmethylxanthine (IMX) at the time of the final medium change (day 2). Affinity of cells for crystals was assessed after replacing the medium with PBS and measuring the amount of added [14C]COM crystals that bound to cells during a 2-min assay. Supplementation of the medium for 1 day with DBcAMP (1 mM, A) or IMX (100 µM, B) significantly reduced binding of crystals. Four-hour exposure to DBcAMP was sufficient to decrease crystal adhesion (A). Specific activity of COM crystals was 375 cpm/µg (A) and 650 cpm/µg (B). *P < 0.001 vs. control.

Fig. 6. PGE2 regulation of COM crystal adhesion to MDCK cells. Culture medium was supplemented with flurbiprofen on the day cells were plated (day 0) and at the time of each medium change (days 1 and 2). On day 3, PGE2 (B), or PGE2 plus an inhibitor (C) was added to the culture medium, and 6 h later the affinity of cells for crystals was assessed by replacing the medium with PBS and measuring the amount of [14C]COM crystals added to PBS that bound to cells during a 2-min assay. A: flurbiprofen (F) at concentrations >0.1 µM, used to suppress endogenous PG production, enhanced COM crystal adhesion to cells, with a maximal stimulation of 62% at 10 µM. B: on day 3 of exposure to flurbiprofen, addition of PGE2 (>0.1 mM) for 6 h reduced COM crystal adhesion, with a maximal decrease of 58% compared with flurbiprofen alone when 1.0 mM PGE2 was added. C: addition of actinomycin D (+A, 2.5 µg/ml), cycloheximide (+C, 1 µg/ml), or tunicamycin (+T, 500 ng/ml) together with PGE2 abolished its inhibitory effect, so that crystal binding returned to values observed with flurbiprofen alone. Specific activity of COM crystals was 650 cpm/µg. *P < 0.001 vs. control (A) or vs. flurbiprofen alone (B and C).
attributed to gastric PGE2 including increased mucous mimicked by DBcAMP (21). Diverse effects have been lular second messenger pathways via cAMP, and can be protect the gastric mucosa (43). The capacity of cAMP blood flow, and actions on leukocytes and mast cells to production and bicarbonate secretion, augmented local mucosal ulceration, although the mechanism of action is controversial (41). Of the different prostaglandins, PGE2 has relatively high cytoprotective activity, binds to cell surface EP1 and EP2 receptors to trigger intracel- plets appears to require gene transcription, new protein synthesis, and N-glycosylation. AA may act via a differ- ent mechanism(s) to inhibit crystal binding, because its effect was not blocked by inhibitors of AA metabolism and was mimicked by the nonmetabolizable analog ETYA. As COM crystals bind to sialic acid-containing glycoproteins on the renal cell surface, PGE2 could exert its action by regulating expression or exposure of these cell surface molecules. If intrarenal PGs inhibit binding of COM crystals to tubular cells along the collecting duct in vivo, as they do to renal cells in culture, then PGs could serve a protective function by preventing retention of crystals in the kidney.

PGs have long been known to protect against gastric mucosal ulceration, although the mechanism of action is controversial (41). Of the different prostaglandins, PGE2 has relatively high cytoprotective activity, binds to cell surface EP1 and EP2 receptors to trigger intracel- lular second messenger pathways via cAMP, and can be mimicked by DBcAMP (21). Diverse effects have been attributed to gastric PGE2 including increased mucus production and bicarbonate secretion, augmented local blood flow, and actions on leukocytes and mast cells to decrease their activation (41); changes in mucus properties and production appear to contribute to its ability to protect the gastric mucosa (43). The capacity of cAMP to regulate cell surface molecules has also been explored in neutrophils (7). Agents that raise intracellular cAMP either directly (DBcAMP, IMX) or via receptor stimulation (β-adrenergic receptor agonists) decreased adhesion of neutrophils to bronchial epithelial cells. This effect of cAMP required only 30 min, and may have been mediated by decreased expression of Mac-1, a specific cell surface molecule important for neutrophil adhesion (7).

PGE2 protects cultured proximal renal epithelial cells from noxious stimuli, such as hypoxia (31) and quinone-thioether-mediated cytotoxicity (42). Protection of primary cultures of rat proximal tubular cells from hypoxia was shared by the PGE_2 agonist misoprostol and prostacyclin, whereas AA was not effective (31). Misoprostol also preserved glomerular filtration rate in rat models of cross-clamp- and mercuric chloride-induced acute tubular necrosis (31). Important differences were observed in the quinone-thioether cytotoxicity study because PGE2 appeared to act via a pharmacologically distinct cell surface receptor (not EP_{1–4}), was mediated by protein kinase C activation and not intracellular cAMP, and the effect was not shared by PGE1. In the present study the anti-crystal binding effects of PGE1 and PGE2 were similar (Fig. 2A) and appeared to be mediated by intracellular cAMP, suggesting a role for a cell-surface EP2 or EP4 receptor (8). Therefore, the mechanisms by which PGE2 decreased crystal binding to distal nephron-derived MDCK cells in the current study (Fig. 4) and protected proximal nephron-derived LLC-PK1 cells during quinone-thioether exposure (42) are not likely to be the same, although similar mechanisms could underlie PGE2-mediated protection against crystal adhesion and hypoxia (31).

DISCUSSION
This study provides new evidence that binding of COM crystals to renal cells is regulated by physiological signals. The capacity of PGs and other agents that raise intracellular cAMP to decrease adhesion of crystals appears to require gene transcription, new protein synthesis, and N-glycosylation. AA may act via a different mechanism(s) to inhibit crystal binding, because its effect was not blocked by inhibitors of AA metabolism and was mimicked by the nonmetabolizable analog ETYA. As COM crystals bind to sialic acid-containing glycoproteins on the renal cell surface, PGE2 could exert its action by regulating expression or exposure of these cell surface molecules. If intrarenal PGs inhibit binding of COM crystals to tubular cells along the collecting duct in vivo, as they do to renal cells in culture, then PGs could serve a protective function by preventing retention of crystals in the kidney.

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Supplementation of the medium of MDCK cells with AA leads to changes in the fatty acid composition of the plasma membrane (22), including incorporation of AA (45), and to increased production of PGE2 in response to stimuli such as bradykinin (22) or phorbol esters (45). However, direct incorporation of AA into plasma membrane phospholipids might not have directly mediated its anti-crystal adhesion effect in the current study, because supplementation with linoleic acid or AA resulted in similar changes in the fatty acid composition of MDCK cell membranes (22), but only AA decreased crystal binding (Fig. 1A). Furthermore, blockade of crystal binding by AA was not prevented by the cyclooxygenase inhibitor flurbiprofen (Fig. 2) but was mimicked by the nonmetabolizable AA analog ETYA (Fig. 3) suggesting that generation of PGE2 from AA is not the pathway by which AA exerts its effect. As membrane fluidity can influence COM crystal binding to cultured renal cells and since AA and ETYA have similar effects on membrane fluidity (2), it is possible that changes in the physical properties of the membrane may in part explain the effect of AA. Interestingly, exposure to PGE1 increases membrane fluidity of synaptosomal mem-brane vesicles (16), suggesting a potentially similar mechanism of action for both AA and PGE. Another possibility is that peroxidation of AA in vitro could
generate free radicals (18), and free radical damage could augment crystal adhesion (15). However, in MDCK cultures, we did not see evidence of cell death, the free radical scavenger BHA did not reverse the action of AA, and crystal adhesion decreased rather than increased with AA exposure (Fig. 1). It will require additional study to define the mechanisms by which AA decreases COM crystal adhesion to MDCK cells.

The series of events by which freshly nucleated crystals are retained in the kidney and initiate nephrolithiasis are poorly understood; however, binding of microcrystals to the apical surface of tubular cells (23, 26) and subsequent cellular processing of the crystals (25, 26) could be important determinants of intranephronal calcification. Polyanions in tubular fluid including glycosaminoglycans, glycoproteins (nephrocalcin, uropontin), and citrate are potent inhibitors of COM crystal nucleation, aggregation (3, 17, 35, 36, 44) and adhesion to renal cells (24, 38) and appear to act by binding to the crystalline surface. Studies in cultured monkey kidney epithelial cells (23), canine renal epithelial cells (39), and primary cultures of rat IMCD cells (32, 33) each suggest that COM crystals adhere to specific cell surface receptors, such as sialic acid-containing glycoproteins (23) and anionic phospholipids (4, 29). Adhesion of crystals is followed by internalization (26–28, 39), and the internalized particulates may dissolve within lysosomal bodies (25). However, the detailed mechanisms by which crystals that bind to the apical surface of renal cells are retained in the nephron and form the nidus of a kidney stone are not yet defined.

The current study suggests that intrarenal PGE₂, as well as other agents that increase intracellular cAMP, could act on tubular cells to decrease crystal adhesion via a mechanism that requires gene transcription, new protein synthesis, and N-glycosylation. In addition, N-glycolylation blockade prevented the time-dependent decline of crystal binding in culture (Fig. 7) and blocked crystal-adhesion inhibition by PGE₂ (Fig. 6). As PGE₂ is known to stimulate mucin production by gastric and gall bladder epithelial cells (21, 43), it is possible that in an analogous fashion PGE₂ stimulates production of a cell surface glycoprotein(s) that blocks crystal adhesion. Alternatively, exogenous PGE₂ may promote removal of potential cell surface crystal-binding molecules such as glycoproteins (23) or phospholipids (5), cause their redistribution from the apical to basolateral surface of the cell (32), or perhaps mediate repeated glycolylation of membrane proteins and thereby sterically block crystal receptor sites. Local production of PGE by cultured renal cells could be the signal that mediates the decline in crystal adhesion that was observed over time (Fig. 7).

Administration of exogenous PGE₂ to humans in-


