NaCl transport stimulates prostaglandin release in cultured renal epithelial (MDCK) cells

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KURTZ, ARMIN, JOSEF PFEILSCHIFTER, COLIN D. A. BROWN, AND CHRISTIAN BAUER. *NaCl transport stimulates prostaglandin release in cultured renal epithelial (MDCK) cells.* Am. J. Physiol. 250 (Cell Physiol. 19): C676–C681, 1986.—Prostaglandins (PGs) can modulate a variety of renal functions, including Na^+ and Cl^- reabsorption. However, it is not known if a direct interdependence exists between PG synthesis and transport activity. The present study was done to find out whether or not the rate of NaCl transport has an influence on PG synthesis in renal tubular cells. For our studies we used cultures of so-called high-resistance MDCK cells, which were originally derived from canine kidney. This cell type has a loop diuretic- and ouabain-sensitive NaCl transport that can be enhanced by activation of the adenylate cyclase (AC). In MDCK cell cultures we found that each state of increased NaCl transport during stimulation of AC by either epinephrine (10^{-6} M), isoprenaline (10^{-5} M), or forskolin (10^{-5} M) was accompanied by a twofold increase in PG release. During inhibition of NaCl transport by furosemide (10^{-4} M) or ouabain (2×10^{-4} M), stimulation of AC failed to increase PGE_2 release, whereas basal PG production was not inhibited by either furosemide or ouabain. Furthermore, PG formation during activation of AC was dependent on the concentration of extracellular Na^+, whereas PG formation in the absence of activators of AC was independent of extracellular Na^+. These results suggest that increased NaCl transport stimulates PG formation in cultures of high-resistance MDCK cells.

renal cells; MDCK cells; ouabain; adenylate cyclase

THERE IS SOME EXPERIMENTAL EVIDENCE indicating that an increased NaCl load to the kidney is capable of enhancing the release of renal prostaglandins (PGs) (13, 18, 22). Because PGs have been found to have a natriuretic effect on the kidney (cf. 5, 6), an increased renal PG release during increased NaCl load could be of physiological importance for the Na^+ homeostasis of the organism (24). The mechanism by which a NaCl load could stimulate renal PG release in the kidney is, as yet, unclear. Assuming that increased renal NaCl load implies enhanced NaCl delivery to renal tubular cells and in consequence enhanced NaCl transport by tubular cells, it may be hypothesized that increased PG synthesis could be linked to enhanced tubular NaCl transport. We were therefore interested in finding out whether or not an increase in NaCl transport is capable of stimulating PG synthesis in a renal epithelial cell. For this study we used cultures of high-resistance MDCK cells, a substrain of MDCK cells (1, 15). MDCK cells have been derived from canine kidney and these cells have been found to share functional and structural similarities with renal tubular cells (16, 20). Moreover, high-resistance MDCK cells were shown to possess a furosemide- and ouabain-sensitive NaCl transport that can be stimulated by activation of the adenylate cyclase (3, 21). We found in this study that each state of enhanced NaCl transport was accompanied by enhanced PG formation. Moreover, we obtained clear evidence for a causal link between increased NaCl transport and enhanced PG formation.

MATERIALS AND METHODS

Cell culture. MDCK cells (60–72 serial passage) were obtained from Flow, Irvine Scotland. Cells were grown on Petri dishes or on Millipore filters (1) in a humidified atmosphere containing 10% CO_2 in air. Compositions of culture media are listed below.

Electrical measurements. Electrical measurements were made as described in Ref. 3. Briefly, cell monolayers grown on Millipore filters were mounted in Ussing chambers (0.75-cm window radius, 1.76 cm^2 exposed monolayer area) thermostatically controlled at 37°C for measurement of potential difference and resistance. An automatic voltage clamp was connected to the chamber via matched calomel half-cells, Ag-AgCl half cells, and saturated KCl salt bridges. Resistance measurements under open-circuit conditions were made by passing 2-μA hyperpolarizing current pulses across the cell monolayer and measuring the subsequent voltage deflection. The experiments were carried out in *buffer 1* (see below).

PGE_2 release. PGE_2 release was determined using subconfluent cultures grown in Petri dishes or confluent cultures grown on Millipore filters. For determination of PGE_2 release of cells grown in dishes, cultures were washed twice with L-15 medium (Boehringer). At the onset of the experiments, 1 ml of fresh L-15 medium with or without agents was added followed by a 5-min incubation. For the experiments in which the effect of NaCl was investigated, *buffers 2, 3,* and *4* (see below) were used instead of L-15 medium. Medium or buffers were withdrawn, frozen in liquid nitrogen, and stored at

−80°C until assay for PGE_2. For determination of PGE_2 release from cells grown on Millipore filters, the filters were mounted in a perfusion chamber (vol 1 ml) in which the perfusate had access to both the basal and apical membranes of the cells. Filters were perfused with L-15 medium at a rate of 5 ml/min. One-minute samples were frozen and stored at −80°C until assay for PGE_2. PGE_2 concentrations were determined by radioimmunoassay for PGE_2 (New England Nuclear). For protein determination, cells were lysed in 0.5 M NaOH, and protein was determined according to the method of Lowry et al. (12).

Determination of pattern of released PG. Cells were labeled with 5 μCi/ml [^{3}H]arachidonic acid (Amersham) for 24 h. Medium was removed, and cells were washed twice with L-15 medium containing 0.3 mg/ml fatty acid-free bovine serum albumin (Sigma Chemical). Then 1 ml of L-15 medium with or without agents was added. Medium was removed after 5 min. PGs were extracted from the medium using columns of octadecylsilyl-silica (10 × 10 mm, Sep Paks, Waters Associates) as described by Ref. 14. PGs were separated by high-pressure liquid chromatography (HPLC) using an Ultropak prepacked column (LichroSorb RP-18, 5 μm, 4 × 250 mm) and precolumn (LKB). PGs were eluted isocratically with 0.1% acetic acid:acetonitril (64:36, vol/vol). Retention times determined with PG standards were 4.06, 8.10, and 9.97 min for 6-keto $PGF_{1\alpha}$, $PGF_{2\alpha}$, and PGE_2, respectively. Eluted radioactivity was counted in a β-scintillation counter using Aquasol-2 (New England Nuclear).

O_2 consumption measurements. O_2 consumption rates were determined in a commercial Gilson chamber (vol 1.5 ml) with a Clark O_2 electrode thermostated at 37°C. As incubation medium, *buffer 2* (see below) was used. The chamber has been equilibrated with air, and experiments were started by the addition of cell suspension. After linear decrease of the O_2 tension was reached, forskolin, furosemide, or ouabain was added to the chamber in a small volume (50 μl max) through a fine channel using a Hamilton syringe. After the experiments, the cells were pelleted using an Eppendorf centrifuge and lysed in 0.5 M NaOH for protein determination according to Lowry et al. (12).

Chemicals and composition of media and buffers. PG standards, epinephrine, isoprenaline, furosemide, and ouabain were purchased from Sigma Chemical. Forskolin was from Hoechst, FRG. The solvent system for HPLC was obtained from Merck (FRG).

Culture media. Dulbecco's Modified Eagle's medium (high glucose) and 10% fetal bovine serum were from Flow. Penicillin (100 U/ml), streptomycin (100 μg/ml), 4 mM glutamine, and nonessential amino acids (1:100) were also used. *Buffer 1* (in mM): 137 NaCl, 5.4 KCl, 2.8 $CaCl_2$, 1.2 $MgSO_4$, 0.3 NaH_2PO_4, 0.4 KH_2PO_4, 12 HCl, 12 tris(hydroxymethyl)aminomethane base, 10 glucose, 2% vol/vol donor horse serum; *buffer 2* (in mM): 134 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1 $MgCl_2$, 10 glucose, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.3; *buffer 3* (in mM): 4 NaCl, 130 choline chloride, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, pH 7.3; *buffer 4* (in mM): 134 methylglucamine, 120 HCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, pH 7.3.

RESULTS

The transport characteristics of high-resistance MDCK cells have already been studied in great detail (cf. Ref. 21). It has been demonstrated that activation of the adenylate cyclase (AC) enhances short-circuit current (SCC) by increasing the Cl^- conductance in the apical membrane of high-resistance MDCK cells. To ensure that the cell preparation we used had in fact the known transport characteristics, we tested the influence of epinephrine (10^{-6} M) in the presence and absence of furosemide (10^{-4} M) on SCC and transepithelial resistance of the cultured MDCK cells grown on filters. Figure 1 shows that epinephrine stimulated SCC and reduced transepithelial resistance. Furosemide caused a rapid inhibition of the epinephrine-stimulated SCC but had little effect on the epinephrine-stimulated conductance.

We then determined the PGE_2 releasing rate of subconfluent cultures of MDCK cells grown in Petri dishes in the presence of epinephrine (10^{-6} M), isoprenaline (10^{-5} M), and forskolin (10^{-5} M). These agents are known to stimulate a furosemide-sensitive NaCl transport in MDCK cells by activating the AC (17, 21). Figure 2 shows that in the presence of epinephrine, isoprenaline, and forskolin the PGE_2 releasing rate was enhanced by a factor of about two. Furthermore, it is evident that furosemide (10^{-4} M) prevented this rise in PGE_2 release, whereas furosemide itself had no inhibitory effect on basal PGE_2 release.

To gain information about the time kinetics of PGE_2 formation in the presence of forskolin and furosemide, we superfused confluent monolayers of MDCK cells grown on filters and assayed PGE_2 production in consecutive 1-min samples. Figure 3 shows the results of a typical superfusion experiment. It can be seen that addition of forskolin (10^{-5} M) led to an immediate rise in PGE_2 release. Successive addition of furosemide (10^{-4} M) led to a subsequent fall in PGE_2 formation to control values. Removal of furosemide from the superfusate in the continued presence of forskolin resulted in an immediate rise in PGE_2 formation, indicating that the inhibitory effect of furosemide was fully reversible. Removal of forskolin from the superfusate resulted in a decrease in PGE_2 formation to control values with a delay of 2 min.

If the enhanced rate of PG release is in fact due to a stimulated transport rate of NaCl, then replacing Na^+ by choline or methylglucamine should prevent the rise in PG release in response to forskolin. Removal of Na^+ is known to inhibit NaCl movement across this epithelium, since uphill Cl^- movement across the basolateral membrane through the Na^+-K^+-$2Cl^-$ cotransport mechanism is highly Na^+ dependent (3, 4). Figure 4 shows that the basal PGE_2 release was not dependent on the concentration of Na^+. The stimulatory effect of forskolin, however, was clearly found to be dependent on the concentration of extracellular Na^+.

It is known that inhibition of the Na^+-K^+-ATPase by ouabain also inhibits NaCl transport in MDCK cells (3, 19). We therefore tested the effect of this compound on PGE_2 formation in the presence and absence of forskolin

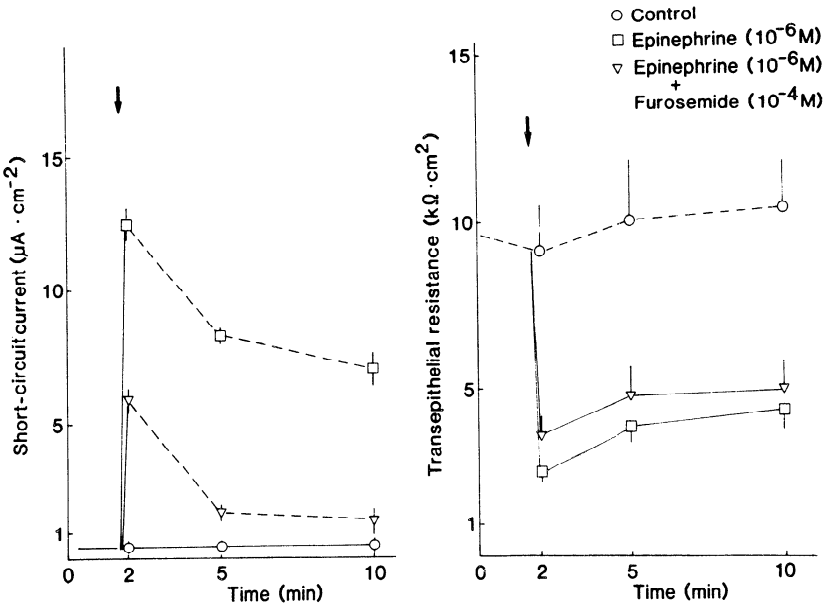

FIG. 1. Short-circuit current (*left panel*) and transepithelial resistance (*right panel*) of confluent cultures of high-resistance MDCK cells in the presence of epinephrine and epinephrine + furosemide. Agents were added as indicated by *arrows*. Data are means ± SD of 10 experiments.

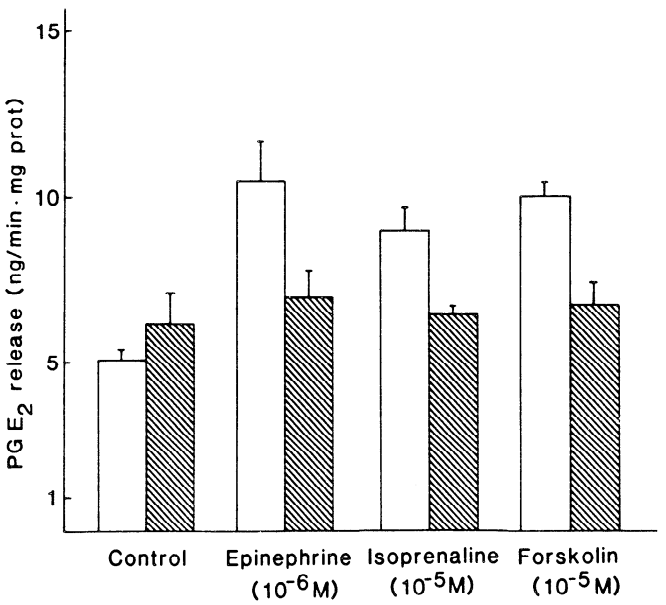

FIG. 2. Effect of epinephrine, isoprenaline, and forskolin on PGE_2 release by high-resistance MDCK cells in the absence (*open bars*) and presence (*striped bars*) of furosemide (10^{-4} M). Data are means ± SEM of 6 experiments.

(10^{-5} M). It can be seen in Fig. 5 that ouabain itself had a significant stimulatory effect on basal PGE_2 release. The stimulatory effect of forskolin, however, was absent in the presence of ouabain.

To find out whether or not the stimulation of NaCl transport by forskolin favored the release of PGE_2 only or that of other prostaglandins as well, we determined the pattern of main prostanoid metabolites (8, 11) of [^{3}H]arachidonic acid in the supernatants of subconfluent high-resistance MDCK cells. By using HPLC, we found a pattern of incorporated radioactivity of 1:0.15:0.04 for PGE_2, 6-keto $PGF_{1\alpha}$, and $PGF_{2\alpha}$, respectively, after stimulation with forskolin. The pattern for cells in the absence of forskolin was 1:0.13:0.04. Experiments were repeated twice. The variation coefficient between the experiments was <5%.

DISCUSSION

This study was designed to find out whether or not NaCl transport has any effect on PG release in high-resistance MDCK cells. Under nonstimulated conditions, MDCK cells show low transport activities of Na^+ and Cl^- ions and generally have a small net NaCl absorption. Stimulation of AC by various agents (epinephrine, isoprenaline or forskolin) results in a net rheogenic secretion of Cl^- ions, which, under open-circuit conditions, will be accompanied by a similar movement of Na^+. NaCl secretion is achieved as the result of the uphill movement of Cl^- across the basolateral membrane through a Na^+-K^+-$2Cl^-$ cotransport mechanism that is inhibitable by the loop diuretic furosemide and is dependent on a functioning Na^+-K^+-ATPase to maintain a favorable Na^+ gradient. At the apical membrane, Cl^- is thought to exit the membrane down its electrochemical gradient via a gated Cl^- channel (10). Our findings (Fig. 1) that epinephrine greatly enhanced SCC and reduced transmembrane resistance is in agreement with previous results and indicates that the cell preparation we used had the well-documented transport characteristics. Furthermore, we found that furosemide caused a rapid inhibition of the epinephrine-stimulated SCC but had little effect on the epinephrine-induced conductance in the apical membrane, implying that furosemide inhibited Cl^- secretion by inhibiting the entry of Cl^- into the cell and not by blocking an apically induced conductance. This finding also confirms previous results and indicates that the stimulated NaCl transport can be blocked by furosemide (17, 21).

We obtained three lines of evidence to indicate that NaCl transport stimulates PG formation in these cells. First, each state of increased NaCl transport during stimulation of AC by either epinephrine, isoprenaline, or forskolin was accompanied by an increased PGE_2 release (Fig. 2). The possibility cannot be ruled out that epinephrine could have stimulated PG synthesis via α_1-adrenoreceptors (7). Well-defined activators of the AC,

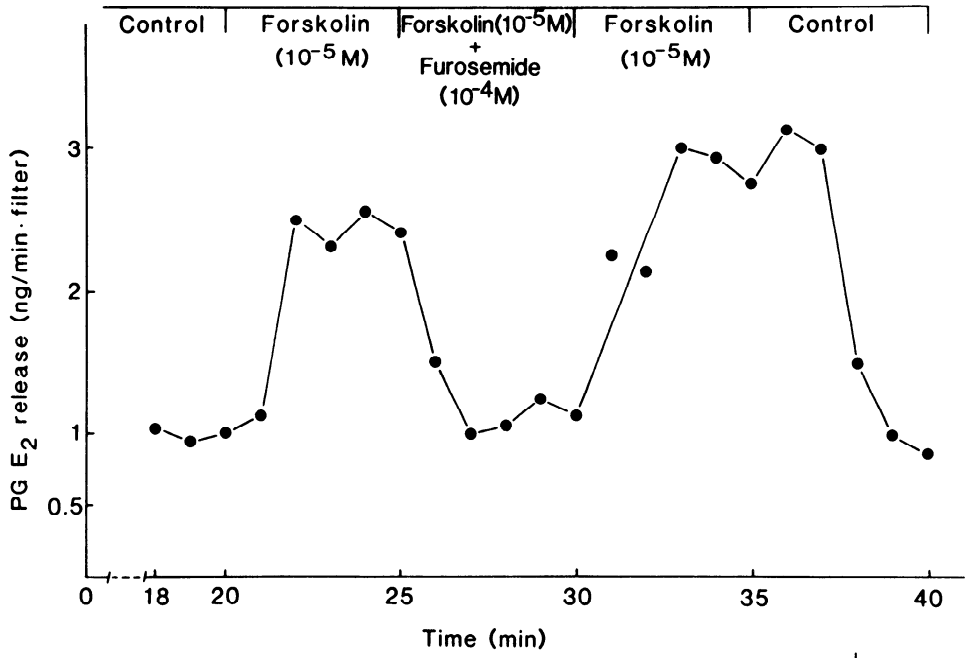

FIG. 3. PGE_2 release of confluent cultures of high-resistance MDCK cells during superfusion. Forskolin and furosemide were added to perfusion buffer as indicated in figure. Perfusate was collected in 1-min samples.

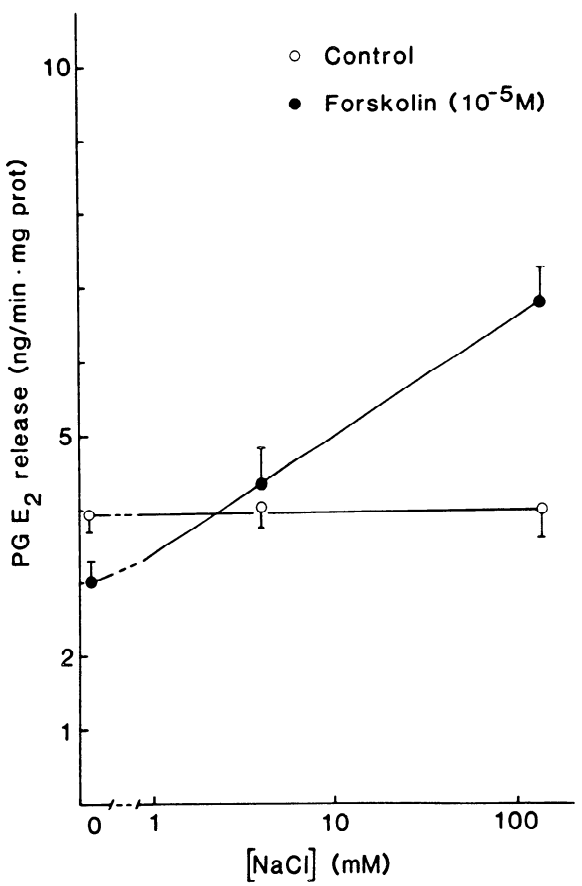

FIG. 4. Effect of Na^+ concentration on PGE_2 release from high-resistance MDCK cells in the presence and absence of forskolin. For experiments done in the absence of Na^+, *buffer 4* was used, and in the presence of 4 mM Na^+ *buffer* 3 (see MATERIALS AND METHODS) was used. Data are means ± SE of 6 experiments.

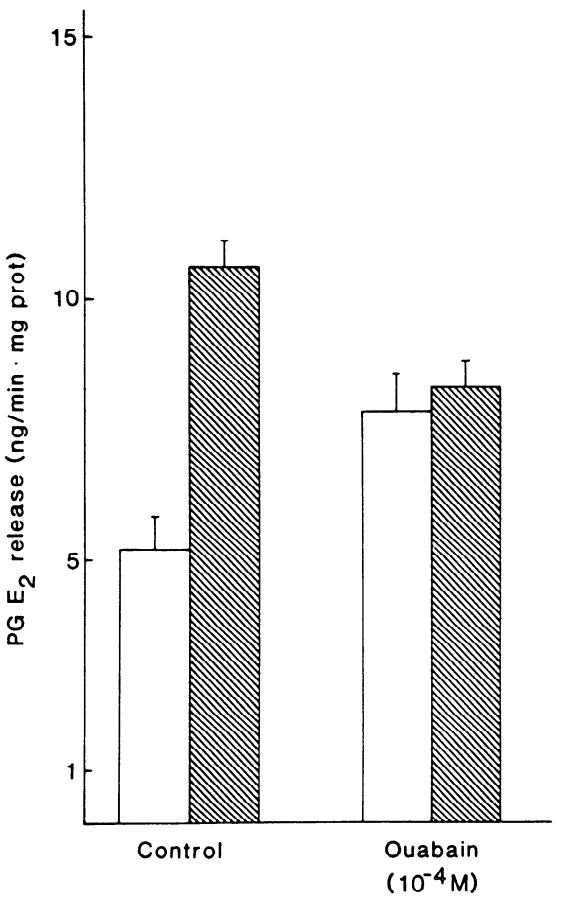

FIG. 5. Effect of ouabain on PGE_2 release from high-resistance MDCK cells in the absence (*open bars*) and presence (*striped bars*) of forskolin (10^{-5} M). Data are means ± SE of 6 experiments.

such as isoprenaline and forskolin, however, are unlikely to stimulate PG formation directly. In contrast, it has been shown that adenosine 3′,5′-cyclic monophosphate (cAMP) impairs PG formation by inhibiting both phospholipase A and cyclooxygenase in MDCK cells (9). A second line of evidence for an interdependence between NaCl transport and PG formation is the finding that inhibition of NaCl transport by furosemide and ouabain prevented the rise in PG release induced by activation of the AC (Figs. 2 and 5). The inhibitory effect of

TABLE 1. *O_2 consumption of high-resistance MDCK cells during stimulated and inhibited NaCl transport*

Condition	O_2 Consumption, $\mu l\ O_2 \cdot h^{-1} \cdot mg\ protein^{-1}$
Control	18.0±1.2
Forskolin (10^{-5} M)	38.2±4.1
Forskolin (10^{-5} M) + furosemide (10^{-4} M)	17.7±1.9
Forskolin (10^{-5} M) + ouabain (2×10^{-4} M)	15.7±3.4

Values are means ± SE of 3 experiments.

furosemide on PG release was fully reversible (Fig. 3), as is its effect on NaCl transport (3, 4). Moreover, we found that furosemide and ouabain had no inhibitory effect on basal PGE_2 release, ruling out a general inhibition of PG formation by these agents. The finding that ouabain led to a significant increase in PG formation is most likely due to the enhanced transmembrane influx of Ca^{2+} (2). A third piece of evidence implicating a connection between NaCl transport and PG formation is our finding that the enhancement of PG release by stimulators of NaCl transport was clearly dependent on the extracellular concentration of Na^+, whereas basal PG release was not (Fig. 4). Moreover, we found that in the absence of Na^+ activation of AC had an inhibitory effect on PG formation (Fig. 4). This finding is in agreement with the results of Hassid (9), who has clearly shown that activation of AC inhibits PG formation in low-resistance MDCK, which have a small NaCl transport that cannot be stimulated by cAMP (1).

In summary, we conclude from the results discussed above that enhanced NaCl transport stimulates PG formation in MDCK cells. The mechanism by which NaCl transport could stimulate PG formation is not clear yet. From the finding that the pattern of PG produced, which is very similar to that observed by Hassid (8) and Lewis and Spector (11), was not changed during enhanced transport, we conclude that the release of arachidonic acid, which is the rate-limiting step of PG formation, is enhanced during stimulated NaCl transport. Furthermore, we found that stimulation of NaCl transport by epinephrine (10^{-6} M) and forskolin (10^{-5} M) led to a twofold increase in O_2 consumption, which could be prevented by furosemide (10^{-4} M) and ouabain (2×10^{-4} M) (Table 1). Furthermore, in a previous set of experiments we obtained clear evidence that hypoxia, which is known to stimulate PG formation in the kidney (23), is also capable of stimulating PG synthesis two to threefold in cell cultures of high-resistance MDCK cells (unpublished results). In view of these findings, one could speculate that enhanced NaCl transport causes a kind of intracellular hypoxia via enhanced O_2 consumption. The mechanisms by which enhanced NaCl transport and hypoxia lead to an enhanced release of arachidonic acid are currently under investigation in our laboratory.

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