Prostaglandin E2 inhibits sodium transport in rabbit cortical collecting duct by increasing intracellular calcium.

R L Hébert, … , H R Jacobson, M D Breyer

J Clin Invest. 1991;87(6):1992-1998. https://doi.org/10.1172/JCI115227.

Research Article

The mechanism by which prostaglandin E2 (PGE2) inhibits sodium absorption (JNa) in the rabbit cortical collecting duct (CCD) was explored. PGE2 activates at least three signaling mechanisms in the CCD: (a) by itself PGE2 increases cAMP generation (b) PGE2 also inhibits vasopressin-stimulated cAMP accumulation, and (c) PGE2 raises intracellular calcium([Ca++]i). We tested the contribution of these signaling pathways to PGE2's effect on Na+ absorption, measuring 22Na flux (JNa) and [Ca++]i (using fura-2) in microperfused rabbit CCDs. In control studies PGE2 reduced JNa from 28.2 +/- 3.4 to 15.6 +/- 2.6 pmol.mm-1.min-1. Lowering bath calcium from 2.4 to 45 nM did not by itself alter JNa but in this setting PGE2 failed to inhibit JNa (28.6 +/- 5.4 to 38.5 +/- 4.0). In separate tubules, PGE2 raised [Ca++]i in a spike-like fashion followed by a sustained elevation. However, in 45 nM bath Ca++, PGE2 failed to produce a sustained [Ca++]i elevation. While pretreatment of CCDs with pertussis toxin blocked PGE2 inhibition of vasopressin-stimulated water permeability, it did not block the effect of PGE2 on JNa. To see if cAMP generation contributes to the effect of PGE2 on JNa, we tested the effect of exogenous cAMP, (8-chlorophenylthio(CPT)cAMP) on JNa. 0.1 mM 8-CPTcAMP reduced JNa from 35.75 +/- 2.3 to 21.6 +/- 2.2. However, the addition of PGE2 further blunted JNa to 15.9 […]

Prostaglandin E_2 Inhibits Sodium Transport in Rabbit Cortical Collecting Duct by Increasing Intracellular Calcium

Richard L. Hébert, Harry R. Jacobson, and Matthew D. Breyer
Division of Nephrology, Vanderbilt University and Veterans Affairs Medical Center, Nashville, Tennessee 37212

Abstract

The mechanism by which prostaglandin E_2 (PGE_2) inhibits sodium absorption (J_{Na}) in the rabbit cortical collecting duct (CCD) was explored. PGE_2 activates at least three signaling mechanisms in the CCD: (a) by itself PGE_2 increases cAMP generation (b) PGE_2 also inhibits vasopressin-stimulated cAMP accumulation, and (c) PGE_2 raises intracellular calcium ($[Ca^{++}]_i$). We tested the contribution of these signaling pathways to PGE_2's effect on Na^+ absorption, measuring ^{22}Na flux (J_{Na}) and $[Ca^{++}]_i$ (using fura-2) in microperfused rabbit CCDs. In control studies PGE_2 reduced J_{Na} from 28.2±3.4 to 15.6±2.6 pmol · mm^{-1} · min^{-1}. Lowering bath calcium from 2.4 to 45 nM did not by itself alter J_{Na} but in this setting PGE_2 failed to inhibit J_{Na} (28.6±5.4 to 38.5±4.0). In separate tubules, PGE_2 raised $[Ca^{++}]_i$ in a spike-like fashion followed by a sustained elevation. However, in 45 nM bath Ca^{++}, PGE_2 failed to produce a sustained $[Ca^{++}]_i$ elevation. While pretreatment of CCDs with pertussis toxin blocked PGE_2 inhibition of vasopressin-stimulated water permeability, it did not block the effect of PGE_2 on J_{Na}. To see if cAMP generation contributes to the effect of PGE_2 on J_{Na}, we tested the effect of exogenous cAMP, (8-chlorophenylthio(CPT)cAMP) on J_{Na}. 0.1 mM 8-CPTcAMP reduced J_{Na} from 35.75±2.3 to 21.6±2.2. However, the addition of PGE_2 further blunted J_{Na} to 15.9±1.3. In CCDs pretreated with indomethacin, 8-CPTcAMP did not significantly decrease J_{Na} 33.6±2.8 vs. 28.4±2. However, superimposed PGE_2 reduced J_{Na} to 19.0±3.0. We conclude that PGE_2 inhibits sodium transport predominantly by increasing intracellular calcium. This action is not mediated by a pertussis toxin-sensitive G protein. Finally, cAMP, through a cyclooxygenase-dependent mechanism, also inhibits CCD J_{Na} and may contribute to the effects of PGE_2 on J_{Na} in the rabbit CCD. (*J. Clin. Invest.* 1991. 87:1992–1998.) Key words: prostaglandin E_2 • collecting duct • sodium • intracellular calcium

A portion of this study was presented at the annual meeting of the American Federation for Clinical Research, Washington, DC., and appeared as an abstract in 1990. Clin. Res. 38:492.

Address correspondence and reprint requests to Dr. Matthew D. Breyer, Division of Nephrology and Department of Medicine, Vanderbilt University, F425-Acre Building, Department of Veterans Affairs Medical Center, Nashville, TN 37232.

Received for publication 12 July 1990 and in revised form 28 December 1990.

J. Clin. Invest.

0021-9738/91/06/1992/07 $2.00
Volume 87, June 1991, 1992–1998

Introduction

Abundant evidence suggests that prostaglandin E_2 (PGE_2)[1] is a major regulator of salt transport in the rabbit cortical collecting duct (CCD) PGE_2 infusion into the renal artery of the dog increases Na^+ and K^+ excretion (1, 2) without any major renal hemodynamic effect, suggesting a direct tubular action. Stokes et al. were the first to demonstrate that PGE_2 had a direct epithelial action by showing that PGE_2 decreased sodium transport in the rabbit (CCD) (3). Iino and Imai confirmed that PGE_2 reduced Na^+ absorption and the lumen negative voltage in rabbit CCD when added to the peritubular surface (4).

The cellular mechanism mediating the decrease in Na^+ absorption is unknown. Recently, Sonnenburg et al. (5) showed that by itself, high concentrations of PGE_2 increase cyclic AMP generation in immunodissected rabbit CCD cells. However, when administered together with AVP, PGE_2 inhibits AVP-stimulated cAMP production in this same preparation. This inhibitory action of PGE_2 was reversed by pertussis toxin. These latter findings are consistent with PGE_2-mediated inhibition of adenylyl cyclase via a pertussis toxin-sensitive guanine nucleotide binding protein (G_i) (5). These biochemical studies suggest that pertussis toxin pretreatment might reverse the inhibitory effect of PGE_2 on AVP-induced water flow and perhaps sodium transport.

We recently showed that PGE_2 also increases intracellular calcium ($[Ca^{++}]_i$) in the rabbit CCD (6). Evidence from a number of different epithelia suggests that changes in $[Ca^{++}]_i$ may regulate transepithelial sodium transport. Maneuvers that increase $[Ca^{++}]_i$ inhibit sodium transport in frog skin (7) and the toad urinary bladder (8, 9). Taylor et al. have suggested that cell calcium might be involved in a feedback mechanism that links the rate of Na^+ entry across the luminal membrane with Na^+ extrusion across the basolateral membrane of isolated perfused proximal renal tubules (10). Thus, increased $[Ca^{++}]_i$ may also contribute to PGE_2's inhibitory effects on sodium absorption.

The main purpose of this study is to determine if PGE_2's effects on cell calcium are required to inhibit sodium transport in isolated perfused rabbit cortical collecting duct. In addition, we wished to test if PGE_2-mediated inhibition of Na^+ transport could be coupled to its demonstrated stimulatory and inhibitory effects on cyclic AMP generation in rabbit CCD.

1. *Abbreviations used in this paper:* $[Ca^{++}]_i$, intracellular calcium ion concentration; CCD, cortical collecting duct; J_{Na}, sodium transport; L_p, hydraulic conductivity; PGE_2, prostaglandin E_2; R, fluorescence ratio; V_T, transepithelial voltage.

Methods

General microperfusion methods

In vitro microperfusion of isolated cortical collecting ducts was performed as previously described (11). Briefly, rabbits weighing 1.5–2.5 kg were killed by using an intramuscular injection of ketamine (44 mg/kg) and xylazine (11 mg/kg) for anesthesia followed by decapitation. The left kidney was quickly removed, and 1- to 2-mm coronal slices were placed in chilled dissection dishes for freehand dissection at 4°C. Tubules were perfused at 37°C at 2–3 nl/min to maximize sensitivity in measurement of changes in isotopic Na. Bath solution was continuously exchanged by an infusion pump (Sage, Orion Research Inc., Cambridge, MA) at 0.5 ml/min. Perfusate, containing ^{22}Na (Dupont-NEN, Boston, MA) was collected in a constriction pipette of known volume (between 23 and 26 nl). Transepithelial voltage (V_T) was measured via a Ringer's agarose bridge connected to the perfusion pipette and a calomel electrode. A similar bridge connected the bath to another calomel electrode and completed the circuit. V_T, in mV, was measured with an electrometer (model 602; Keithley Instruments, Inc., Cleveland, OH) and continuously recorded on a strip-chart recorder (Primeline model R-02; Soltec Co., Sun Valley, CA). The composition of standard bath medium, dissection medium, and isotonic perfusate were as follows (in millimolar): NaCl, 105; $NaHCO_3$, 25; Na acetate, 10; $NaHPO_4$, 2.3; KCL, 5; $CaCl_2$, 2.4; $MgSO_4$, 1.0 glucose, 8.3; and alanine, 5; (osmolality, 300 mosmol). The perfusate contained 0.2 mg/ml Food, Drug, and Cosmetic dye No. 3 (Analine and Chemical Co., Chicago, IL) to detect cell damage and perfusate leak (12).

Measurement of sodium transport in isolated perfused CCDs. Experiments examining CCD sodium transport (J_{Na}) were conducted according to the following time schedule: after a 30-min period of equilibration in isotonic bath and perfusate, ^{22}Na (25 μCi/ml), and ^3H-inulin (75 μCi/ml) were added to the lumen. After an additional 75 min of equilibration, four collections were made for determination of basal lumen-to-bath J_{Na}. Then in the experimental period, PGE_2 or 8-*p*-chlorophenylthio (CPT) cAMP was added to the bath and after a 10-min equilibration period, four additional timed collections were made to determine sodium flux. J_{Na} was defined as the mean of the last three collections made during exposure to the different agonists used. Changes in lumen-to-bath J_{Na} likely reflect changes in net sodium absorption since bath-to-lumen J_{Na} is low and unaffected by most experimental maneuvers. In all experiments, a final period of J_{Na} measurements was taken with 10^{-4} M ouabain in the bath. Ouabain reduces net sodium absorption to a value close to zero (13), thus the lumen-to-bath J_{Na} post-oubain, is likely passive and equal to bath-to-lumen J_{Na}.

Measurement of cell calcium $[Ca^{++}]_i$ in isolated perfused CCD. CCDs were perfused in vitro as described above with the following differences: the bath was a special low volume (0.150 ml) chamber to allow for rapid fluid exchange. The bath solution was preheated in a water-jacketed line and flow rate was maintained between 0.5 to 2.5 cm^3/min. Tubules were bathed with 2.5 μM acetoxymethyl ester of fura-2 (fura-2-AM) (Molecular Probes, Eugene, OR) for 45 min at 30°C. After tubules were loaded, the bath temperature was increased to 37°C, the flow rate was increased to 2.5 cm^3/min, and tubules were allowed to equilibrate for 20–30 min. CCD fluorescence was measured using continuous rapidly alternating excitation (20 ms per reading) from dual monochromators set at 340 and 380 nm, respectively (Deltascan; Photon Technology International, New Brunswick, NJ). The monochromator output was coupled to the inverted microscope using a 400-nm dichroic mirror and a 100× lens (Nikon fluor oil immersion). Fluorescent emission of light greater than 435 nm was measured by photon counting. Before loading with fura-2, CCD autofluorescence and background light were measured (less than 10% of fluorescent emission in fura-2-loaded tubules) and this value was continuously subtracted from all measurements. The corrected emission intensity ratio, using 340- and 380-nm excitation (340/380 ratio, R), was monitored continuously. After fura-2 loading and equilibration, a baseline reading of 100 to 200 s was taken in standard bath medium. At the end of each experiment an in situ calibration of $[Ca^{++}]_i$ was performed. The bath medium was changed to a Ca^{++} and Mg^{++}-free isotonic bath medium containing 2 mM ethyleneglycol-*bis*-B-aminoethylether N,N,N',N'-tetraacetic acid (EGTA) and 10 μM 4Br-A23187. After a stable 340/380 ratio (minimum fluorescence ratio, R_{min}) was achieved, the bath was changed back to normal bath medium (2.4 mM Ca^{++}) and 10 μM of 4Br-A23187 and the ratio was again allowed to stabilize (maximum fluorescence ratio, R_{max}).

Measurement of hydroosmotic water flow (hydraulic conductivity $[L_p]$. Each experiment measuring osmotic water flow was conducted on an identical time schedule. All experiments were performed at 37°C. The perfusate, which contained ^3H inulin (75 μCi/ml) as a volume marker, was collected into a constriction pipette of known volume (between 90 and 130 nl) and counted for ^3H (New England Nuclear). The perfusion rate was maintained between 12–20 nl/min by adjusting the hydrostatic pressure. At this perfusion rate osmotic equilibration between bath and lumen did not occur. During the first 45 min of equilibration all tubules were perfused with an isotonic solution similar to the bath. Subsequently, the perfusate was changed to hypotonic perfusate. In control studies, 30 min of further equilibration were allowed and then, three collections were made for determination of basal L_p. Tubules with a negative basal L_p were discarded. 10 μU/ml AVP was then added to the bath, and after a 15 min equilibration period three to four timed collections were made to determine L_p. A stable L_p was usually observed 20–50 min after the addition of AVP. Subsequently, either PGE_2 + AVP was added or AVP alone was continued. After a 15-min equilibration six more timed collections were made. In each period the three collections with the greatest calculated L_p were averaged to calculate mean L_p for this period.

Experimental protocols

1. The role of intracellular calcium in PGE_2's action on CCD Na^+ transport:

1a. Effect of PGE_2 on J_{Na} in CCDs. After four basal collections the CCD was exposed to 10^{-7} M bath PGE_2 and J_{Na} was determined. Afterwards, 10^{-4} M ouabain was then added to the bath and J_{Na}, presumably equivalent to the passive component of the lumen-to-bath, ^{22}Na flux was assessed.

1b. Effect of PGE_2 on $[Ca^{++}]_i$ in CCDs pretreated with 45 nM bath calcium and TMB-8. Measurements of cell calcium were determined with 2.4 mM bath calcium. The bath was then exchanged to 45 nM $[Ca^{++}]$ + 10^{-5} M TMB-8 and PGE_2 was then added. Finally, the bath was changed to 2.4 mM calcium solution and $[Ca^{++}]_i$ was measured in the continued presence of PGE_2.

1c. Effect of PGE_2 on J_{Na} in CCDs pretreated with 45 nM bath calcium and TMB-8. We examined the effect of a 45 nM calcium buffer + 10 μM TMB-8 on J_{Na}. The calcium ion concentration was calculated using a computerized ion affinity table taking into account temperature, Mg^{++} and Ca^{++}, and EGTA concentration (14). We estimate that 1 mM Ca^{++} + 2 mM EGTA yields a free calcium activity of 45 nM. The basal J_{Na} collections were made using 2.4 mM calcium in the bath. The bath was then exchanged for 45 nM Ca^{++} + 10^{-5} M TMB-8 and ^{22}Na flux was measured. The CCDs were then exposed to PGE_2 (in the continued presence of 45 nM Ca^{++} + TMB-8) and J_{Na} was again determined. Finally, ouabain was added to the bath and J_{Na} was measured. In these studies TMB-8 was used to blunt any rise in $[Ca^{++}]_i$ induced by PGE_2. TMB-8 inhibits cell Ca^{++} released from intracellular stores by an, as yet, uncharacterized mechanism (15).

2. Pertussis toxin sensitivity of PGE_2's effects on osmotic water flow, J_{Na}, and $[Ca^{++}]_i$:

2a. Effect of pertussis toxin pretreatment on PGE_2 inhibition of AVP-stimulated L_p in CCDs. CCDs were pretreated with 0, 100, or 500 ng/ml pertussis toxin for 60 min before basal collections. After basal collections CCDs were exposed to 10 μU/ml AVP and L_p was determined. Finally, PGE_2 + AVP were added to the bath and peak L_p was determined.

2b. Effect of PGE_2 on J_{Na} in CCDs pretreated with pertussis toxin. Pertussis toxin (500 ng/ml) was added to the bath medium for 60 min before basal J_{Na} collections. After basal collections the CCD was exposed to PGE_2 and J_{Na} was again determined. Finally, ouabain was added to the bath and J_{Na} was determined.

2c. Effect of PGE_2 on $[Ca^{++}]_i$ in CCDs pretreated with pertussis toxin. The tubules were pretreated with pertussis toxin 500 ng/ml for 1 h at 37°C. A baseline reading of 100 to 150 s was taken in standard bath medium. The tubules were then exposed to 10^{-7} M PGE_2 for 100 to 150 s.

3. Role of 8-CPTcAMP in mediating PGE_2's effects on J_{Na}:

3a. Effect of 8-CPTcAMP pretreatment on PGE_2 inhibition of J_{Na} in CCDs. Basal ^{22}Na lumen-to-bath flux was measured. After basal collections J_{Na} was determined while tubules were exposed to 10^{-4} M 8-CPTcAMP alone. This was followed by the addition of PGE_2 + 8-CPTcAMP and J_{Na} was again determined. Finally, ouabain was added to the bath and J_{Na} was measured.

3b. Effect indomethacin pretreatment on 8-CPTcAMP + PGE_2 inhibition of J_{Na} in CCDs. The tubules were pretreated with 5 μM indomethacin for 15 min before basal collections. Then, the CCDs were exposed to 8-CPTcAMP and J_{Na} was determined. PGE_2 was then added together with 8-CPTcAMP and J_{Na} was calculated. Finally, ouabain was added to the bath and J_{Na} was measured.

Calculations

Lumen to bath *^{22}Na flux.* Net volume flux (J_v) was uniformly less than 1% of V_0. This negligible J_v discounted. The perfusion rate (V_i) was thus equal to the collection rate (V_0). J_{Na} (l-b) was calculated from the rate of disappearance of tracer from the perfusate, using the following equation:

$$J_{Na}(l - b) = (1 - C_0^*/C_i^*) \times 145 \times V_0/L = \text{pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1},$$

where C_i^* and C_0^* are perfused and collected fluid concentrations of ^{22}Na (cpm/nl), assuming constant specific activity along the tubule length (L). Perfusion rates were adjusted so that only a small amount of perfusate tracer was lost along the tubule, ensuring relative axial uniformity of tracer specific activity.

Intracellular calcium concentration. Cell Ca^{++} ($[Ca^{++}]_i$), was calculated by: $[Ca^{++}]_i = K_d(R - R_{min})/(R_{max} - R)$ (380 min/380 max), assuming that the K_d value for the fura-2-Ca^{++} complex is 224 nM at 37°C (16). Since this apparent K_d might display shifts in the intracellular environment (17), the data is represented not only as $[Ca^{++}]_i$ but also as percentage increase in $[Ca^{++}]_i$ above basal levels, which is independent of K_d.

Hydraulic conductivity. Net volume flux (J_v) was calculated from $J_v = (V_i - V_0)/L$ where V_i is the perfusion rate (nl/min), V_0 is the collection rate (nl/min), and L is the tubule length. V_0 was measured directly, and V_i was calculated from $V_i = V_0$ (cpmo/cpmi), where cpmo and cpmi are perfusate and collected fluid ^3H-counts $\times$ min^{-1} $\times$ nl^{-1}, respectively. Hydraulic conductivity (L_p; cm $\times$ atm^{-1} $\times$ s^{-1}) was determined according to Dubois et al. (18).

$$L_p = (1/RTS) \cdot (1/O_b)^2 \cdot [O_b \cdot (V_i - V_0)$$
$$+ O_i \cdot V_i \cdot \text{Ln}\{(O_b - O_i) \cdot V_i/(O_b \cdot V_0 - O_i \cdot V_i)\}],$$

where R is the gas constant, T is °K, S is the tubule lumen surface area (assumed luminal diameter of 20 μm), and O_b and O_i represent the osmolality of the bath and perfusate respectively.

Statistics. Data are presented as mean ± SE and statistical analyses were made using paired t test or one way analysis of variance (ANOVA) whenever appropriate. Differences with $P < 0.05$ were considered statistically significant.

Reagents. AVP, EGTA, PGE_2, 8-chloro-phenylthio-cyclicAMP, indomethacin, and pertussis toxin were purchased from Sigma Chemical Co., St. Louis, MO. FURA-2AM and 4Br-A23187 were purchased from Molecular Probes.

Results

1. The role of intracellular calcium in PGE_2's action on CCD Na^+ transport:

1a. PGE_2 decreases J_{Na} in CCDs. We first confirmed in the control group the effect of PGE_2 on sodium transport. In the control periods in the absence of PGE_2, lumen-to-bath ^{22}Na flux was 28.2±3.4. As expected, addition of 10^{-7} M PGE_2 to the bath caused J_{Na} fall to 15.6±2.6 pmol/mm per min ($P < 0.0005$, $n = 6$) (Fig. 1 left). The addition of 10^{-4} M of ouabain, as expected, further decreased J_{Na} to 8.3±2.5 $\text{pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$. Thus, PGE_2 inhibits sodium transport in rabbit CCDs.

1b. Effect of PGE_2 on $[Ca^{++}]_i$ in CCDs bath in 45 nM calcium + TMB-8. In the control bath (Fig. 2 *a*), PGE_2 increases $[Ca^{++}]_i$, resulting in an abrupt spike-like increase followed by a sustained elevation in $[Ca^{++}]_i$. Fig. 2 *b* shows the effect of reducing ambient calcium concentration to 45 nM in the presence of 10^{-5} M TMB-8, a substance that inhibits calcium release from the intracellular stores (15). It is clear that the effect of PGE_2 on $[Ca^{++}]_i$ is blunted. PGE_2 produces a small and transient increase in $[Ca^{++}]_i$, but there is no sustained increase. However, when bath calcium is returned to 2.4 mM in the continued presence of PGE_2, a large and sustained increase in $[Ca^{++}]_i$ is observed again.

1c. Effect of PGE_2 on J_{Na} in CCDs pretreated with 45 nM calcium + TMB-8. We tested the effect of PGE_2 on J_{Na} in the setting of the low Ca^{++} buffer + TMB-8. It can be seen that lowering extracellular calcium to 45 nM plus 10^{-5} M TMB-8 by itself, had no effect on lumen-to-bath ^{22}Na flux (27.8±2.6 vs. 28.6±5.4 pmol/mm per min; Fig. 3). However, when PGE_2 was then added, a significant increase in J_{Na} was seen to 36.4±3.9 pmol/mm per min ($n = 5$). Subsequent administration of 10^{-4} M ouabain decreased J_{Na} to 12.8±1.6 pmol/mm per min (Fig. 3). Thus, the low Ca^{++} bath + TMB-8 blocked PGE_2's capacity to produce a sustained increase in intracellular calcium, and reversed the inhibitory effect of PGE_2 on lumen-to-bath ^{22}Na flux.

2. Pertussis toxin sensitivity of PGE_2's effects on J_{Na} and hydraulic conductivity:

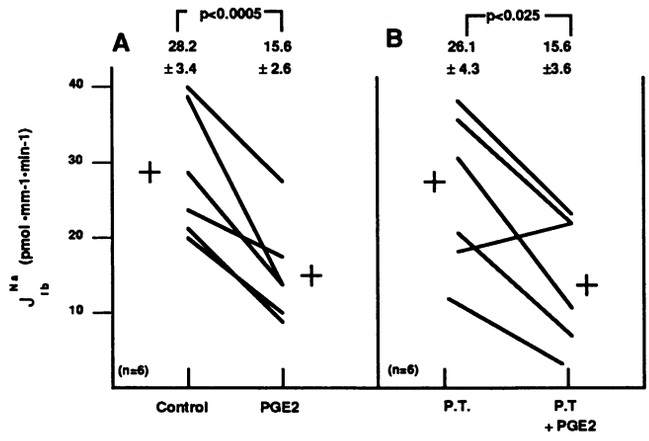

Figure 1. (*A*) 10^{-7} M PGE_2 inhibits J_{Na} ($P < 0.0005$ control compared with PGE_2). (*B*) Pertussis toxin pretreatment does not block the inhibition of sodium transport J_{Na} by 10^{-7} M PGE_2 ($P < 0.025$ control compared with PGE_2). n, Number of CCDs.

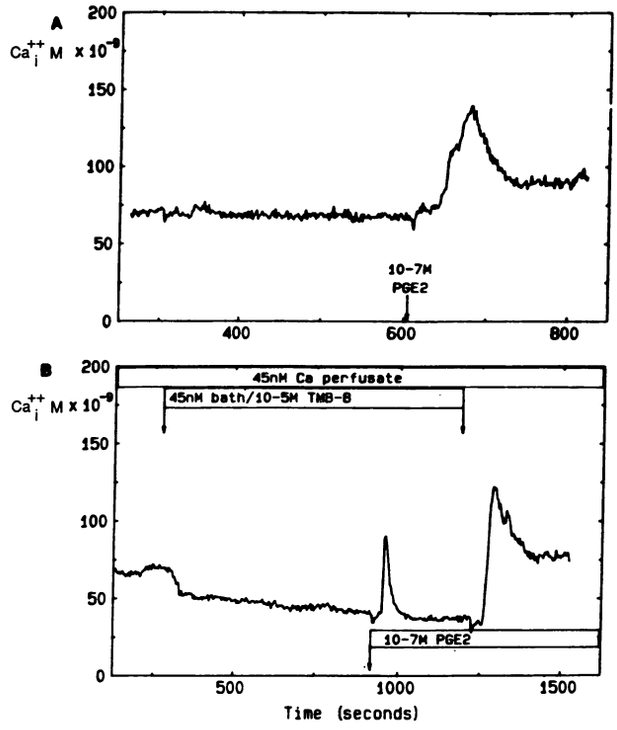

Figure 2. (*A*) 10^{-7} M PGE_2 produces a peak followed by a lower but sustained increase in $[Ca^{++}]_i$. (*B*) In the presence of low bath calcium 45 nM + TMB-8, PGE_2 produces a small transient spike without any sustained elevation. Readdition of 2.4 mM calcium bath resulted in a sustained elevation in cell calcium.

2a. Pertussis toxin reverses the inhibitory effect of PGE_2 on AVP-stimulated L_p. To test the role of an inhibitory G protein (G_i) in the action of PGE_2 to suppress AVP-mediated water transport, we pretreated CCDs with pertussis toxin for 1 h, pertussis toxin irreversibly ADP ribosylates G_i (19, 20). 100 ng/ml pertussis toxin partially reversed the inhibitory effect of 10^{-7} M PGE_2 on AVP-induced L_p from 91.4±14.1 (PGE_2 alone) to 134.5±19.2 PT + PGE_2 (Fig. 4). This reversal of PGE_2 inhibition was more pronounced when the CCDs were pretreated with pertussis toxin 500 ng/ml in the bath: AVP-stimulated L_p was initially 223.9±9.8 (AVP alone) and fell only to 204.3±11.0 with PT + AVP + PGE_2 (NS, n = 7), (Fig. 4). Thus, 500 ng/ml pertussis toxin potently reversed the inhibitory effect of PGE_2 on AVP-stimulated L_p.

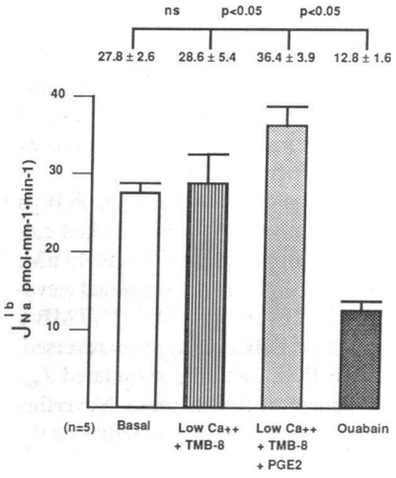

Figure 3. 45 nM calcium bath + 10^{-5} M TMB-8 have no effect on J_{Na} compared with 2.4 mM calcium bath. In the presence of 45 nM Ca^{++} + 10^{-5} M TMB-8, PGE_2 increase J_{Na} ($P < 0.05$ compared with 45 nM + TMB-8). Error bars = standard error. n, Number of experiments.

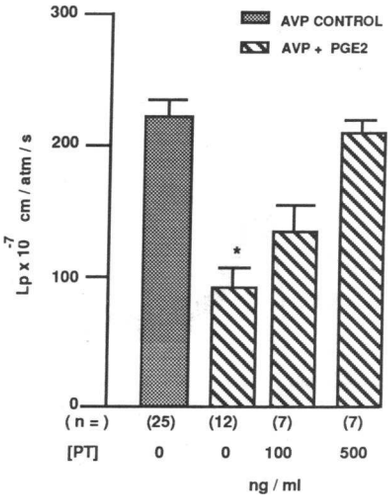

Figure 4. Pertussis toxin reverses the inhibitory effect of PGE_2 on 10 μU/ml AVP-stimulated hydraulic conductivity. (*$P < 0.0005$ compared with PGE_2 alone). Error bars = standard error. n, Number of experiments.

2b. Effect of PGE_2 on J_{Na} in CCDs pretreated with pertussis toxin. To determine whether the inhibitory effect of PGE_2 on J_{Na} in the rabbit CCDs was mediated by a pertussis toxin-sensitive G protein, we examined the effect of PGE_2 on J_{Na} in tubules that had been pretreated with 500 ng/ml of pertussis toxin for 60 min. Pretreatment with pertussis toxin failed to reverse the inhibitory effect of PGE_2 on lumen-to-bath J_{Na}. Thus, sodium flux fell from 26.1±4.3 to 15.6±3.6 pmol/mm per min ($P < 0.025$, n = 6) (Fig. 1 right). Further addition of 10^{-4} M of ouabain decreased J_{Na} to 6.9±2.0 pmol/mm per min. Pertussis toxin also failed to block the action of PGE_2 to depolarize lumen negative V_T in these tubules, (V_T − 16 mV pre-PGE_2 and −5 mV post-PGE_2). These results argue against a significant role for a pertussis toxin sensitive G protein in mediating the inhibitory effect of PGE_2 on sodium transport in the rabbit CCD.

2c. Effect of PGE_2 on $[Ca^{++}]_i$ in CCD pretreated with pertussis toxin. CCDs were exposed for 60 min to pertussis toxin 500 ng/ml. Subsequent addition of 10^{-7} M PGE_2 to fura-2-loaded CCD resulted in a rapid increase in cell calcium. Ca^{++} transiently increased to 244±17% (n = 6) of basal levels. Peak $[Ca^{++}]_i$ occurred within 30–60 s after PGE_2 addition, and was followed by a fall to a new sustained $[Ca^{++}]_i$ 104% above base line levels. (Fig. 5). These results are no different from those observed in the absence of pertussis toxin. Thus, neither PGE_2's inhibition of J_{Na} nor the Ca^{++} spike were blocked by pertussis toxin.

3. Role of cyclic AMP:

3a. PGE_2 reduces J_{Na} in 8-CPTcAMP pretreated CCDs. We next examined the effect of the cell permeable cyclic AMP analogue 10^{-4} M 8-CPTcAMP on sodium transport. We noted that while the first collection routinely showed 8-CPTcAMP slightly stimulated J_{Na}, subsequent collections revealed inhibition of J_{Na}. The last three collections were used to assess J_{Na} after 8-CPTcAMP. In the control period lumen-to-bath ^{22}Na flux was 35.8±2.3 and decreased to 21.6±2.2 pmol/mm per min in the presence of 8-CPTcAMP ($P < 0.0005$, n = 13) (Fig.

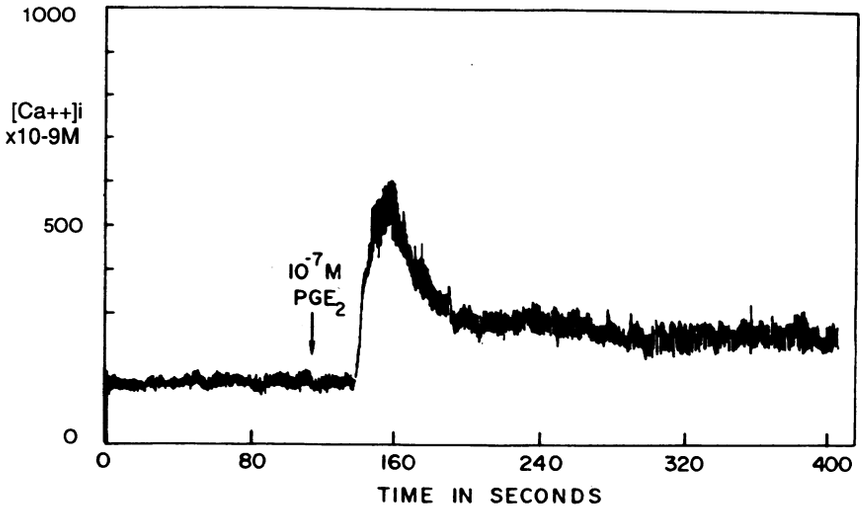

Figure 5. Pretreatment of pertussis toxin 500 ng/ml for 1 h does not block the effect of 10^{-7} M PGE_2 on $[Ca^{++}]_i$.

6). Superimposition of PGE_2 in the presence of 8-CPTcAMP caused J_{Na} to fall further to 15.9±1.3 pmol/mm per min ($P <$ 0.005). These results demonstrate that while 8-CPTcAMP inhibits J_{Na}, PGE_2 further decreases sodium flux in the rabbit CCD.

3b. Effect of 8-CPTcAMP on J_{Na} in CCDs pretreated with indomethacin. Since Holt and Lechene (21) found that cyclooxygenase inhibition reversed the inhibitory effect of vasopressin on Na^+ absorption in rabbit CCDs, we tested the effect of 8-CPTcAMP on sodium transport in tubules that had been pretreated with 5 μM indomethacin. Indomethacin pretreatment almost completely blocked the effect of 8-CPTcAMP on J_{Na} (33.6±2.85 vs. 28.4±2.8 pmol/mm per min) (n = 7) (Fig. 6). This suggests that the inhibition of J_{Na} by 8-CPTcAMP is mediated by cyclooxygenase metabolite(s). Furthermore, when PGE_2 was superimposed on 8-CPTcAMP in indomethacin-pretreated tubules, there was significant inhibition of J_{Na} from 28.4±2.8 to 19.05±3.0 pmol/mm per min ($P < 0.025$, n = 7) (Fig. 6). These results demonstrate PGE_2's capacity to inhibit sodium transport in the rabbit CCDs is also independent of its capacity to raise cyclic AMP.

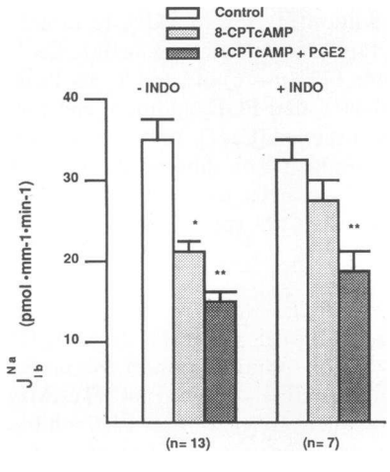

Figure 6. 10^{-4} M 8-CPTcAMP inhibits J_{Na} (*$P < 0.0005$ control compared with 8-CPTcAMP). Superimposition of PGE_2 in the continued presence of 8-CPTcAMP further reduces J_{Na} (**$P < 0.005$ compared with 8-CPTcAMP alone). Pretreatment with 5 μM indomethacin reverses the inhibition of 8-CPTcAMP on J_{Na}. However, in the presence of 8-CPTcAMP, PGE_2 still inhibits J_{Na}, (**$P < 0.025$ compared with 8-CPTcAMP alone). Error bars = standard error. n, Number of experiments.

Discussion

These studies are the first to investigate the mechanism by which PGE_2 inhibits sodium reabsorption (J_{Na}) in the rabbit cortical collecting duct. Based on the data from separate in vitro perfusion studies of Stokes et al. and Iino et al. (3, 4), it is clear that PGE_2 inhibits sodium transport in the rabbit CCD in addition to its well known modulation of vasopressin-stimulated water permeability (6, 22, 23). Studies examining the interaction of PGE_2 with vasopressin and cyclic AMP-stimulated water permeability, suggest that PGE_2 interacts with at least three different signaling mechanisms in the rabbit CCD: (*a*) a pathway linked to stimulation of the hydraulic conductivity via increased cAMP accumulation; (*b*) a second pathway coupled through G_i by which PGE_2 inhibits hydrosmotic water flow in response to vasopressin by decreasing cAMP accumulation; (*c*) and finally, a third pathway by which PGE_2 can also release cell calcium from intracellular stores. The purpose of these studies was, therefore, to characterize which of these three signaling mechanisms, if any, is used by PGE_2 to inhibit sodium transport. First, the ambient calcium concentration was lowered into the nanomolar range to block the effect of sustained increases in intracellular calcium as a mechanism for PGE_2 action. Second, a role for G_i in the action of PGE_2 was examined using pertussis toxin. Finally, the possible role of increased cell cyclic AMP was examined using exogenous 8-CPTcAMP alone and in the presence of PGE_2.

Initial studies examined the relationship between PGE_2's capacity to increase $[Ca^{++}]_i$ and inhibit J_{Na}. We blocked calcium influx by lowering bath calcium concentration to 45 nM. We found that not only was the PGE_2-induced sustained elevation in $[Ca^{++}]_i$ completely blocked by the 45-nM Ca^{++}/TMB-8 buffer, but the inhibitory effect of PGE_2 on J_{Na} was reversed. Indeed, under these conditions PGE_2 actually stimulated J_{Na}. We did not pursue the mechanism of this increase. Nevertheless, it is clear that calcium plays a critical role in mediating the inhibitory effect of PGE_2.

The mechanism by which increased $[Ca^{++}]_i$ inhibits J_{Na} in the CCD is only partially characterized. Inhibition of J_{Na} by increased $[Ca^{++}]_i$ could occur via inhibition of the apical sodium entry step or the basolateral Na^+/K^+ ATPase (24–28). Frindt and Windhager suggested that calcium inhibits Na^+ entry through the apical amiloride-sensitive sodium channel (27). Their data argue against inhibition of J_{Na} by a direct action of Ca^{++} on the basolateral Na^+/K^+-ATPase because ionomycin-induced inhibition of J_{Na} was reversed by amphotericin. There is also evidence that Ca^{++} inhibits apical sodium permeability indirectly through activating protein kinase C (28). Because we have recently shown that PGE_2 inhibits water flow in rabbit CCD, in part through the activation of protein kinase C (PKC) (6), we tested whether PKC inhibition by staurosporine (SSP) or H-7 had any effect on PGE_2's action to inhibit sodium transport. However, both SSP and H-7, by themselves, inhibited J_{Na} in rabbit CCD (data not shown). Thus, whether PGE_2's inhibition of J_{Na} occurs directly or indirectly through PKC activation still remains to be tested. However, we have clearly shown that preventing a sustained increase in $[Ca^{++}]_i$ blocks the effect of PGE_2 on sodium transport.

We next examined whether pertussis toxin reverses the inhibitory effect of PGE_2 on Na^+ transport as well as vasopressin-stimulated water flow. Studies on freshly isolated CCD cells show that PGE_2 inhibits cAMP accumulation in a manner that is sensitive to pertussis toxin. Treatment of rabbit CCD with pertussis toxin results in ADP ribosylation of a 41-kD protein (20, 29) consistent with the presence of G_i in the collecting duct. Similarly, studies by Watanabe showed copurification of a pertussis toxin sensitive GTP binding protein with a PGE_2 receptor from renal medulla (30). Our observation that pertussis toxin reverses the effect of PGE_2 on AVP-mediated hydroosmotic water flow supports the notion that PGE_2 blocks AVP-stimulated cAMP accumulation through an action involving G_i. However, similar treatment failed to block PGE_2 inhibition of J_{Na} or its capacity to increase $[Ca^{++}]_i$. Thus, PGE_2 inhibition of sodium transport may not be mediated by a pertussis toxin sensitive G protein, such as G_i.

We have previously shown that PGE_2 increased $[Ca^{++}]_i$ in rabbit cortical collecting duct consistent with stimulation of IP_3 production (6). PGE_2 recently has been shown to raise $[Ca^{++}]_i$ in UMR-106 cells and MDCK cells (31, 32). In both these cell types PGE_2 increased IP_3 production confirming stimulation of phospholipase C (PLC) by PGE_2. Agonist-induced PLC activation has been shown to be mediated by both pertussis toxin-sensitive (33) and pertussis toxin-insensitive G proteins in a variety of tissues. In chromaffin cells a PGE receptor is thought to be linked to phospholipase C via a pertussis toxin insensitive G protein (34, 35). In Swiss 3T3 cells, bombesin has been shown to cause a rapid transient rise in $[Ca^{++}]_i$ as a consequence of inositol 1,3,4-trisphosphate generation (36, 37), and this is also pertussis toxin insensitive. Under the conditions of our experiments, there is no evidence for a pertussis toxin-sensitive G protein involved in calcium signaling by PGE_2. Indeed, in data not shown, extension of the incubation period with pertussis toxin to 3 h did not prevent PGE_2-induced Ca^{++} changes.

We next examined the possible role of cAMP in mediating PGE_2's effect. Several biochemical studies have shown that in purified freshly isolated cortical collecting duct cells, PGE_2 (0.1–10 μM) stimulates cyclic AMP accumulation, suggesting that in these cells there is a PGE_2 receptor coupled to the activation of adenylyl cyclase (5). Functional studies by Grantham et al., Nadler et al., and Hébert et al. have confirmed that PGE_1 or PGE_2 by themselves increase CCD hydraulic conductivity presumably through stimulation of cAMP production (6, 22, 23). To test whether PGE_2 might inhibit J_{Na} via its capacity to increase cyclic AMP, we examined the effect of the cell permeable cyclic AMP analogue, 10^{-4} M 8-CPTcAMP on sodium transport in the rabbit cortical collecting duct. In these studies, we found a major inhibitory effect of 8-CPTcAMP on sodium transport. Schuster had previously found that using hypotonic perfusate (55 mM), 8-BrcAMP depolarized the tubules but had no effect on lumen-to-bath sodium flux (13). In contrast, using isotonic perfusate, we found that both 8-BrcAMP and 8-CPTcAMP decrease sodium transport. These data are in perfect agreement with those of Kimmel et al. who showed that 8-CPTcAMP inhibited lumen-to-bath Na transport with isotonic perfusate (38), but when they used hypotonic perfusate, 8-CPTcAMP had no effect on lumen-to-bath sodium flux. While cAMP could potentially mediate the effect of PGE_2 on J_{Na}, we found that PGE_2 further significantly decreased J_{Na} in CCDs already treated with 8-CPTcAMP. This clearly shows an inhibitory effect of PGE_2 on J_{Na} independent of cAMP generation.

The effect of cAMP on J_{Na} is similar to the effect of AVP on J_{Na} in the rabbit CCD. Whereas AVP produces a persistent increase in J_{Na} in the rat (39, 40), it only transiently stimulates J_{Na} and V_T in the rabbit (21, 27, 41). The biphasic effect of vasopressin action on V_T and J_{Na} in the rabbit CCD has been attributed to prostaglandin production in the rabbit CCD (21). Holt and Lechene found that pretreatment of rabbit collecting tubules with meclofenamate prevented the inhibitory effect of vasopressin on J_{Na}. They speculated that endogenous prostaglandin synthesis, stimulated by vasopressin, inhibits J_{Na} (21). Because of the possibility that cyclic AMP might also inhibit sodium transport through an effect involving stimulation of endogenous prostaglandin synthesis, we tested the effect of 8-CPTcAMP on sodium flux in tubules that had been pretreated with the cyclooxygenase inhibitor, indomethacin. Inhibition of lumen-to-bath sodium flux by cyclic AMP was diminished in CCDs pretreated with indomethacin, suggesting an important role for product(s) of the cyclooxygenase pathway. However, when PGE_2 was superimposed on cAMP in the presence of indomethacin, there was further significant inhibition of sodium transport. This strongly supports the previous findings obtained with PGE_2 addition to 8-CPTcAMP in the absence of indomethacin.

The mechanism by which endogenous prostaglandin synthesis could be stimulated by cAMP is unclear. However, because cAMP raises CCD $[Ca^{++}]_i$ (42), this could stimulate phospholipase activity generating free arachidonic acid leading to prostaglandin production (43). Alternative mechanisms for cAMP inhibition of J_{Na} could exist, including direct activation of phospholipases by cAMP (28). Finally, some comments should be made regarding the possible contribution of cAMP to the calcium response of CCD to PGE_2. This is pertinent because it has been shown recently that cAMP increases $[Ca^{++}]_i$ in rabbit CCD (42). The rise in cell calcium induced by cAMP requires extracellular calcium, whereas PGE_2 clearly raises cell calcium by both promoting release from intracellular stores and by promoting entry from the extracellular fluid. It is pres-

ently unknown what contribution cAMP generation makes to calcium entry stimulated by PGE_2. Nevertheless, both the inhibition of J_{Na} and the PGE_2-mediated increase in $[Ca^{++}]_i$ can clearly occur by cAMP-independent mechanisms (6).

In summary, these studies have shown for the first time that PGE_2-mediated increases in cytosolic calcium are responsible for the inhibitory action of PGE_2 on sodium transport. While stimulation of cAMP production by PGE_2 may contribute to the inhibition of Na^+ transport, it is not required since in the presence of 8-CPTcAMP, PGE_2 still decreases sodium transport in the rabbit cortical collecting duct. Finally, the effect of PGE_2 on J_{Na} is pertussis toxin insensitive and it is thus unlikely to be mediated by G_i. We conclude that PGE_2 inhibits sodium transport in rabbit cortical collecting ducts predominantly by increasing intracellular calcium.

Acknowledgments

The authors thank Miss Barbara H. Nadeau and M. Shea Tuberty for their expert technical assistance.

Richard L. Hébert received a fellowship from le Fonds de la Recherche en Santé du Québec. Dr. Matthew D. Breyer is the recipient of a Veterans Administration Research Associate Award. This work was funded in part by National Institutes of Health grants AM-37097 and 1P50DK-38667, and a VA merit grant to Dr. Brayer and Dr. Jacobson.

References

1. Hébert, R. L., C. Lamoureux, P. Braquet, P. Sirois, and G. E. Plante. 1985. Potentiating effects of Leukotriene B_4 and Prostaglandin E_2 on urinary sodium excretion by the dog kidney. *Prostaglandins, Leukotrienes Med.* 18:69–80.

2. Hébert, R. L., C. Lamoureux, P. Sirois, P. Braquet, and G. E. Plante. 1987. Interaction between Prostaglandin E_2 and Leukotriene D_4 on the excretion of electrolytes by the dog kidney *in vivo*. *Prostaglandins.* 33:301–314.

3. Stokes, J. B., and J. P. Kokko. 1977. Inhibition of sodium transport by prostaglandin E_2 across the isolated perfused rabbit collecting tubule. *J. Clin. Invest.* 25:1099–1104.

4. Iino, Y., and M. Imai. 1978. Effects of prostaglandin on Na transport in isolated collecting tubules. *Pfluegers Arch.* 373:125–132.

5. Sonnenburg, W. K., and W. L. Smith. 1988. Regulation of cyclic AMP metabolism in rabbit cortical collecting tubule cells by prostaglandins. *J. Biol. Chem.* 263:6155–6160.

6. Hébert, R. L., H. R. Jacobson, and M. D. Breyer. 1990. PGE_2 inhibits AVP induced water flow in cortical collecting ducts by protein kinase C activation. *Am. J. Physiol.* 259:F318–F325.

7. Grinstein, S., and D. Erlij. 1978. Intracellular calcium and the regulation of sodium transport in the frog skin. *Proc. R. Soc. Lond. B. Biol. Sci.* 202:1071–1074.

8. Chase, H. S., and Q. Al-Awqati. 1981. Regulation of the sodium permeability of the luminal border of toad bladder by intracellular sodium and calcium. *J. Gen. Physiol.* 77:693–712.

9. Taylor, A., E. Eich, A. S. Brem, and E. Q. Peeper. 1987. Cytosolic calcium and the action of vasopressin in toad urinary bladder. *Am. J. Physiol.* 252:F1028–F1041.

10. Taylor, A., and E. E. Windhager. 1979. Possible role of cytosolic calcium and Na-Ca exchange in regulation of transepithelial sodium transport. *Am. J. Physiol.* F505–F512.

11. Breyer, M. D., J. P. Kokko, and H. R. Jacobson. 1986. Regulation of net bicarbonate transport in rabbit cortical collecting tubule by peritubular pH, carbon dioxyde tension, and bicarbonate concentration. *J. Clin. Invest.* 77:1650–1660.

12. Ando, Y., H. R. Jacobson, and M. D. Breyer. 1987. Phorbol myristate acetate, dioctanoylglycerol, and phosphatidic acid inhibit the hydroosmotic effect of vasopressin on rabbit cortical collecting tubule. *J. Clin. Invest.* 80:590–593.

13. Schuster, V. L. 1985. Mechanism of bradykinin, ADH, and cAMP interaction in rabbit cortical collecting duct. *Am. J. Physiol.* 249:F645–F653.

14. Perrin, D. D., and I. G. Sayce. 1967. Computer calculation of equilibrium concentrations in mixtures of metal ions and complexing species. *Talanta.* 14:833–842.

15. Malagodi, M. H., and C. Y. Chiou. 1974. Pharmacological evolution of a new Ca^{++} antagonist, 8-(N,N-diethylamine) octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB-8): studies in skeletal muscles. *Pharmacology.* 27:20–31.

16. Grynkiewicz, G., M. Poenie, and R. Y. Tsien. 1985. A new generation of Ca^{++} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440–3450.

17. Scanlon, M., D. A. Williams, and F. S. Fay. 1987. A Ca^{++}-insensitive form of Fura-2 associated with polymorphonuclear leukocytes. *J. Biol. Chem.* 262:6308–6312.

18. DuBois, R., A. Verniory, and M. Abramow. 1976. Computation of the osmotic water permeability of perfused tubule segments. *Kidney Int.* 10:478–479.

19. Rodbell, M. 1980. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature (Lond.).* 284:17–22.

20. Ribeiro-Neto, F., R. Mattera, J. Hildebrandt, J. Codina, J. Field, L. Birnbaumer, and R. Sekura. 1985. ADP-ribosylation of membrane components by pertussis and cholera toxin. *Methods Enzymol.* 109:566–572.

21. Holt, W. F., and C. Lechène. 1981. ADH-PGE_2 interactions in cortical collecting tubule. 1. Depression of sodium transport. *Am. J. Physiol.* 241:F452–F460.

22. Grantham, J. J., and J. Orloff. 1968. Effect of Prostaglandin E_1 on the permeability response of the isolated collecting tubule to Vasopressin, Adenosine 3′, 5′-Monophosphate, and Theophylline. *J. Clin. Invest.* 47:1154–1161.

23. Nadler, S. P., S. C. Hebert, and B. M. Brenner. 1986. PGE_2, forskolin, and cholera toxin interactions in rabbit cortical collecting tubule. *Am. J. Physiol.* 19:F127–F136.

24. Taylor, A. 1975. Effect of quinidiine in the action of vasopressin. *Fed. Proc.* 34:285.

25. Pfeiffer, D. R., R. W. Taylor, and H. A. Lardy. 1978. Ionophore A23187: cation binding and transport properties. *Ann. NY Acad. Sci.* 307:402–403.

26. Taylor, A., M. Pearl, B. Barber, and B. Crutch. 1984. Role of cytosolic calcium in vasopressin-sensitive epithelia. Vol. 1. *In* Nephrology. R. R. Robinson, editor. Springer-Verlag, New York.

27. Frindt, G., and E. E. Windhager. 1990. Ca^{++}-dependent inhibition of sodium transport in rabbit cortical collecting tubules. *Am. J. Physiol.* 258:F568–F582.

28. Ling, B., and D. C. Eaton. 1989. Effects of luminal Na^+ on single Na^+ channels in A6 cells, a regulatory role for protein kinase C. *Am. J. Physiol.* 256:F1094–F1103.

29. Pedrosa-Ribeiro, C., F. Ribeiro-Neto, J. B. Field, and W. N. Suki. 1987. Prevention of alpha$_2$-adrenergic inhibition on ADH action by pertussis toxin in rabbit CCT. *Am. J. Physiol.* 253:C105–C112.

30. Watanabe, T., K. Umegaki, and W. L. Smith. 1986. Association of a solubilized prostaglandin E_2 receptor from renal medulla with a pertussis toxin-reactive Guanine Nucleotide Regulatory Protein. *J. Biol. Chem.* 261:13430–13439.

31. Yamaguchi, D. T., T. J. Hahn, T. G. Beeker, C. R. Kleeman, and S. Muallem. 1988. Relationship of cAMP and calcium messenger systems in prostaglandin stimulated UMR-106 cells. *J. Biol. Chem.* 263:10745–10753.

32. Aboolian, A., M. Vander Molen, and E. P. Nord. 1989. Differential effects of phorbol esters on PGE_2 and bradykinin-induced elevation of $[Ca^{++}]_i$ in MDCK cells. *Am. J. Physiol.* 256:F1135–F1143.

33. Moriarty, T. M., E. Padrell, D. J. Carty, G. Omri, M. Landau, and R. Iyengar. 1990. G_0 protein as signal transducer in the pertussis toxin-sensitive phosphatidylinositol pathway. *Nature (Lond.).* 343:79–82.

34. Yokohama, H., T. Tanaka, S. Ito, M. Negishi, H. Hayashi, and O. Hayaishi. 1988. Prostaglandin E receptor enhancement of catecholamine release may be mediated by phosphoinositide metabolism in bovine adrenal chromaffin cells. *J. Biol. Chem.* 263:1119–1122.

35. Yokohama, H., M. Negishi, K. Sugama, H. Hayashi, S. Ito, and O. Hayaishi. 1988. Inhibition of prostaglandin E_2-induced phosphoinositide metabolism by phorbol ester in bovine adrenal chromaffin cells. *Biochem. J.* 255:957–962.

36. Mendoza, S. A., J. A. Schneider, A. Lopez-Rivas, J. W. Sinnett-Smith, and E. Rozengurt. 1986. Early events elicited by bombesin and structurally related peptides in Quiescent Swiss 3T3 cells. Changes in Na^+/K^+ pump activity, and intracellular pH. *J. Cell Biol.* 6:2223–2233.

37. Takuwa, N., Y. Takuwa, W. E. Bollag, and H. Rasmussen. 1987. The effects of bombesin on polyphosphoinositide and calcium metabolism in Swiss 3T3 cells. *J. Biol. Chem.* 262:182–188.

38. Kimmel, P. L., and S. Goldfard. 1984. Effects of isoproterenol on potassium secretion by the cortical collecting tubule. *Am. J. Physiol.* 246:F804–F811.

39. Tomita, K., J. J. Pisano, and M. A. Knepper. 1985. Control of sodium and potassium transport in the cortical collecting duct of the rat. Effects of bradykinin, vasopressin, and deoxycorticosterone. *J. Clin. Invest.* 76:132–136.

40. Reif, M. C., S. L. Troutman, and J. A. Schafer. 1986. Sodium transport by rat cortical collecting tubule. Effects of vasopressin and deoxycorticosterone. *J. Clin. Invest.* 77:1291–1298.

41. Frindt, G., and M. B. Burg. 1972. Effect of vasopressin on sodium transport in renal cortical collecting tubules. *Kidney Int.* 1:224–231.

42. Breyer, M. D., D. Fredin, and R. L. Hebert. 1990. Cyclic AMP stimulates Na^+ dependent calcium influx and inhibits Na^+ transport in the rabbit cortical collecting duct. *J. Am. Soc. Nephrol.* 1:681*a*. (Abstr.)

43. Craven, P. C., and F. R. DeRubertis. 1983. Calmodulin-dependent release of arachidonic acid for renal medullary prostaglandin synthesis. *J. Biol. Chem.* 258:4814–4823.