Interactions among Prostaglandin E_2, Antidiuretic Hormone, and Cyclic Adenosine Monophosphate in Modulating Cl^- Absorption in Single Mouse Medullary Thick Ascending Limbs of Henle

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ABSTRACT This paper describes the inhibitory effect of prostaglandin E_2 (PGE_2) on antidiuretic hormone (ADH)-stimulated net Cl^- absorption and spontaneous transepithelial voltage (V_e) in single medullary thick ascending limbs of Henle (TALH, thick ascending limb; mTALH, medullary segment; cTALH, cortical segment) obtained from mouse kidney. The experimental data indicate that PGE_2 reduced the ADH-dependent values of net Cl^- absorption (J_{Cl}^{net}, eq cm^{-2} s^{-1}) and V_e (mV) in a dose-dependent manner; that increasing concentrations of peritubular ADH reversed the PGE_2-mediated reductions in the ADH-dependent moiety of V_e in the mouse mTALH; that PGE_2 had no effect on cyclic AMP-stimulated increments in V_e in the mouse mTALH; and that PGE_2 had no effect on V_e in the cTALH, where V_e is unaffected either by ADH or by cyclic AMP.

These effects might be obtained because of a direct competition between ADH and PGE_2 for receptor binding on basolateral membranes. Alternatively, PGE_2 might have reduced the affinities between ADH-receptor units and a component(s) of the series of processes leading to adenyl cyclase activation. The latter argument requires that basolateral membranes of the mouse mTALH exhibit receptor reserve, i.e., at the minimum concentration of ADH required to enhance V_e and J_{Cl}^{net} maximally, a fraction of basolateral membrane ADH receptors were unoccupied. According to this view, increasing peritubular ADH concentrations might reverse the PGE_2-mediated reduction in ADH-dependent salt transport by increasing the number of basolateral membrane receptors occupied by ADH.

INTRODUCTION

This paper describes the inhibitory effect of prostaglandin E_2 (PGE_2)[1] on antidiuretic hormone (vasopressin, ADH)-stimulated net Cl^- absorption (J_{Cl}^{net}) and spontaneous transepithelial voltage (V_e) in single medullary thick ascending limbs of Henle (TALH, thick ascending limb; mTALH, medullary segment; cTALH, cortical segment) obtained from mouse kidney. In most mammalian species, salt absorption from the water-impermeable TALH (1–12) provides a means for diluting tubular fluid entering early distal convolutions. In the renal medulla, this process enriches medullary osmolality, thereby contributing to urinary concentrating power (13, 14). In certain species, like the mouse, salt absorption (11) and adenyl cyclase activity (15, 16) in mTALH segments are enhanced by ADH. Thus, in the mouse renal medulla, ADH-mediated increments in conservative NaCl absorption by the mTALH may complement ADH-mediated increments

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[1] *Abbreviations used in this paper:* ADH, antidiuretic hormone; BSA, bovine serum albumin; cAMP, cyclic AMP; CCT, cortical collecting tubule; $[Cl^-]^f$, chloride concentration in the collected fluid; $[Cl^-]^p$, chloride concentration in the perfusate; cTALH, cortical segment of TALH; db-cAMP, dibutyryl analogue of cAMP; J_{Cl}^{net}, net chloride absorption; J_v, net fluid transport; KRB, Krebs-Ringer bicarbonate; KRP, Krebs-Ringer phosphate; mTALH, medullary segment of TALH; PGE, prostaglandins of the E series; R_v ADH receptor; TALH, thick ascending limb of Henle; $\dot{V}^o$, perfusion rate; V_e, spontaneous transepithelial voltage.

in the water permeability of mammalian collecting ducts.

In the isolated mouse mTALH, NaCl absorption involves the coupled entry of Cl^-, and possibly K^+ (17, 18), to Na^+ entry across apical plasma membranes. In other words, Cl^- transport in this nephron segment is a secondary active transport process, driven in part by the Na^+ gradient between luminal fluid and cell interior (11, 17). Conservative NaCl absorption in this nephron segment is enhanced considerably, and in a dose-dependent manner, by ADH, operating through the second messenger cyclic (c)AMP (11). Conversely, conservative NaCl absorption in the mouse mTALH is suppressed significantly by increases in peritubular osmolality independently of transepithelial osmotic pressure gradients (19).

The mouse mTALH, like the rabbit cTALH (6, 20) and mTALH (7), behaves as a hybrid epithelium: the mouse mTALH is electrically leaky (11, 17) and rather permeable to Na^+ and to Cl^- (11, 17), yet is virtually water impermeable either in the presence or absence of ADH (9–11). Consequently, as medullary peritubular salt concentrations vary, there occur varying degrees of dissipative NaCl backleak from interstitium to lumen through the paracellular pathway. Thus, for the mouse mTALH, maintenance of diluting power in the presence of varying NaCl concentrations in peritubular media depends, at least in part, on the interplay between ADH and peritubular osmolality in modifying conservative NaCl transport (11, 19). We will argue in this paper that PGE_2 represents yet another factor that modulates the latter in the mouse mTALH.

In the amphibian urinary bladder, Orloff et al. (21) found that prostaglandins inhibited the hydroösmotic effect of ADH but did not change the response of the epithelium to cAMP. They inferred that prostaglandins had acted at a locus proximal to hormone-dependent accumulation of cAMP within the cell, with little or no discernible prostaglandin action on transport events beyond cAMP accumulation. Based on in vitro studies in the isolated rabbit collecting duct, Grantham and Orloff (22) also suggested that prostaglandins might modulate renal urinary concentrating systems by opposing the hydroösmotic effect of ADH, but not cAMP, on the mammalian collecting tubule.

Under in vitro conditions, prostaglandins of the E series have been noted to antagonize a number of ADH-mediated increments in salt and/or water transport processes. As indicated above, prostaglandins of the E series inhibit the hydroösmotic effect of amphibian epithelia to ADH, but not to cAMP (21, 23). In the single rabbit cortical collecting tubule (CCT), Grantham and Orloff (22) found that PGE_1 reduced ADH-mediated increments in osmotic water permeability. Stokes (24) reported a decrease in both ADH-stimulated water permeability and the Na efflux coefficient in the isolated, perfused rabbit CCT exposed to PGE_2. In other studies with mammalian nephron segments, Iino and Imai (25) and Stokes and Kokko (26) found that exogenously applied PGE_2 suppressed Na^+ transport in isolated rabbit CCT.

In both the CCT and in the toad urinary bladder, ADH has also stimulated the endogenous production of PGE_2 (27, 28). In these two tissues, inhibition of endogenous PGE production with prostaglandin synthetase inhibitors, such as indomethacin or meclofenamate, has increased the rate of sodium transport or the rate of osmotic water permeation (28–31). For example, Holt and Lechene (31) demonstrated inhibition of ADH-stimulated Na^+ transport in isolated rabbit CCT by prostaglandins produced within that tubule. Thus, the ADH effects on transport in these tissues have been modulated by an inhibitor synthesized in situ, and whose production is stimulated by ADH.

In the rabbit CCT and the toad urinary bladder, prostaglandins have appeared to inhibit the ADH-stimulated accumulation of cAMP within the cell, and to have exerted little or no inhibitory action on transport events beyond the accumulation of cAMP within the cell (21–24). By way of contrast, Schlondorff et al. (32) have argued that the antagonism between prostaglandins and ADH in modulating the hydroösmotic properties of amphibian epithelia might also depend on a direct action of prostaglandins on the factors that alter the water permeability characteristics of apical membranes in amphibian epithelia, that is, by "post-cAMP" effects.

There are additional observations consistent with the view that prostaglandins may alter urinary concentrating ability by affecting either water abstraction from collecting ducts and/or salt absorption in the TALH.

Studies conducted in vivo have demonstrated increased sodium excretion following infusions of prostaglandins of the E series (33–35), and, in hydropenic dogs, decreased absorption of free water (34, 35). Inhibition of endogenous renal prostaglandin synthesis, either with indomethacin or with meclofenamate, has resulted in antinatriuresis (36, 37) and in an enhanced urinary concentrating ability in response to administration of vasopressin (36, 38, 39). In addition, Ganguli et al. (40) demonstrated an increase in medullary NaCl content following prostaglandin synthesis inhibition even in the absence of any discernible change in papillary blood flow.

In rat micropuncture experiments, Higashihara et al. (41) have demonstrated a decrease in NaCl delivery to distal tubule sites and an increase in papillary NaCl

content following prostaglandin synthesis inhibition; they concluded that prostaglandins might inhibit NaCl transport in the TALH. Complementary studies conducted by Kaukker (42), using micropuncture techniques, and by Stokes (43), using the isolated, perfused rabbit mTALH, have also been consistent with the notion that PGE_2 inhibits NaCl absorption by the TALH. Importantly, in none of these studies was an interaction between ADH and PGE_2 examined.

These observations, when taken in conjunction with the report (44) that PGE_2 biosynthesis by renal medullary interstitial cells is enhanced by ADH, make attractive the possibility that prostaglandins of the E series might contribute, in the mouse mTALH, to the modulation of conservative NaCl absorption, and hence to the regulation of tubular fluid diluting capacity. Accordingly, in the present studies, we evaluated the effects of PGE_2 on J_{Cl}^{net} and on the V_e (millivolts) that attends conservative salt absorption in single microperfused TALH segments, both mTALH and cTALH, isolated from mouse kidney (9–12).

The experimental data indicate that PGE_2 reduced the ADH-dependent values of J_{Cl}^{net} and V_e in a dose-dependent manner; that increasing concentrations of peritubular ADH reversed the PGE_2-mediated reductions in the ADH-dependent moiety of J_{Cl}^{net} and V_e; that PGE_2 had no effect on the ADH-independent moiety of V_e in the mouse mTALH; that PGE_2 had no effect on cAMP-stimulated increments in V_e in the mouse mTALH; and that PGE_2 had no effect on V_e in the cTALH, where V_e is unaffected either by ADH or by cAMP (11, 12). The disparity between the effects of PGE_2 on V_e in the mouse cTALH and mTALH therefore indicates another characteristic of the functional heterogeneity between these two nephron segments (11, 12). Furthermore, the interactions among ADH, PGE_2, and cAMP in the mouse mTALH are consistent with the view that, in the latter, PGE_2 reduced the ADH-dependent J_{Cl}^{net} and V_e by inhibiting, in a seemingly competitive manner, ADH-mediated enhancements in conservative salt absorption.

Such an effect might be obtained because of a direct competition between ADH and PGE_2 for receptor binding on basolateral membranes. Alternatively, PGE_2 might have reduced the affinities between ADH-receptor units and a component (or components) of the series of processes leading to adenyl cyclase activation. Clearly, the latter argument requires that basolateral membranes of the mouse mTALH exhibit receptor reserve, i.e., at the minimum concentration of ADH required to enhance V_e and J_{Cl}^{net} maximally, a fraction of basolateral membrane ADH receptors were unoccupied (45). According to this view, increasing peritubular ADH concentrations reversed the PGE_2-mediated reduction in ADH-dependent salt transport by increasing the number of basolateral membrane receptors occupied by ADH (46). A preliminary report of these observations has appeared elsewhere (47).

METHODS

Isolated nephron segments from mouse kidney, either cTALH or mTALH, were dissected and perfused using methods previously employed in this laboratory and detailed elsewhere for both rabbit kidney (48, 49) and mouse kidney (11, 12). Exceptions to these methods are described below.

20–25-d-old male Swiss White mice (Timco Breeding Laboratories, Houston, TX), each weighing ~25 g, were maintained on standard chow with free access to water. The animals were killed by rapid decapitation with no prior treatment; the zero reference time for all experiments was considered to be the time of decapitation. The kidneys were removed, sectioned into quarters by making midlongitudinal and midcross-sectional cuts, and immersed in a cold (0–5°C) Krebs-Ringer phosphate (KRP) solution containing (mM): 145 NaCl, 2.8 KH_2PO_4/K_2HPO_4, 1.0 $CaSO_4$, 1.2 $MgSO_4$, 5 L-alanine, 5.5 glucose, and 0.4 g/100 ml exhaustively dialyzed bovine serum albumin (BSA; fraction V, Reheis Chemical Co., Phoenix, AZ). The solution was equilibrated with 100% O_2 and was adjusted to pH 7.40 and 300 mosmol/Kg H_2O.

Segments of either cortical or medullary thick ascending limbs of Henle, measuring 0.5–1.0 mm in length, were isolated by freehand dissection as reported previously (11, 12). For isolation of mTALH, the cortex was excised and discarded, and tubule segments were dissected from the remaining medullary tissue. Cortical thick ascending limbs (cTALH) were dissected from the cortical tissue freed from the underlying medulla. As noted previously (11, 12), the cortical segments were less opaque in appearance, and when perfused had a thinner epithelium: 3 μm cell height for cTALH segments and 5 μm for mTALH segments.

The tubule segments were transferred to a lucite chamber, volume ~1.2 ml, fitted to the stage of an inverted microscope, and then mounted between two sets of concentric pipettes as described previously (11, 12, 48, 49). The tubules were perfused at rates of 3–15 nl/min using hydrostatic pressure. The temperature was maintained at 37°±0.5°C by a YSI temperature regulator (Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Composition of solutions. Table I lists the constituents of the bathing and perfusing solutions used in these experiments. All solutions were equilibrated with 100% O_2, for phosphate-buffered, HCO_3^--free solutions, and with 95% O_2/5% CO_2 for HCO_3^--containing solutions. All solutions were adjusted to pH 7.40, 300 mosm/kg H_2O. The perfusing and bathing solutions were identical except for the addition of 0.4 gm/100 ml BSA to bathing solutions and the isoosmotic substitution of urea in bathing solutions for L-alanine and glucose in perfusing solutions. KRP solutions and a 100% O_2 gas phase were used to perfuse and bathe mTALH segments, since V_e and NaCl transport in the mouse mTALH are unaffected by the absence of HCO_3^- (11, 50). HCO_3^--containing KRB solutions and a 95% O_2/5% CO_2 gas phase were used in all experiments with cTALH segments, since the latter exhibit (CO_2 + HCO_3^-)-stimulated NaCl transport (11, 12).

ADH (synthetic arginine vasopressin, grade V, Sigma Chemical Co. St. Louis, MO), where indicated, was added

TABLE I
Composition of Solutions

Solute	KRP		KRB	
	Perfusate	Bath	Perfusate	Bath
	mM			
NaCl	145	145	109	109
$NaHCO_3$	—	—	25	25
Na acetate	—	—	10	10
KCl	—	—	5	5
NaH_2PO_4/Na_2HPO_4	—	—	1.2	1.2
KH_2PO_4/K_2HPO_4	2.8	2.8	—	—
$CaCl_2$	—	—	1.0	1.0
$CaSO_4$	1.0	1.0	—	—
$MgSO_4$	1.2	1.2	1.2	1.2
L-Alanine	—	5	—	5
Glucose	—	5.5	—	5.5
Urea	11	—	11	—
BSA *(g/100 ml)*		0.4		0.4

to peritubular media at the concentrations given in the text. The dibutyryl analogue (db-cAMP) of cyclic adenosine monophosphate (Sigma Chemical Co.), was stored at −4°C and was added to the bathing solutions immediately prior to each experiment. PGE_2 (Sigma Chemical Co.) was reconstituted in 95% ethanol, stored at −20°C, and added to bathing or perfusing solutions immediately before each experiment; PGE_2 is stable when stored under these conditions (51). The amount of ethanol vehicle added to solutions to give the PGE_2 concentrations used in these studies caused a rise of <6 mosmol/kg H_2O in the overall osmolality of the solution. In a series of control experiments with both cTALH and mTALH, the alcohol vehicle without PGE_2 had no detectable effects on the transport properties of these segments.

Measurement of V_e. V_e (millivolts, lumen with respect to bath) was measured as described previously (11, 12, 50). Glass 0.9% NaCl/4% agar bridges were placed in both perfusing and bathing solutions, were joined via Tygon tubing filled with 3 M KCl to calomel half cells, and were connected to the input of a differential instrumentation amplifier. The voltages were recorded on a strip chart recorder. Since the perfusing and bathing solutions were almost identical in ionic composition, and only negligible amounts of BSA were present in bathing solutions, no corrections for liquid junction or Donnan voltages were necessary (11, 12, 17). Because the length constant for the mTALH is ~0.13 mm (11, 17), and the tubule segments were ~0.5–1.0 mm in length, V_e values recorded during measurement of J_{Cl}^{net} provided information only about electrical events in close proximity to the perfusion pipette.

Measurement of net water flux. The rates of net fluid transport (J_v, nl min^{-1} mm^{-1}) were measured as described in detail previously (11, 12, 48, 49) using exhaustively dialyzed ^3H-labeled inulin (New England Nuclear, Boston, MA, sp act 419 mCi/g). As in previous studies (11, 12, 48, 49), we utilized only those experiments in which the rate of perfusate leakage into bathing solutions was <5% of the perfusion rate.

Measurement of J_{Cl}^{net}. As described previously (11, 12, 50), samples of fluid for Cl^- determination, either initial perfusate or collected fluid, were collected between oil blocks to reduce evaporative loss. Immediately following each collection, Cl^- concentrations were measured electrotitrametrically in triplicate (11), using the method of Ramsey et al. (52). The mean of these three determinations was used to calculate J_{Cl}^{net}. Since J_v for the mouse mTALH is virtually zero during net Cl^- absorption (cf. Results and references 9–11), J_{Cl}^{net} (eq s^{-1} cm^{-2} luminal surface area) was calculated from the expression:

$$J_{Cl}^{net} = \frac{([Cl^-]^P - [Cl^-]^{cf})\dot{V}^o}{A_t}, \quad (1)$$

where $[Cl^-]^P$ and $[Cl^-]^{cf}$ are the chloride concentrations in the perfusate and collected fluid, respectively; $\dot{V}^o$ (cm^3 s^{-1}) is the perfusion rate; and A_t (cm^2) is luminal surface area, measured from photographs.

Statistical analyses. Two to four measurements per experimental condition were made in a given tubule. All measurements in a given tubule for a given set of experimental conditions were used to compute a mean value for that tubule. The mean values for individual tubules were then used to calculate a mean±standard error of the mean for the indicated number of tubules. *P* values for mean paired differences were computed from the Student's *t* test by comparing the difference to zero. Regression coefficients for linear fit were calculated using the sum of least-squares method. Statistics for the intercept and slope of the linear regression were calculated using the standard error of estimate as described by Steel and Torrie (53).

RESULTS

Effect of PGE_2 on the ADH-dependent V_e: mTALH. Fig. 1 illustrates a representative experiment showing the effect of adding graded concentrations of PGE_2 to the bath on V_e in an mTALH segment exposed to 10 μU/ml ADH in the bath; the latter is the minimal ADH concentration required for maximal enhancement of V_e in the mouse mTALH (11; cf. also Fig. 4). These data show clearly that the maximal ADH-stimulated V_e declined progressively with increasing bath concentrations of PGE_2; that the reduction of V_e to ~50% of the maximal ADH-stimulated value occurred at a bath PGE_2 concentration of 10^{-7} M PGE_2; and that 10^{-5} M PGE_2 had no further inhibitory effect on V_e. Although the data are not shown in Fig. 1, we noted that the PGE_2 effect was reversible, i.e., V_e returned to the maximal ADH-stimulated value when PGE_2 was removed from the bath; and that adding identical volumes of 95% ethanol (the PGE_2 solvent) to the bath, but without PGE_2 had no detectable effect on V_e.

We assessed in more detail the relations between peritubular PGE_2 concentrations and the ADH-stimulated spontaneous V_e. Fig. 2 summarizes the results of these experiments in which each tubule served as its own control. To evaluate the effects of graded concentrations of PGE_2 on inhibiting the ADH-stimulated V_e, we measured the relation between $\Delta V_e/\Delta V_{e_{max}}$ and

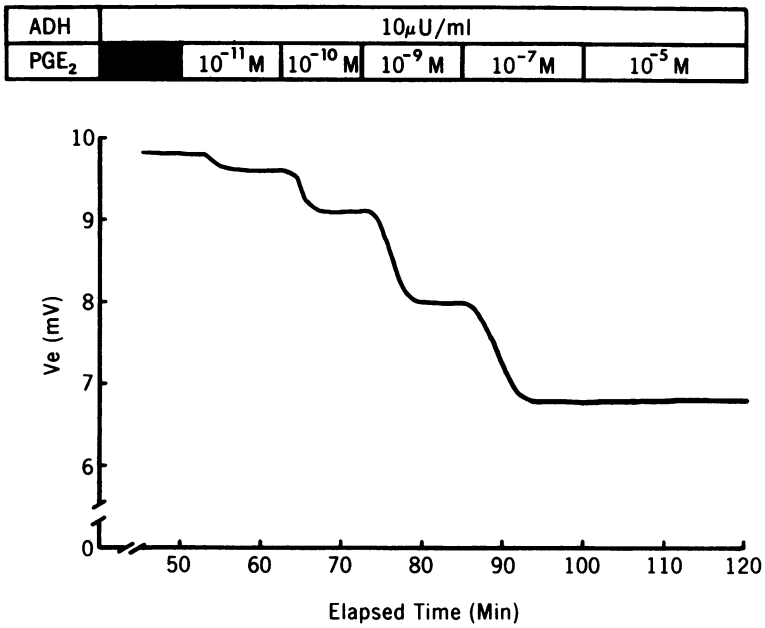

FIGURE 1 A representative experiment illustrating the relation between varying peritubular PGE_2 concentrations and the ADH-stimulated V_e in the mouse mTALH.

the concentration of a given agent in each tubule: $\Delta V_{e_{max}}$ was defined as the maximum change in V_e produced by a given agent in a given tubule, and ΔV_e was defined as the change in V_e produced by a specified concentration of the same agent in that tubule. This protocol is identical to that used previously (11) in evaluating the effects of graded concentrations of ADH or cAMP analogues on V_e in the mTALH, and was chosen to permit sequential, paired observations on the effects of PGE_2 on V_e in each tubule. The ab-

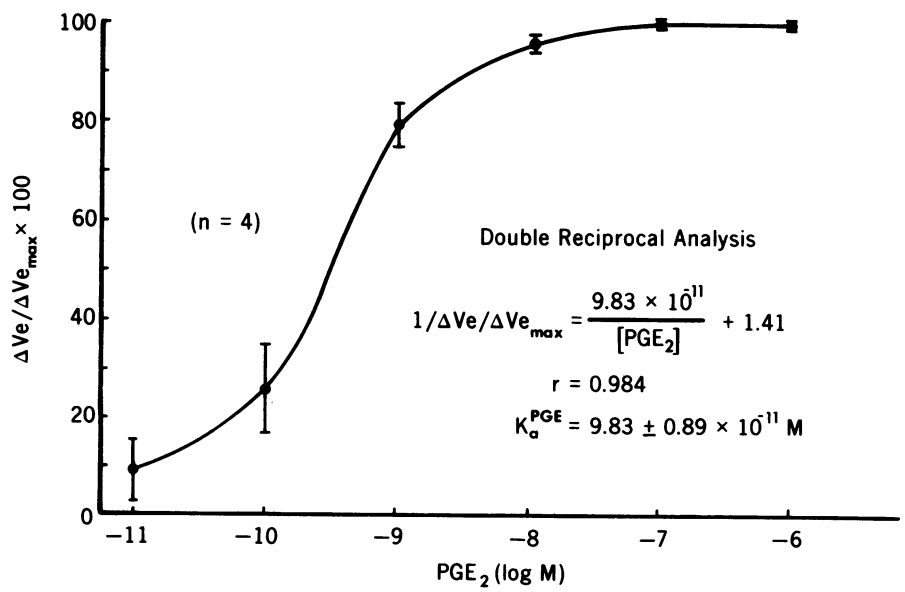

FIGURE 2 The effect of varying peritubular concentrations of PGE_2 on $\Delta V_e/V_{e_{max}}$; the latter term is described in the text. The data are expressed as means±SEM.

solute values±standard error of the mean of the V_e data for each set of experiments, both with and without ADH, have been provided in the text.

The results plotted in Fig. 2 indicate that 10^{-7} M bath PGE_2 produced a maximal reduction in the ADH-dependent V_e, and that 10^{-6} M PGE_2 had no further effect on the ADH-dependent V_e. The control ADH-dependent value of V_e was 9.4±1.3 mV, and the nadir value of V_e in the presence of PGE_2 was 5.6±0.9 mV ($P < 0.01$); thus 10^{-7} M PGE_2 produced ~ a 50% decline in the ADH-dependent values of V_e in the mTALH.

Now the relation between $\Delta V_e/\Delta V_{e_{max}}$ and bath PGE_2 concentrations may be expressed in reciprocal form as

$$1/(\Delta V_e/\Delta V_{e_{max}}) = K_a^{PGE} \cdot \frac{1}{[PGE_2]} + 1, \qquad (2)$$

where K_a^{PGE} is the apparent association constant for the interactions among PGE_2 and ADH-dependent processes in the mTALH that resulted in a reduction in the ADH-dependent V_e. The inset in Fig. 2 indicates the results of such a double reciprocal analysis of the experimental data shown in Fig. 2 and expressed in terms of Eq. 2. The double reciprocal analysis yielded a highly significant linear regression ($r = 0.984$); and the apparent K_a^{PGE} value for the PGE_2-mediated inhibition of the ADH-dependent V_e was 9.83×10^{-11} M.

It was relevant to evaluate in further detail the effects of 10^{-6} M PGE_2 on V_e in the ADH-stimulated mTALH. The results, shown in Fig. 3, indicate that 10^{-6} M PGE_2 reduced the ADH-dependent V_e to a value nearly equal to the ADH-independent V_e. One might surmise from these data that PGE_2 interfered with ADH-mediated increments in V_e, and by inference in conservative salt absorption, and that PGE_2 had no significant effect on ADH-independent salt transport. These latter possibilities will be addressed explicitly in Tables II and III.

Finally, it should be noted that, in 4 of the 12 tubules reported in Fig. 3, 10^{-6} M PGE_2 was added to the perfusate rather than to the bath. The latter data, indicated by dashed lines were obviously indistinguishable from those denoted by solid lines, in which PGE_2 was added to the bath. Thus, we conclude that 10^{-6} M PGE_2 had the same effect on the ADH-dependent V_e, whether added to luminal or peritubular fluids.

Effect of PGE_2 on the ADH-independent V_e: mTALH. It has been established previously (11, 50) that net Cl^- transport in the mouse mTALH includes an ADH-independent component and a component enhanced either by ADH or by cAMP (cf. Figs. 3, 4, 7, and 8). Given the results shown in Fig. 3, it might be argued that PGE_2 interfered with the ADH-stimulated components of V_e and net Cl^- absorption rather than with the ADH-independent moieties of V_e and J_{Cl}^{net}. To test this point directly, we evaluated the effects of 10^{-6}–10^{-3} M PGE_2 (which, from Figs. 1 and 2, are vastly in excess of 10^{-7} M PGE_2 and produced a maximal reduction in the ADH-dependent component of V_e). The results, shown in Table II, involved paired comparisons in the same tubules of the ADH-independent components of V_e in the presence and absence of PGE_2. The data show that the mean paired V_e difference (plus vs. minus PGE_2) was negligible. In keeping with the data shown in Fig. 3, the results in Table II are consistent with the view that PGE_2 affected exclusively the ADH-dependent component of V_e in the mouse mTALH.

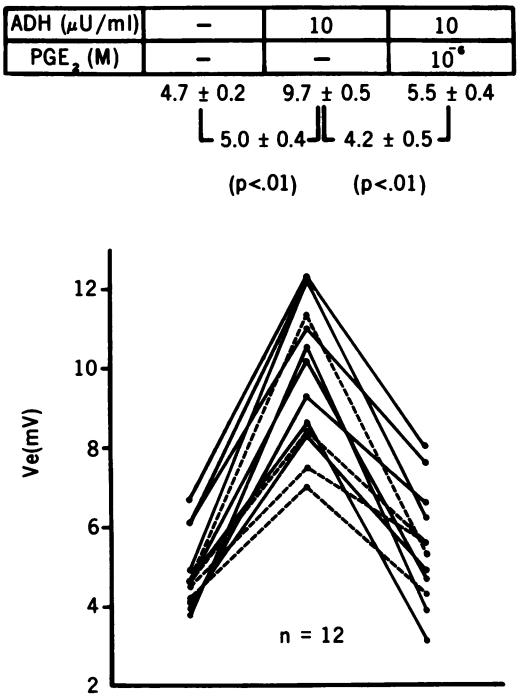

FIGURE 3 The effects of ADH on enhancing V_e and of PGE_2 on reducing the ADH-stimulated V_e. ADH, when present, was added to peritubular media. PGE_2, when present, was added either to the bath (solid lines) or to the lumen (dashed lines). The lines connect data points on individual tubules. The results are expressed as means±SEM.

Effect of PGE_2 on J_{Cl}^{net}: mTALH. It is generally considered (6, 7, 20) that the spontaneous V_e in isolated rabbit TALH segments, either cortical or medullary, attends conservative salt absorption, although there are divergent views (6, 7, 20) about the mechanism by which salt absorption results in a lumen-positive transepithelial voltage. The same considerations apply to the relations between conservative salt absorption and

TABLE II
Effect of PGE_2 on the ADH-independent V_e in mTALH

Control ADH-independent V_e	PGE_2 V_e	Mean paired difference	
4.3±0.6	4.1±0.6 (n = 4)	0.2±0.1	NS

The ADH-independent V_e in these tubules was measured as described previously (11). Then PGE_2 was added to the peritubular media at concentrations of 10^{-6} M (two tubules), 10^{-5} M (one tubule) or 10^{-3} M (one tubule). Since the results were the same at each concentration, the results are pooled.

V_e in the mouse mTALH (8–11, 17), where it is clear that, under a wide variety of circumstances, there is a close and statistically meaningful relation between the spontaneous V_e and J_{Cl}^{net} (11, 50). Thus, one may deduce from the data shown in Fig. 3 that PGE_2, at a luminal or bath concentration of 10^{-6} M, interfered with ADH-stimulated conservative NaCl absorption in the mouse mTALH.

To evaluate this question more explicitly, we assessed the effects of 10^{-6} M peritubular PGE_2 on ADH-mediated net Cl^- absorption [which, for this tubule segment, is approximately equal to the rate of net NaCl absorption (11), a conclusion also deduced for the in vivo TALH by Wallin et al. (54)] under conditions of a constant perfusion rate. These experiments were intended to complement those presented in Fig. 3.

The results of these experiments are summarized in Table III, which tabulates paired observations in the same tubules in which we measured the effect of 10^{-6} M bath PGE_2 on the ADH-dependent value of J_{Cl}^{net}. To exclude the possibility that variations in J_{Cl}^{net} were referable to variations in perfusion rate (11, 50), the latter was maintained constant, within the limits of experimental error, during control and experimental periods. The data reported in Table III indicate clearly that 10^{-6} M bath PGE_2 reduced the ADH-dependent J_{Cl}^{net} by ~50%, i.e., to approximately the same degree as the PGE_2-mediated reduction in V_e (Figs. 1–3). Thus, the results in Table III, when taken in conjunction with those in Figs. 1–3, indicate that, in the mTALH, PGE_2 reduced the ADH-dependent V_e concomitant with a PGE_2-mediated reduction in ADH-dependent conservative Cl^- absorption.

Effect of varying peritubular ADH concentrations on the PGE_2-inhibited, ADH-dependent V_e. Fig. 4 shows the results of two different groups of experiments assessing the effect of varying peritubular ADH concentrations on $\Delta V_e/\Delta V_{e_{max}}$ in the mouse mTALH. In one series, indicated by the solid curve, peritubular PGE_2 was absent while in the second series, indicated by the dashed curve, 10^{-6} M PGE_2 was present in the bath. Each series of experiments, as in prior studies (11) and in those reported in Fig. 2, involved measurements of V_e in the same tubule at varying ADH concentrations. But because of technical constraints, i.e., the time required for a given set of measurements, the curves with ADH alone and with ADH plus 10^{-6} M peritubular PGE_2 were obtained on different groups of tubules. Additional paired control observations for these group comparisons will be presented below (cf. Fig. 5).

The results in Fig. 4 show clearly that, when the peritubular ADH concentration was raised sufficiently, V_e was stimulated to the same value even with 10^{-6} M PGE_2 in the bath. In the absence of PGE_2, a maximal ADH-dependent V_e of 10.7±0.5 mV was obtained at a peritubular ADH concentration of 15 μU/ml; in the presence of 10^{-6} M PGE_2 in the bath, V_e rose to 10.0±0.8 mV, but only when the peritubular ADH concentration was raised to 250 μU/ml. Importantly, these two values of V_e—10.7±0.5 mV at 15 μU/ml ADH and 10.0±0.8 mV at 250 μU/ml ADH plus 10^{-6} M PGE_2—are statistically indistinguishable.

TABLE III
Effect of PGE_2 on ADH-stimulated J_{Cl}^{net} at a Constant Perfusion Rate in mTALH

Condition	$[Cl^-]^p$	$[Cl^-]^{cf}$	J_{Cl}^{net}	$\dot{V}^o$
		mM	*peq s^{-1} cm^{-2}*	*nl min^{-1}*
ADH, 10 μU/ml	145	121.3±3.9	7,940±1,465	6.2±0.9
ADH, 10 μU/ml + PGE_2, 10^{-6} M	145	134.5±3.2	3,190±545	5.2±0.9
Mean paired difference		12.7±3.4	4,755±1,390	1.0±0.7
P		<0.02	<0.02 (n = 6)	NS

ADH and PGE_2, when present were added to bathing solutions. Paired observations of $[Cl^-]^{cf}$ and J_{Cl}^{net} were carried out as described in Methods. The results are expressed as means±SEM.

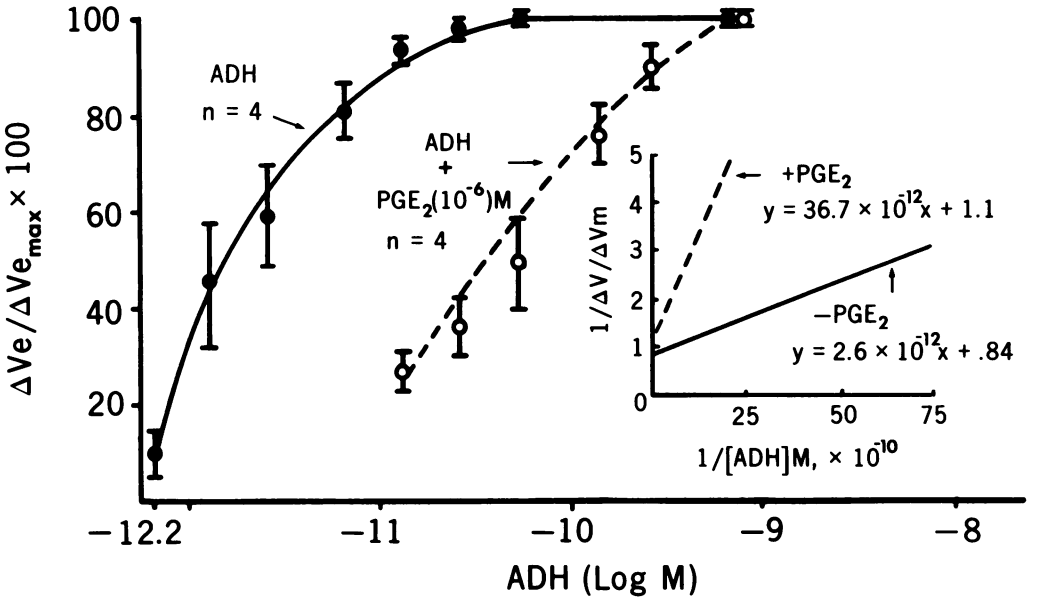

FIGURE 4 The effect of varying peritubular concentrations of ADH in enhancing $\Delta V_e/\Delta V_{e_{max}}$ in the presence (dashed curve) and absence (solid curve) of 10^{-6} M PGE_2. The data are expressed as means±SEM. The inset is a double-reciprocal plot of the data according to Eq. 3.

One may argue from these data that the interactions between ADH and PGE_2 in modulating V_e (Fig. 4), and concomitantly conservative salt absorption (Table III), were seemingly competitive.

A simple way of illustrating the latter argument is to plot the data in Fig. 4 in double-reciprocal fashion. In this instance, Eq. 2 becomes

$$1/(\Delta V_e/\Delta V_{e_{max}}) = K_a^{ADH} \cdot \frac{1}{[ADH]} + 1, \qquad (3)$$

where K_a^{ADH} refers to the association constant between peritubular ADH concentrations and ADH-dependent processes in the mouse mTALH which resulted in a rise in the ADH-dependent V_e. The results, shown in the inset of Fig. 4, indicate that PGE_2 increased the K_a^{ADH} for ADH-mediated increases in the spontaneous V_e in the mTALH from 2.63×10^{-12} M ADH to 36.7×10^{-12} M ADH (the former K_a^{ADH} is virtually identical to the one reported previously by us [11] for the stimulating effect of ADH on V_e in the mTALH). The fit of the experimental data to Eq. 3 is indicated by the highly significant regression coefficients (+PGE_2, $r = 0.98$; −PGE_2, $r = 0.97$) and the zero intercepts shown in Fig. 4 (+PGE_2, 1.10; −PGE_2, 0.84), which were indistinguishable from unity.

The comparisons reported in Fig. 4 were obtained on separate groups of tubules. Consequently, we wished to demonstrate, by paired observations on the same tubules, that sufficiently high concentrations of peritubular ADH could reverse entirely the PGE_2-mediated reduction in the ADH-dependent moiety of V_e. These data are shown in Fig. 5. When the bath contained 10 μU/ml ADH, V_e was 7.4±1.0 mV and was reduced by ~50% to 3.9±0.7 mV ($P < 0.01$) when 10^{-6} M PGE_2 was added to the bath. But when the bath ADH was raised to 250 μU/ml in the presence of 10^{-6} M bath PGE_2, V_e rose to 6.7±0.7 mV, a value indistinguishable from the control V_e, i.e., that obtained with 10 μU/ml ADH and no peritubular PGE_2. In other words, these data, involving paired observations on the same tubules, affirm explicitly the conclusions derived from the results (cf. Fig. 4) obtained among groups of tubules.

Interactions among db-cAMP, PGE_2, V_e, and J_{Cl}^{net}. Fig. 6 illustrates the results of a series of experiments having protocols comparable to those shown in Fig. 4 with the exception that the experiments in Fig. 6 were designed to evaluate the effects of 10^{-6} M bath PGE_2 on db-cAMP-mediated (11) increments in V_e. As was the case with the data shown in Fig. 4, the results shown in Fig. 6 involved comparisons among groups of tubules. The solid curve represents tubules exposed to varying concentrations of db-cAMP alone; and the dotted curve represents tubules exposed to varying concentrations of db-cAMP and 10^{-6} M peritubular PGE_2. Inspection of the results in Fig. 6 shows clearly that PGE_2 had no discernible effect on the V_e-enhancing ability of db-cAMP. More concretely, the

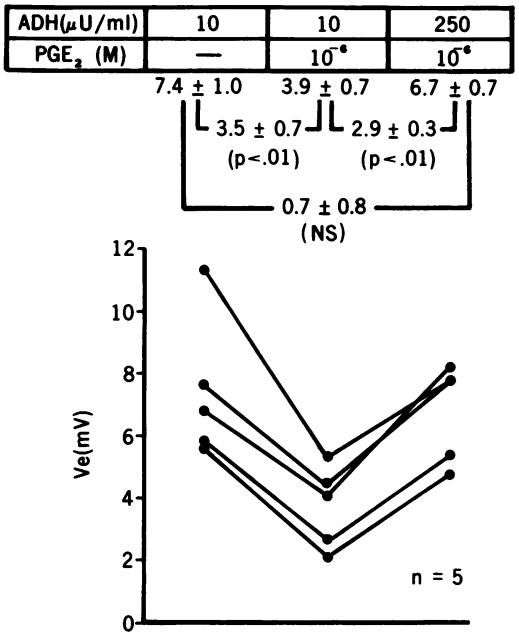

FIGURE 5 The effect of increasing peritubular ADH concentrations from 10 to 250 μU/ml on the reduction in the ADH-mediated V_e produced by 10^{-6} M peritubular PGE_2. The lines connect data points in individual tubules. The data are expressed as means±SEM.

maximal V_e obtained with 10^{-3} M db-cAMP alone was 10.7±0.8 mV, while that obtained with the same concentrations of cyclic nucleotide and 10^{-6} M PGE_2 was 10.8±0.5 mV; these two values of V_e are indistinguishable statistically.

For a double reciprocal analysis of the data shown in Fig. 6, Eq. 3 becomes

$$1/(\Delta V_e/\Delta V_{e_{max}}) = K_a^{cAMP} \cdot \frac{1}{[\text{db-cAMP}]} + 1, \qquad (4)$$

where K_a^{cAMP} refers to the affinity constant for the interactions between db-cAMP-dependent processes in the mouse mTALH that resulted in a rise in V_e. The inset in Fig. 6 shows the data plotted according to Eq. 4. The results indicate that the K_a^{cAMP} for db-cAMP-mediated enhancement of V_e in the mTALH was virtually identical whether 10^{-6} M PGE_2 was present or absent from the bath. Moreover, the zero intercepts for both plots, in keeping with the requirements of Eq. 4, were virtually unity and the regression coefficients highly significant ($+PGE_2$, $r = 0.99$; $-PGE_2$, $r = 0.99$).

Finally, by analogy with the results in Table III and Figs. 4 and 5, it was relevant to demonstrate, in paired observations on the same tubules, that the minimum concentration of db-cAMP required to enhance V_e and J_{Cl}^{net} maximally, i.e., 10^{-3} M (cf. Fig. 6 and reference 11), would reverse entirely the inhibitory effect of PGE_2 on the ADH-dependent V_e and on the ADH-dependent moiety of J_{Cl}^{net}. These data, shown in Fig. 7, included simultaneous, paired observations of V_e and J_{Cl}^{net} in the same tubule under varying conditions.

The first two columns of Fig. 7, in keeping with the results in Figs. 1–4 and Table III, show clearly that

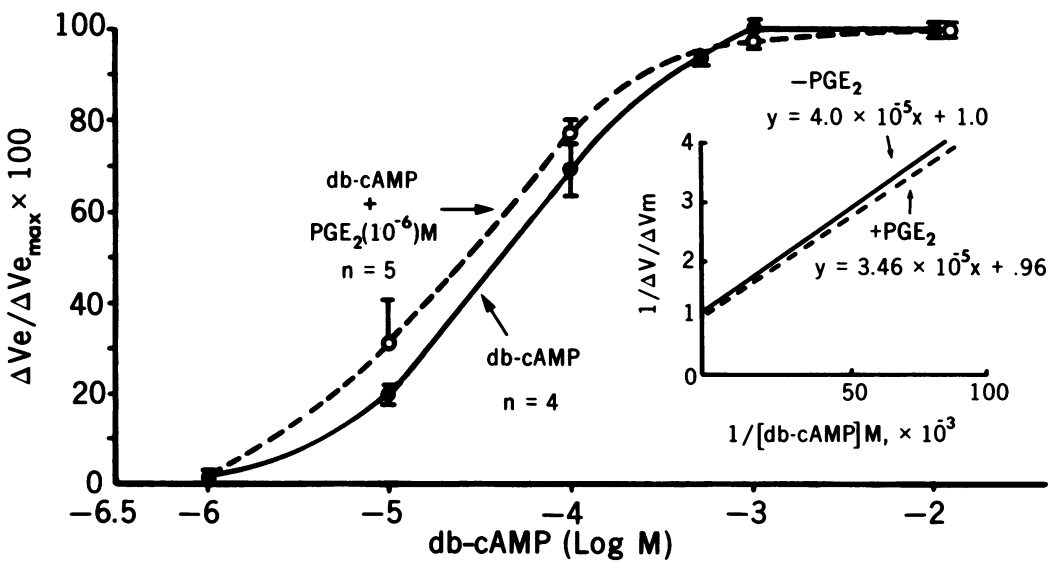

FIGURE 6 The effect of increasing peritubular db-cAMP concentrations on $\Delta V_e/\Delta V_{e_{max}}$ in the absence (solid curves) and presence (dashed lines) of 10^{-6} M peritubular PGE_2. The data are expressed as means±SEM. The inset is a double-reciprocal plot of the data according to Eq. 4.

10^{-6} M PGE_2 produced ~50% reduction in the V_e (lower panel) and J_{Cl}^{net} (upper panel), which occurred when 10 μU/ml ADH was added to the bath. These data are entirely consonant with the paired observations reported in Fig. 3 and Table III. The final column of Fig. 7 shows clearly that 10^{-3} M db-cAMP reversed entirely the inhibitory effect of 10^{-6} M PGE_2 on the ADH-dependent moieties of both V_e and J_{Cl}^{net} in these mTALH segments. It should be noted in this regard that both the V_e and J_{Cl}^{net} values shown in the first and third columns of Fig. 7 are indistinguishable statistically.

Functional heterogeneity of the mouse mTALH and cTALH. Our prior observations (11, 12, 50) on the mouse mTALH and cTALH have provided evidence that the two nephron segments are functionally heterogeneous. In both nephron segments, there is a spontaneous transepithelial voltage that attends conservative salt absorption (11, 12), and the magnitudes of V_e and J_{Cl}^{net} are closely correlated (11, 12). Furthermore, both in the mouse cTALH and mTALH, V_e and J_{Cl}^{net} are abolished by 10^{-4} luminal furosemide, by cooling, by 10^{-3} M peritubular ouabain, or by omission of luminal Na^+, luminal Cl^-, or peritubular K^+ (11, 12, 17, 50).

However, important differences between these two segments also exist. In the mouse mTALH, both V_e and J_{Cl}^{net} are enhanced considerably by peritubular ADH or by db-cAMP (Figs. 3, 7; references 11, 50); but in that segment, the combination (CO_2 + HCO_3^-) has no effect on either V_e or J_{Cl}^{net} (11). In contrast, in the mouse cTALH, neither J_{Cl}^{net} nor V_e is affected perceptibly either by ADH or by cAMP (11), while the combination (CO_2 + HCO_3^-) enhances considerably both V_e and J_{Cl}^{net} in that segment (11, 12).

The data reported in Fig. 8 provide another set of results consistent with the view that the processes regulating conservative NaCl absorption in the mouse cTALH and mTALH are modulated by different factors. As illustrated in Fig. 8, 10^{-6} M PGE_2, which reduced significantly the ADH-dependent moieties of V_e (Fig. 3) and J_{Cl}^{net} (Table III) in the mouse mTALH had no discernible effect on the (CO_2 + HCO_3^-)-dependent V_e in the mouse mTALH.

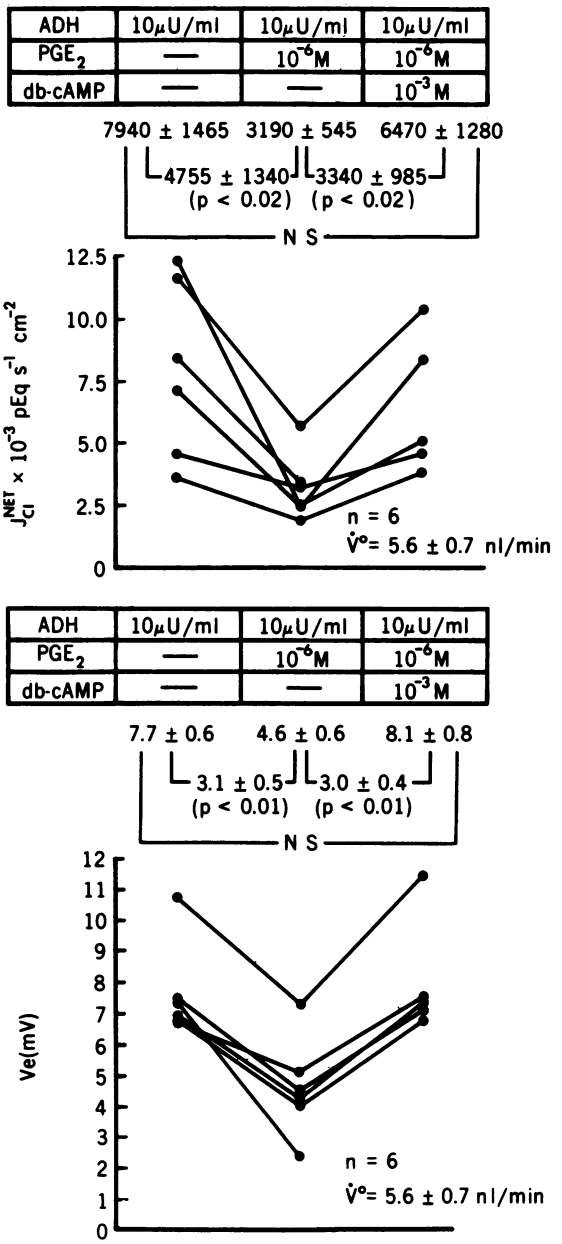

FIGURE 7 Reversal of the 10 μU/ml ADH-dependent V_e and J_{Cl}^{net} values inhibited by 10^{-6} M peritubular PGE_2 by 10^{-3} M db-cAMP. The upper panel shows the J_{Cl}^{net} data and the lower panel the V_e data; both sets of data were obtained simultaneously on the same tubules. The lines connect data points on individual tubules. The results are expressed as means±SEM.

DISCUSSION

Since the isolated mouse mTALH is both electrically leaky (11, 17) and highly permeable to Na^+ and Cl^- (11, 17), it is evident that varying peritubular NaCl concentrations would, all other factors being equal, result in a variable diluting capacity for that water-impermeable (9–11) nephron segment. However, at least two other factors have been identified previously that modulate conservative NaCl absorption by the mouse mTALH, and hence regulate diluting power of that nephron segment. ADH, operating via the second messenger cAMP (11), enhances both V_e and J_{Cl}^{net} in the

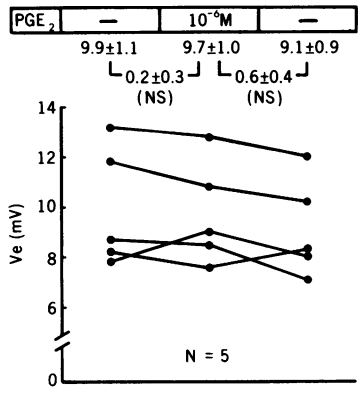

FIGURE 8 The effect of 10^{-6} M peritubular PGE_2 on V_e in the mouse cTALH. The lines connect data points on individual tubules. The data are expressed as means±SEM.

mouse mTALH; and increases in peritubular osmolality diminish the rate of conservative ADH-dependent Cl^- absorption by the mTALH (19). The present studies were designed to evaluate the interactions among db-cAMP, ADH, and PGE_2 in regulating J_{Cl}^{net} and V_e in isolated mouse cTALH and mTALH.

General conclusions. Within the limits of experimental error, the present data permit the following general conclusions. First, when the peritubular media contained 10 μU/ml ADH (the minimal hormone concentration required to stimulate maximally V_e and J_{Cl}^{net} in the isolated mouse mTALH [cf. Fig. 4 and reference 11]), PGE_2 reduced concomitantly the ADH-dependent moieties of V_e and J_{Cl}^{net} to values approximately equal to those obtained in the absence of ADH (Figs. 1 and 3; Table III). The apparent K_a^{PGE} for reducing the ADH-dependent V_e was $\sim 10^{-10}$ M (Fig. 2), and the effects were indistinguishable whether PGE_2 was present in the lumen or the bath (Fig. 3).

Second, in unpaired experiments, 10^{-6} M peritubular PGE_2 raised K_a^{ADH} from $\sim 2.6 \times 10^{-12}$ M to 36.7×10^{-12} M (Fig. 4). And both in unpaired observations among different groups of tubules (Fig. 4) and in paired observations on the same tubules (Fig. 5), 250 μU/ml ADH restored V_e to the maximally stimulated ADH value, even in the presence of 10^{-6} M peritubular PGE_2. Since ADH has no discernible effects on the dissipative properties of the shunt pathway in the isolated mouse mTALH (11, 17, 50), we may conclude from the data in Fig. 5, taken together with the results in Figs. 1–4 and Tables II and III that PGE_2 inhibited, in a seemingly competitive manner, the ADH-dependent moieties of J_{Cl}^{net} and V_e; that these latter effects obtained by a PGE_2-mediated interference with conservative, rather than with dissipative, salt transport processes; and that PGE_2, at the concentrations tested, had no demonstrable effect on the ADH-independent moiety of V_e in the isolated mouse mTALH.

Third, in unpaired groups of observations among different sets of tubules, 10^{-6} M PGE_2 had no effect on K_a^{cAMP} (Fig. 6). In paired simultaneous observations on the same tubules, 10^{-3} M peritubular db-cAMP (the minimal db-cAMP concentration required to stimulate maximally V_e and J_{Cl}^{net} in these tubules [cf. Fig. 6; reference 11]) reversed entirely the inhibitory effect of 10^{-6} M PGE_2 on both the ADH-stimulated V_e and the ADH-stimulated J_{Cl}^{net} (Fig. 7). Thus, we conclude that the PGE_2-mediated reduction in the ADH-dependent V_e and J_{Cl}^{net} observed in the mouse mTALH occurred as a consequence of a reduction in the ADH-dependent rate of intracellular cAMP accumulation, rather than by interfering with the mechanisms by which cAMP modulates J_{Cl}^{net} and V_e.

Finally, the data shown in Fig. 8 indicate that 10^{-6} M peritubular PGE_2 had no effect on V_e in the mouse cTALH, a finding in keeping with the fact that, in the mouse cTALH, V_e is unaffected by ADH (11, 12). These data indicate further the functional heterogeneity recognized previously (11, 12) between the factors modulating V_e and salt absorption in the mouse cTALH and mTALH.

It should also be noted in this context that the effects of PGE on V_e and J_{Cl}^{net} in isolated TALH segments may vary among species. Stokes (43) has found that PGE_2 inhibited J_{Cl}^{net} and V_e by ~50% in the ADH-unstimulated rabbit mTALH, while the present results (cf. Tables II and III and Fig. 3) indicate that, in the mouse mTALH, PGE_2 inhibited only the ADH-dependent moieties of V_e and J_{Cl}^{net}. The reasons for the discrepancy between our results and those of Stokes (43) on mTALH segments of these two species are not clear at present, although it is noteworthy in this regard that the adenyl cyclase responsiveness of the isolated rabbit mTALH to ADH is considerably less than that of the isolated mouse mTALH (15, 16). However, for the isolated cTALH, the present data with mouse segments affirm the observations by Stokes (43) that PGE_2 had no effect on V_e in these isolated rabbit segments. By contrast, Fine and Trizna (55) found no effect of PGE_2 on NaCl transport in the ADH-unstimulated rabbit mTALH, a finding that parallels our observations of the lack of effect of PGE_2 on the ADH-independent V_e in mouse mTALH (Table II).

Tentative hypotheses. The proposal that PGE_2 might interfere with the hydroösmotic effect of ADH at loci that prevented intracellular cAMP accumulation was first set forth by Orloff et al. (21) and by Grantham and Orloff (22). Subsequent studies utilizing cellular preparations of individual nephron segments have demonstrated directly an inhibition of ADH-

stimulated increases in intracellular cAMP by PGE_2. Torikai and Kurokawa (56) reported that incubation of rat mTALH with ADH caused a sharp rise in cellular cAMP. The addition of PGE_2 to the incubation medium resulted in a marked inhibition of the ADH stimulation of cellular cAMP; but PGE_2 had no effect on cAMP production when added to the rat mTALH in the absence of ADH. These biochemical studies are thus in close accord with our results, which demonstrate a PGE_2 inhibition of ADH-mediated transport events (Fig. 3; Table III) and a lack of PGE_2 on ADH-independent transport events (Table II).

Similar results were obtained by Edwards et al. (57) in rat papillary collecting duct cells. Dunn et al. (58) have reported arachidonic acid stimulation of PGE_2 in cells of rabbit papillary collecting duct and, simultaneously, arachidonic acid inhibition of ADH-stimulated cellular cAMP. Both effects of arachidonic acid were reversed by inhibition of prostaglandin synthesis, suggesting an action of prostaglandins produced in situ to inhibit ADH enhancement of intracellular cAMP.

The data in the present paper are obviously consistent with the possibility that, in the mouse mTALH, PGE_2 inhibited the rate of ADH-mediated cytoplasmic cAMP accumulation. Accordingly, it is prudent to consider the possible nature of these interactions in the mouse mTALH.

The fact that the effects of db-cAMP on V_e and J_{Cl}^{net} were indistinguishable with and without PGE_2 is consistent with the view (21, 22) that PGE_2 did not modify significantly the interactions between db-cAMP, and presumably cAMP, leading to perturbations in membrane transport processes. Rather, the seemingly competitive interactions between ADH and PGE_2 (Figs. 4, 5) reported in this paper are more in accord with the notion (21, 22) that PGE_2 inhibited the ADH-dependent moieties of V_e and J_{Cl}^{net} by interfering either with the rate of cytosolic cAMP formation and/or by accelerating the rate of cytosolic cAMP dissipation.

There are two general ways, operating singly or in concert, in which PGE_2 might have diminished the rate of cytosolic cAMP formation. PGE_2 might have competed directly for the ADH receptor (R). Alternatively, PGE_2 might have reduced the overall affinity for adenyl cyclase activation by ADH-R units. The possible mechanisms for such an action of PGE_2 include, at a minimum: a PGE_2-mediated decrease in cell membrane fluidity; a PGE_2-mediated inhibition of enzyme activation occurring at the guanine nucleotide regulatory component of adenyl cyclase; or a PGE_2-mediated inhibition of the activation of the catalytic subunit of the adenyl cyclase.

Since supramaximal concentrations of ADH reversed the PGE_2-mediated inhibition of the ADH-dependent V_e and J_{Cl}^{net}, a reduced affinity for adenyl cyclase activation by ADH-R units requires two tacit assumptions: basolateral membranes contained spare receptors for ADH at levels of maximal stimulation of V_e and J_{Cl}^{net} in the absence of PGE_2; and in the presence of PGE_2, a greater fractional occupancy of R units by ADH was required to enhance V_e and J_{Cl}^{net} maximally than in the absence of PGE_2.

The seemingly competitive interactions (Figs. 4, 5) between ADH and PGE_2 might also have been the result of a PGE_2-dependent increase in the rate of cAMP dissipation. Thus PGE_2 might have accelerated cAMP hydrolysis by phosphodiesterase. In that case, the inability of PGE_2 to modify the effects of db-cAMP on J_{Cl}^{net} and V_e (Figs. 6, 7) may have been referable to the resistance of db-cAMP to phosphodiesterase hydrolysis (59). We consider this possibility unlikely since the observations of Edwards et al. (56) indicate that, in a variety of murine nephron segments, prostaglandins have no effect on phosphodiesterase activity. It might also be argued that PGE_2 increased the permeability of cellular membranes to cAMP, leading to a reduced concentration of intracellular cAMP at any given peritubular ADH concentration. Such a possibility, although not excluded by the present data, seem improbable since there is no evidence available that indicates that PGE_2 alters significantly the permeability of epithelial membranes to cAMP.

Thus, when taken together, the data in this paper affirm the fact that PGE_2, like ADH (11, 50) and peritubular osmolality (19), serves as a significant modulator of the ADH-dependent rate of net Cl^- absorption, and consequently of diluting capacity, in the isolated mouse mTALH. It is plausible to argue that this effect obtained because of a PGE_2-mediated reduction in the rate of cytosolic cAMP accumulation that occurred in response to ADH, either by a direct competition between PGE_2 and ADH for receptor units, or by diminishing the affinity for adenyl cyclase activation by ADH-R units. Obviously, the applicability of these findings to other species, and to the in vivo mammalian urinary concentrating process, requires further evaluation.

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