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Research Article

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ABSTRACT The effect of prostaglandin E_1 (PGE_1) on the water permeability response to vasopressin, theophylline, and cyclic adenosine 3′,5′-monophosphate (C-AMP) of isolated, perfused collecting tubules of the rabbit was investigated in vitro. Prostaglandin is a naturally occurring substance present in a number of tissues, including kidney. It has been implicated in the action of a variety of hormones, many of which are known to exert their physiological effects through the intermediacy of the C-AMP system. In the collecting tubule, PGE_1 (10^{-7} M) elicited a minimal increase in net water absorption along an osmotic gradient. However, when administered in association with a concentration of vasopressin (2.5 μU ml^{-1}) selected to induce a submaximal increment in water absorption, the effect of the latter was reduced by approximately 50%. Theophylline (5×10^{-3} M) also increased net water absorption, an effect not previously demonstrated in renal tissue. This effect was potentiated by the simultaneous addition of PGE_1. In contrast, PGE_1 did not influence the increase in net water absorption induced by C-AMP (10^{-2} M). Since C-AMP is responsible for the permeability effects of vasopressin in renal tissue, the present results are consistent with the view that PGE_1 interferes with the action of the octapeptide by competing with it at a site which influences the generation of C-AMP. In addition it is proposed that prostaglandin may be an important modulator of the action of vasopressin. The tubule is exquisitely sensitive to the hormone, responding to as little as 0.25 μU ml^{-1}. It is conceivable that in the intact animal prostaglandin may serve to dampen the effects of small amounts of residual hormone and thereby prevent overshoots in permeability which might otherwise occur.

INTRODUCTION

Prostaglandin E_1 (PGE_1), a naturally occurring substance, is known to modify the action of a variety of hormones on their specific receptor tissues (1, 2). It has been suggested that some of these effects may be a consequence of interference with hormone-induced accumulation of cyclic 3′,5′-AMP in the tissue (3). In earlier studies it had been established that PGE_1 inhibited the increase in the permeability to water of isolated toad bladders produced by vasopressin (4). The neurohypophyseal hormone is presumed to augment the permeability to water of this tissue by increasing the intracellular concentration of C-AMP (5, 6). Similar conclusions relative to the role of C-AMP in the action of vasopressin have been derived from studies in isolated collecting tubules of the rabbit (7). The purpose of our present study was

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to examine the effect of PGE_1 on the response to vasopressin in the kidney. Although reports of in vivo effects of PGE_1 on renal function have appeared (8, 9), in none is it possible to exclude the influence of associated vasomotor effects of prostaglandin on the results. In order to obviate these difficulties, direct examination of the effects of PGE_1 on the water permeability response of isolated, perfused segments of rabbit collecting tubule to vasopressin, theophylline, and C-AMP has been performed. The results are consistent with the view that prostaglandin may serve as an intracellular modulator of vasopressin action in the kidney by interfering with the accumulation of C-AMP normally induced by the polypeptide.

METHODS

Fragments of collecting tubules were dissected from slices of rabbit kidney cortex in oxygenated Ringer's solution and perfused as described previously (10). The outside bathing medium contained 115 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 10 mM Na acetate, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 1.0 mM $CaCl_2$, 5% v/v calf serum (Microbiological Associates), and 5.5 mM dextrose. This solution was 290 milliosmolal. The "hypotonic" perfusion solution was modified from previous studies (7) and contained 60 mM NaCl, 2.5 mM K_2HPO_4, 1.2 mM $MgSO_4$, and 1.0 mM $CaCl_2$, with pH adjusted to 7.4 with HCl; this solution was 125 milliosmolal. Tubules, 1.2–5.0 mm long, were transferred from the dissecting dish to a chamber that contained approximately 2.5 ml of the outside bathing solution through which a gas mixture (95% O_2 and 5% CO_2) was bubbled. All experiments were done at room temperature (23–24°C). Albumin was added to the perfusion solution as a volume marker. In order to remove preservatives, approximately 1 ml of albumin labeled with ^{131}I (Albumotope, E. R. Squibb & Sons, N. Y., 200–500 $\mu c\ ml^{-1}$) was dialyzed in 200 ml of the outside bathing medium. A portion of the dialyzed material was added to the perfusion solution, with the final concentration at 10–25 $\mu c\ ml^{-1}$. During tubule perfusion the bathing medium was analyzed for radioactivity in a gamma well detector and was found to contain less than 1% of the perfused radioactivity. The absolute perfusion rate was then calculated from the known concentration of radioactivity in the perfusion solution and the amount of labeled albumin recovered in the collecting pipette. Perfusion rate was essentially constant in each experiment at 3–13 nl min^{-1}. Net water absorption ($\mu l\ min^{-1}$) was measured from the difference between the perfusion and collection rates and expressed as per square centimeter of plane luminal surface ($\mu l\ cm^{-2}\ min^{-1}\ osmol^{-1}$). All tabulated values for net water absorption are averages of two or more 10–30 min collection periods in the steady state. In order to reduce the variation in results between experiments due to differences in tubule length and perfusion rate, absolute absorption was expressed as per unit of osmotic difference ($\mu l\ cm^{-2}\ min^{-1}\ osmol^{-1}$) across the tubule wall. Owing to the fact that in collecting tubules fluid is absorbed without appreciable loss or gain of solute in the lumen,[1] the osmotic difference across the tubule wall decreases with increasing tubule length. For purposes of calculation it was assumed that for a constant perfusion rate, the rise in osmolality was a linear function of tubule length. From the change in albumin concentration, the osmolality of the collected fluid was calculated and this and the initial value (125 milliosmolal) of the perfusion fluid were averaged to derive the mean transtubular osmotic difference.

All test agents were added to the outside bathing medium. A single lot of vasopressin (Pitressin, Parke, Davis & Co., Detroit, Mich.) was used. In the dose-response experiments, the hormone was diluted immediately before use to a final concentration of 0.025, 0.25, 2.5, 25, or 250 $\mu U\ ml^{-1}$. In the remainder of the experiments a concentration found to give a submaximal response, 2.5 $\mu U\ ml^{-1}$, was generally used. Solutions containing C-AMP (Calbiochem), with a final concentration of 10^{-2} M, or theophylline (Nutritional Biochemicals Corp.) at a final concentration of 5×10^{-3} M were adjusted to 290 milliosmolal with distilled water and pH at 7.4 with 0.15 M NaOH. Pure prostaglandin E_1 (PGE_1), obtained as a dry powder from Dr. Sune Bergstrom, was dissolved in a small volume of 80% ethanol. Aliquots of this were diluted to final concentrations of 10^{-7} and 10^{-9} M. Final ethanol concentrations in these solutions were 3×10^{-4} and 3×10^{-6} M, respectively. In preliminary studies we found that ethanol in these concentrations had no effect on permeability.

As noted earlier the values for net water absorption are the mean of two or more collection periods, each approximately 10–30 min long during the steady state. In order to evaluate the effect of PGE_1, vasopressin, theophylline, and C-AMP on water absorption, as well as the effect of PGE_1 on the permeability response to the three other test agents, the following procedure was adopted. In experiments 1–3, 7, 14, 15, and 21, PGE_1 was added after the initial control periods. When the response had stabilized and two or more collections had been made, a combination of PGE_1 and vasopressin, theophylline, or C-AMP was then added. Two or more measurements of absorption were performed and PGE_1 was removed from the bathing solution. When water absorption had stabilized, we obtained two or more additional collections and followed these in some studies with a final control period. In experiments 4–6, 10–13, 16–20, and 22 the sequence of administration of test agents was reversed. Either vasopressin, theophylline, or C-AMP was added first, followed by a combination of one of these three agents with PGE_1. The effect of PGE_1 alone was then tested. Finally in some of the studies prostaglandin was removed and net water absorption determined in the after control period. The mean difference betwen the initial and final control periods in the nine studies on which measurements were made was not significantly different from zero; 0.32 ± 0.52 (SEM) $\mu l\ cm^{-2}\ min^{-1}\ osmol^{-1}$. In

[1] Unpublished studies.

Tables I, II, and III only the initial control values are recorded.

RESULTS

Collecting tubules incubated in vasopressin-free media became relatively impermeable to water after 150–200 min of perfusion. When no hormone was added, the tubules remained impermeable for several hours as illustrated in Fig. 1. Addition of vasopressin caused a rapid increase in water absorption which was sustained for several hours (Fig. 1). These experiments permitted assessment of the variability of water absorption during the period of relative impermeability and the period of vasopressin-induced high osmotic permeability. In the impermeable condition the standard deviation of mean net water absorption in the steady state was $\pm$ 1.1 μl cm^{-2} min^{-1} osmol^{-1}; during the vasopressin period a comparable standard deviation was observed, $\pm$ 1.6 μl cm^{-2} min^{-1} osmol^{-1}. The response of isolated perfused tubules to graded doses of vasopressin is shown in Fig. 2. In two experiments the smallest dose that elicited a significant response was 0.25 μU ml^{-1} and the dose above which a further increase in absorption could not be detected was 25 μU ml^{-1}. In two studies, a dose of 2.5 μU ml^{-1} elicited 57 and 60% of the maximal permeability response which was also determined in the same tubules.

In order to test the effect of PGE_1 on the permeability response to vasopressin, three tubules were exposed to the hormone and a sustained increase in water absorption was elicited (Fig. 3). The addition of PGE_1 to the solution containing vasopressin decreased water absorption to a value slightly greater than that of the initial control period. No further change was observed in the final period when vasopressin was removed. In four additional studies the sequence was reversed. PGE_1 was added in the first period, followed by a combination of PGE_1 and vasopressin, and then by vasopressin alone. As shown in the representative study in Fig. 4, there was a significant increase in water absorption when PGE_1 was removed from the vasopressin solution. The mean results from steady-state collection periods in each study are summarized in Table I. It is evident that PGE_1 reduced the response to vasopressin by approximately 50%. This amount of inhibition was observed at two concentrations of PGE_1.

As mentioned earlier, vasopressin is thought to increase the formation and accumulation of C-AMP in the kidney, and C-AMP is believed to be responsible for the alteration in water permeability. Methylxanthines, such as theophylline, mimic vasopressin and C-AMP by interfering with the degradation of the intracellular nucleotide.

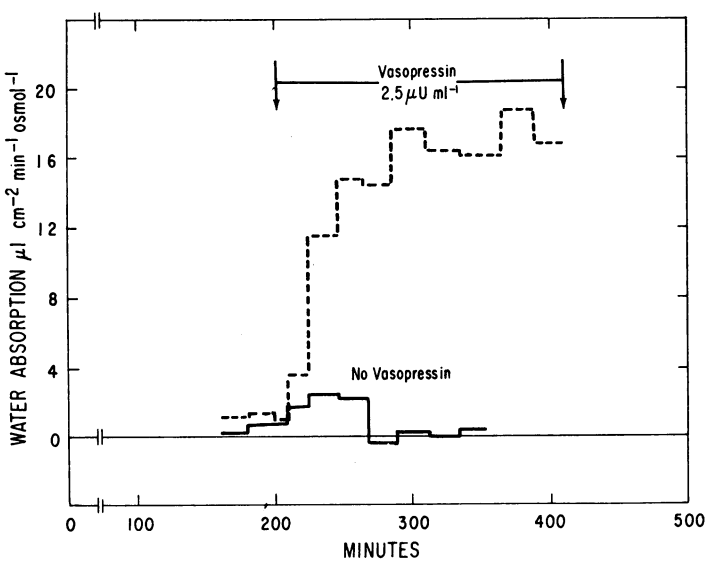

FIGURE 1 Permeability to water of collecting tubules in the steady state preceding and after administration of vasopressin. Results from two tubules are depicted.

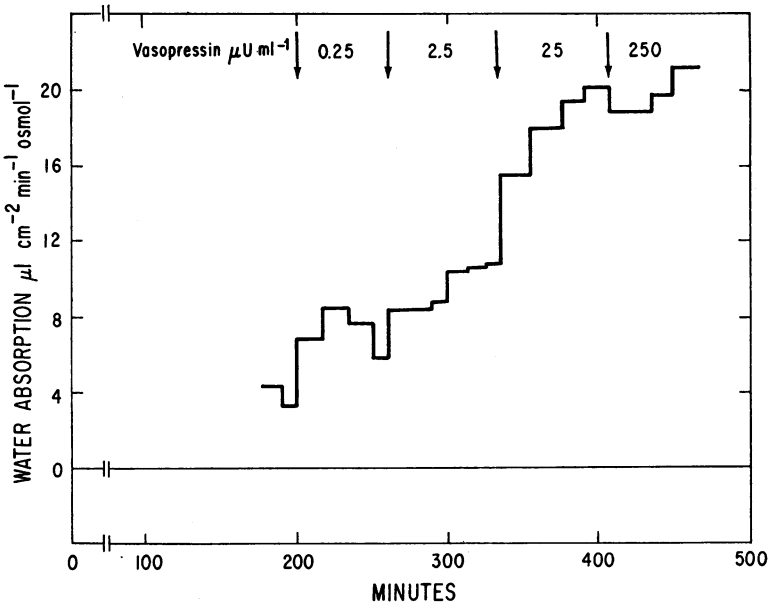

FIGURE 2 Permeability response of a collecting tubule to different concentrations of vasopressin.

Consistent with this view, theophylline, in the present study increased net water absorption, as had been previously demonstrated for vasopressin and C-AMP (7).

The effect of PGE_1 on the theophylline-induced increase in water absorption was tested in the same manner as in the vasopressin studies discussed above. When theophylline was added to an impermeable tubule, water absorption increased, as is shown in Fig. 5. Somewhat surprisingly, when PGE_1 was then added to the solution containing theophylline, water absorption increased further. Selective removal of theophylline from the bathing medium resulted in a sharp decrease in water absorption. The sequence of adding the test agents was reversed and identical results were obtained.

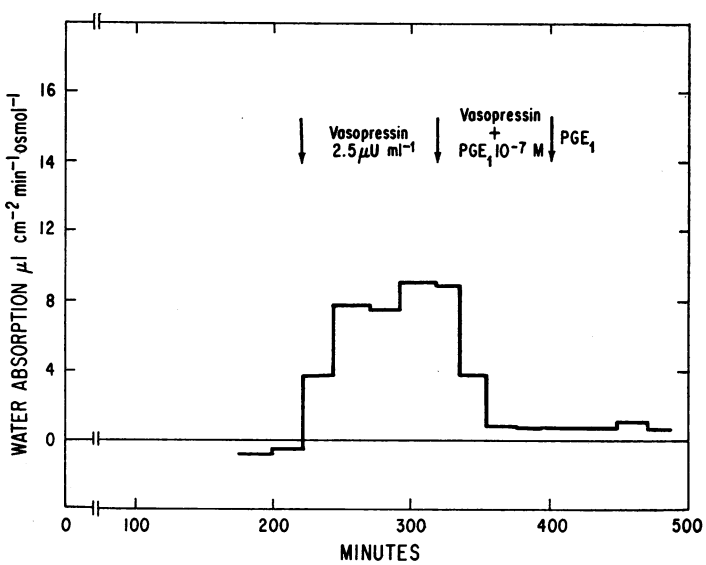

FIGURE 3 Effect of PGE_1 on the permeability response to vasopressin.

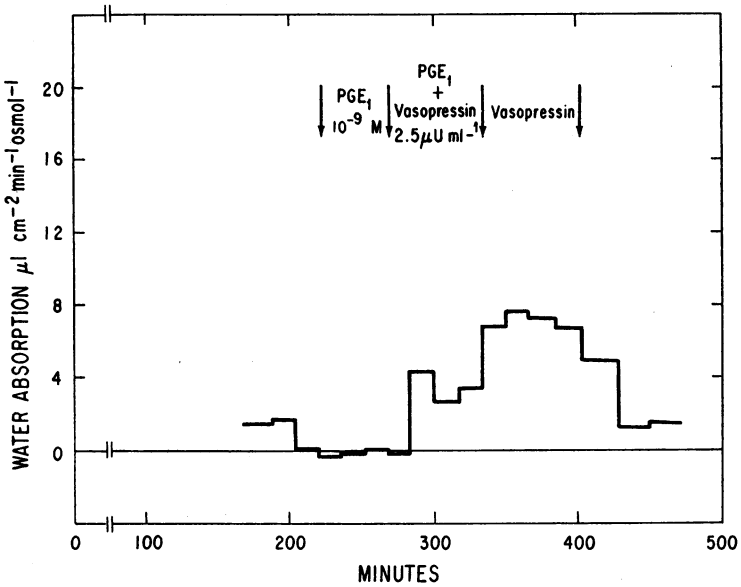

FIGURE 4 Effect of PGE_1 on the permeability response to vasopressin.

All results are summarized in Table II. In seven studies, including one in which 10^{-9} M PGE_1 was used, a combination of PGE_1 and theophylline caused a marked increase in water absorption. This increment was about 55% greater than would be predicted from the additive effect of the two agents.

The effect of PGE_1 on the water permeability response to C-AMP was also tested and the results are listed in Table III. As has been reported previously (7), C-AMP alone caused an increase in water absorption. Although a mean increase in the permeability response to C-AMP was obtained with PGE_1, the effect was small and was not statistically significant.

It should be emphasized that PGE_1 alone

TABLE I
Effect of PGE_1 on Vasopressin-Induced Permeability Response

Experiment	I Control	II Vasopressin, 2.5 μU ml^{-1}	III Vasopressin plus 10^{-7} M PGE_1	II-I	II-III
		μl cm^{-2} min^{-1} osmol^{-1}			
1	3.37	8.86	4.49	5.49	4.37
2	1.61	9.71	8.04	8.10	1.67
3	6.03	19.39	12.57	13.36	6.82
4	4.65	13.26	8.92	8.61	4.34
5	−0.69	8.04	0.79	8.73	7.25
6	1.11	16.47	13.77	15.36	2.70
7	1.00	7.14	3.39*	6.14	3.75
Mean	2.44	11.84	7.42	9.40	4.41
SE				1.38	0.77
P				<0.01	<0.01

Numbers in columns I, II, and III are values of absolute net water absorption, whereas those in the remaining columns represent differences.
* 10^{-9} M PGE_1.

TABLE II
Effect of PGE_1 on Theophylline-Induced Permeability Response

Experiment	I Control	II Theophylline, 5×10^{-3} M	III Theophylline plus 10^{-7} M PGE_1	II-I	II-III
		μl cm^{-2} min^{-1} osmol^{-1}			
8*	2.30	7.53		5.23	
9*	12.95	26.67		13.72	
10	2.02	5.84	10.89	3.82	−5.05
11	0.87	1.45	2.00	0.58	−0.55
12	0	2.67	6.00	2.67	−3.33
13	1.27	2.87	14.64	1.60	−11.77
14	0.42	−0.31	10.55	−0.73	−10.86
15	3.52	4.65	13.18	1.13	−8.53
16	1.30	5.13	14.09‡	3.83	−8.96
Mean	2.74	6.28	10.19	3.54	−7.01
SE				1.42	1.56
P				<0.05	<0.01

* In experiments 8 and 9 the response of single tubules to varying concentrations of theophylline was tested and PGE_1 was not included in the study. Only those results in which 5×10^{-3} M drug was used are included in the table.
‡ 10^{-9} M PGE_1.

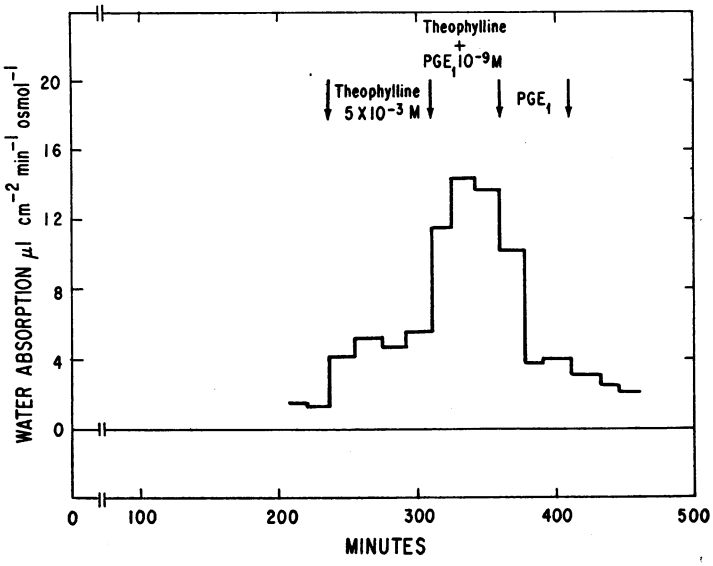

FIGURE 5 Effect of PGE_1 on the permeability response to theophylline.

significantly increases net water absorption in the collecting tubule. As noted in Table IV, PGE_1 (10^{-7} M) increased net water absorption in studies in which PGE_1 was added after the initial control period. Similar conclusions can be derived from the results of studies in which a definite effect of PGE_1 alone was observed following periods of high permeability to water. Net absorption during this period, when only PGE_1 is present, is greater than was net absorption during an after-control period in which no agent was present.

DISCUSSION

It is evident on the basis of these studies that PGE_1 inhibits the hydro-osmotic effect of

TABLE III
Effect of PGE_1 on Cyclic 3',5'-AMP–Induced Permeability Response

Experiment	I Control	II Cyclic AMP, 10^{-3} M	III Cyclic AMP, plus PGE_1 10^{-7} M	II-I	II-III
		µl cm⁻² min⁻¹ osmol⁻¹			
17	0	1.80	2.47	1.80	−0.67
18	1.10	9.25	6.08	8.15	3.17
19	0.36	8.13	9.36	7.77	−1.23
20	2.61	6.31	5.71	3.70	0.60
21	6.57	8.75	11.63	2.18	−2.88
22	0.71	3.31	6.99	2.60	−3.68
Mean	1.89	6.26	7.04	4.37	−0.78
SE				1.17	1.01
P				<0.02	>0.4

TABLE IV
Effect of PGE_1 (10^{-7} M) on Permeability Response

Experiment	I Initial control	II 10^{-7}M PGE_1	III After control	Increase due to PGE_1
		µl cm⁻² min⁻¹ osmol⁻¹		
1	3.37	3.68		0.31
2	1.61	5.58		3.97
3	6.03	7.21		1.18
14	0.42	3.06		2.64
15	3.52	4.97		1.45
21	6.57	8.56		1.99
Mean	3.59	5.51		1.92
SE				0.52
P				<0.02
				(II-III)
13		2.40	1.08	1.32
17		2.06	−0.24	2.30
18		4.27	2.56	1.71
19		5.82	2.56	3.26
20		1.53	1.08	0.45
22		3.90	0	3.90
Mean		3.33	1.17	2.16
SE				0.52
P				<0.01

In experiments 1–3, 14, 15, and 21, PGE_1 was added after the initial control period. In experiments 13, 17–20, and 22 the effect of PGE_1 alone was tested following a period of increased permeability; water absorption measured during the periods in which PGE_1 was in the bath was compared with a control period obtained after the test agent was removed (see Fig. 5).

vasopressin in isolated collecting tubules of the rabbit. The effect is analogous to that previously reported for toad bladder (4). Also, as in the toad bladder studies, no demonstrable alteration in the response of the collecting tubule to C-AMP was noted with PGE_1. Several important differences between the results of the two studies exist, however. Thus, although PGE_1 of itself does not alter the permeability of the toad bladder to water, it causes a significant, albeit small, increase in permeability of the collecting tubule. Furthermore, under certain circumstances, PGE_1 interferes with the hydro-osmotic effect of theophylline in toad bladder. This is not the case with respect to the collecting tubule. In this tissue a striking enhancement of the permeability response to theophylline is observed after addition of PGE_1 to the bathing medium. In view of the two latter sets of findings, one cannot conclude that PGE_1 exerts its inhibitory effect on the action of vasopressin in the collecting tubule merely by diminishing the activity of adenyl cyclase, the enzyme system responsible for the conversion of ATP to C-AMP, as was suggested for toad bladder. Rather, the results are consistent with the hypothesis that PGE_1 and vasopressin both stimulate adenyl cyclase activity and, in addition, compete with one another for a common receptor, presumably the adenyl-cyclase system.[2] Viewed within the context of this thesis, PGE_1 alone increases the permeability to water of the collecting tubule by increasing the concentration of C-AMP in the tissue, but when administered in combination with vasopressin, PGE_1 prevents the interaction of vasopressin with the receptor and thereby blunts the "normal" response to the octapeptide. It is pertinent to note that Butcher and Sutherland[3] have obtained direct evidence that PGE_1 interferes with the vasopressin-induced accumulation of C-AMP in rat renal tissue. The stimulatory effect of PGE_1 also accounts for the potentiation of the effect of theophylline, since the latter agent acts by preventing the degradation of C-AMP, and in this manner permits accumulation of the active nucleotide in the tissue. Butcher, Pike, and Sutherland (3) have reported that PGE_1 potentiates the effect of another methylxanthine, caffeine, on C-AMP accumulation in rat epididymal fat pads. In addition, Butcher and Sutherland (11) have observed an increase in the concentration of C-AMP with PGE_1 alone in adipose tissue and, further, that when given in conjunction with epinephrine, PGE_1 prevents the marked increase in tissue C-AMP concentration usually produced by the catecholamine.

The physiological role of prostaglandin in the kidney is uncertain. Lee, Covino, Takman, and Smith (12) consider that medullin, obtained from an extract of renal tissue, is a prostaglandin and that it may be an important antihypertensive agent. The possible role of prostaglandin as an intracellular modulator of the antidiuretic effect of vasopressin has been discussed briefly in a previous publication from our laboratory (4). At present, we favor the view that prostaglandin may serve to dampen the alterations in permeability of the collecting duct which would result from small changes in the concentration of vasopressin and, in this manner, prevent the wide overshoots in permeability which might otherwise occur. The collecting tubule of the rabbit is extraordinarily sensitive to vasopressin. It responds to as little as 0.25 μU ml^{-1} of the octapeptide, a concentration less than that generally assumed to be present in blood during normal states of hydration (13). Given the sensitivity of the tubule and the gross control of the secretory rate of vasopressin achieved by alterations in plasma osmolality, it is unlikely that the osmoreceptor system, even in combination with a degradative system for inactivation and excretion of the octapeptide alone, would be capable of fine regulation of the water permeability of the collecting tubule. An additional inhibitory effect of another naturally occurring substance (prostaglandin) formed within the pertinent effector cell would, as stated above, serve to dampen the effects of residual hormone and modulate changes in water permeability. In this regard, it is of interest that PGE_1 not only prevents the full effect of vasopressin in the collecting tubule, but reverses an established effect of the hormone. Bergstrom (1) has recently discussed the possibility that the prostaglandins act in a

[2] Admittedly critical evidence in support of this hypothesis requires both measurements of adenyl cyclase activity and a Lineweaver-Burke type analysis of the combined effects of vasopressin and PGE_1. These studies are not feasible at the present time.

[3] Butcher, R. W., and E. W. Sutherland. Personal communication.

similar manner to modulate the effect of other hormones on their receptor tissues.

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