Effects of Ca^{++} and Prostaglandin E_1 on Vasopressin Activation of Renal Adenyl Cyclase

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ABSTRACT Adenyl cyclase activity was assayed in crude homogenates of the renal cortex, medulla, and papilla of the golden hamster. The specific activity (moles C-AMP/unit of time per mg protein of tissue) of the enzyme under basal conditions, was greatest in papilla, somewhat lower in medulla, and least in cortex. On an absolute scale, the sensitivity to vasopressin was greater in the medullary and papillary than in the cortical homogenates. In addition, at concentrations of 0.1–1.0 mM, $CaCl_2$ inhibited the enzyme in the order papilla > medulla > cortex. These results imply the existence of distinct differences in the composition of the adenyl cyclase-receptor complex in various parts of the kidney. We proposed that Ca^{++} inhibits the core enzyme directly since at the minimally inhibitory concentration (0.1 mM), $CaCl_2$ reduced to an equivalent extent (*a*) basal activity, (*b*) the response to graded doses of vasopressin (0.5 to 50.0 mU/ml) and (*c*) the response to maximal stimulatory concentrations of NaF (10 mM). Prostaglandin E_1 ($PGE_1 = 10^{-7}$M) had no effect on either basal adenyl-cyclase activity or the response to 10 mM NaF in medullary and papillary homogenates. 7-Oxa-13-prostynoic acid (10^{-4} M) similarly had no effect under basal conditions or on stimulation with NaF in medullary homogenates. Both fatty acids, however, inhibited the enzymic response to vasopressin, particularly at low concentrations of the peptide. The straight-chain fatty acid, 11-eicosanoic acid (10^{-7} M), was inactive on basal activity or on the response to vasopressin. The possibility that PGE_1 modifies the coupling mechanism between the core enzyme and the hormone-specific receptor is discussed.

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INTRODUCTION

Considerable evidence has been adduced that adenosine 3',5'-phosphate (cyclic AMP) is an intracellular mediator in vasopressin regulation of hydroosmotic flow in target epithelia (e.g., anuran bladder, mammalian kidney (1). It is highly probable that the accumulation of 3',5'-cyclic AMP is primarily a result of vasopressin activation of adenyl cyclase. In the kidney, medullary adenyl cyclase is more sensitive than cortical adenyl cyclase to vasopressin, which is in accord with the medullary site of antidiuretic action (2). Two interesting modulators of the renal response to vasopressin have been identified: Ca^{++} and prostaglandin E_1 (PGE_1).[1]

In the toad bladder, an increase in the Ca^{++} concentration of the media impairs the hydroosmotic and urea permeability but not the active Na^+ transport response to submaximal concentrations of vasopressin (3–5). In contrast, elevation of the Ca^{++} concentration had no effect on the cyclic AMP-dependent increase in osmotic water flow, urea permeability, or active Na^+ transport (5, 6). These findings prompted Petersen and Edelman (5) to propose that vasopressin activates two segregated–adenyl cyclase systems, one of which is Ca^{++} sensitive and coupled to the regulation of osmotic water flow. Vasopressin-mediated increase in the osmotic permeability of kidney slices is also inhibited by raising the Ca^{++} concentration of the media to 10 mmoles/liter (7).

PGE_1, a natural product of many tissues including the mammalian kidney, inhibited the hydroosmotic effect of low concentrations of vasopressin in the isolated toad bladder and in the isolated rabbit kidney collecting tubule (8, 9). Cyclic AMP-dependent effects on osmotic water flow, in contrast, were not modulated by PGE_1. In addition, Grantham and Orloff (9) noted that PGE_1 stimulated water flow and potentiated the hydroosmotic

[1] *Abbreviations used in this paper:* Id, 7-oxa-13-prostynoic acid; PGE_1, prostaglandin E_1.

effect of theophylline. On the basis of these results, they suggested that PGE_1 may compete for the vasopressin receptor site.

The availability of a convenient assay for adenyl cyclase activity in broken cell preparations provided the opportunity of using Ca^{++} and PGE_1 to probe more directly the mechanism by which vasopressin stimulates the generation of cyclic AMP in the kidney, and to provide additional information on the mechanisms involved in the action of these agents. Hamsters were used as experimental animals on the assumption that the relatively large mass of kidney, the long papilla, and their ability to form highly concentrated urine, signifies the presence of abundant amounts of renal adenyl cyclase responsive to vasopressin. In agreement with the interpretation of Bär, Hechter, Schwartz, and Walter (10), we use the term "adenyl cyclase system" to denote a three component complex consisting of a hormone-specific receptor, a receptor-enzyme coupling mechanism, and the core enzyme. The results indicate that Ca^{++} inhibited the basal level of adenyl cyclase activity and vasopressin did not overcome this inhibition. In contrast, PGE_1 had no effect on the basal level of enzyme activity but impaired the response to vasopressin.

METHODS

Female hamsters, 100–140 g body weight, were fasted and thirsted for 14 hr before use. Hydration was achieved by injecting distilled water, 5% of the body weight, intraperitoneally followed in 45 min by a second injection of distilled water, 2% of the body weight, under light ether anesthesia. The hamsters were killed 3 hr after the first injection, which corresponded to the peak of the diuretic response. The kidneys were separated into cortex, medulla, and papilla by sharp dissection, and each segment was separately homogenized gently in a glass-teflon pestle homogenizer in 50 mM tris-HCl buffer (pH = 7.4), 1.3 mM mercaptoethanol, and 1.0 mM EGTA.[2] Adenyl cyclase activity was assayed by the method of Krishna, Weiss, and Brodie (11), slightly modified. The incubation medium contained 50 mM tris HCl buffer (pH = 7.4), 4 mM $MgCl_2$, 0.015% bovine serum albumin, 2.3 mM cyclic AMP and 3 mM α-labeled ATP^{32} (specific activity = 2–10 Ci/mM, obtained from International Chemical & Nuclear Corporation). The crude homogenates were added to the incubation mixture in a final volume of 0.065 ml at 37°C, as described (11). Excess quantities of cyclic AMP rather than theophylline were used to minimize losses of cyclic AMP^{32} due to phosphodiesterase activity. In preliminary trials, we found that the velocity of the reaction was linear for 7 min of incubation and with protein content of the flask. The incubations were terminated at 5 min by the addition of 0.2 ml of solution which contained 88 mM ATP, 12 mM cyclic AMP and 83 mμCi ^{3}H-cyclic AMP, and boiling for 3 min. The ^{3}H-cyclic AMP was used to correct for the losses of cyclic AMP^{32} during chromatography. The boiled extracts were diluted, centrifuged, and subjected to ion exchange chromatography in a 4.5 × 0.55 cm column (200–400 mesh, Bio-Rad analytical grade 50W-X8 Bio-Rad Labs). The cyclic AMP eluates were treated with 8% $ZnSO_4$ titrated to pH = 8.5, with saturated $Ba(OH)_2$ as described previously (11). The barium-zinc precipitates were removed by two cycles of centrifugation, 2,500 × *g* for 15 min and the supernatants were recentrifuged at 2,500 × *g* for 10 min. 2 ml portions of the resultant supernatants were added to 15 ml of Bray's solution (12) and assayed for radioactivity by liquid scintillation spectrometry (Nuclear-Chicago Corp.). The protein content of the final supernatants was determined by the method of Lowry, Rosebrough, Farr, and Randall (13).

The test substances, vasopressin (Pitressin, Parke-Davis & Company) and NaF, were added after the incubation medium and crude homogenates were mixed. PGE_1 was made up in 50 mM Tris-HCl (pH = 7.4), 7-oxa-13-prostynoic acid (Id) [supplied by Dr. Josef Fried, University of Chicago] in 50 mM $NaHCO_3$ solution and 11-eicosanoic acid in 50 mM Tris-HCl (pH = 7.4) and added to the incubation medium just before the addition of the homogenates. When PGE_1 and Id were used together, the Id was made up in the incubation buffer, mixed with the enzyme preparation, and the PGE_1 was then added. The Ca^{++} concentrations of the system were varied by preparing incubation buffers with varying amounts of added $CaCl_2$. In all instances, the reactions were started by the addition of α-$AT^{32}P$ after the reaction mixture reached temperature equilibrium in the water bath (37°C). The reactions were all terminated at 5.0 min under stopwatch control.

RESULTS

The differential effects of varying concentrations of vasopressin on adenyl cyclase activity in homogenates of the cortex, medulla, and papilla prepared from hydrated hamsters, are shown in Fig. 1. In vasopressin-free media the basal level of activity, expressed in micromicromoles/milligrams protein per 5 min (mean ±SEM), was highest in papilla (933 ±100), next highest in medulla (653 ±67) and least in cortex (279 ±25). Enzyme activity was increased monotonically in papilla at concentrations of vasopressin from 0.05 to 5.0 mU/ml, in medulla from 0.1 to 10 mU/ml, and in cortex from 0.5 to 10 mU/ml. The smaller increase in activity in cortex compared to medulla and papilla is in accord with earlier findings of Chase and Aurbach (2) and with the well-defined role of the medullary and papillary segments of the nephron in the concentrating process (9). The concentrations of vasopressin required to activate these renal homogenates, however, are orders of magnitude higher than those found in the circulation under physiological conditions. Similarly, Bär et al. (10) found that the peptide concentrations required for half-maximal stimulation of adenyl cyclase activity in the 600 × g fraction of toad bladder epithelial cells was about 100-fold greater than for the half-maximal hydroosmotic effect in intact bladders. These results imply some modification in the properties of the hormone receptor-enzyme complex during the preparation of the tissue homogenates.

The susceptibility of renal adenyl cyclase to Ca^{++} was assayed over a concentration range of 0.05–1.0 mM (Fig. 2). To normalize the data, the results were expressed as per cent of the control value (i.e., in Ca^{++}-free media).

[2] EGTA denotes 1,2-bis-(2-dicarboxymethylamino ethoxy)-ethane.

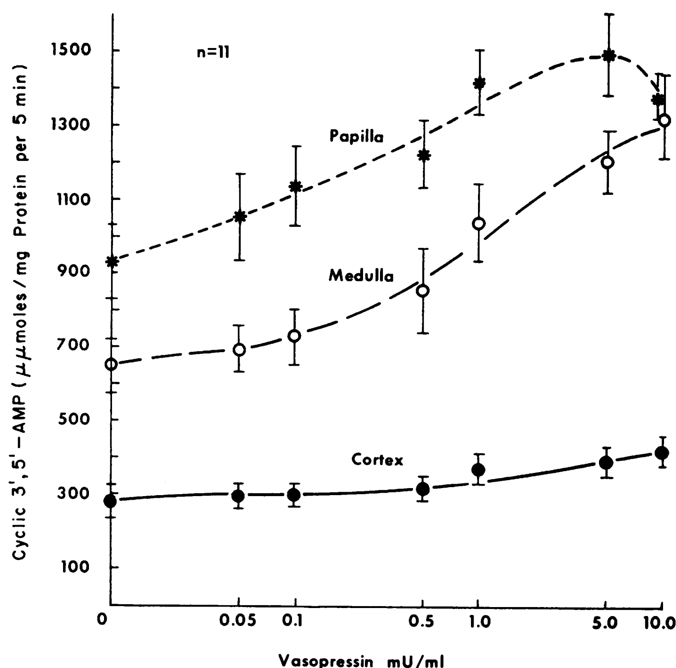

FIGURE 1 Dose-response of renal adenyl cyclase activity to vasopressin. Crude homogenates were prepared from cortex (—●—), medulla, (—○—) and papilla (—*—) of hydrated hamsters. The concentration of vasopressin is displayed on a log scale. Each point is the mean of 11 determinations. The vertical lines correspond to ±1 SEM.

At Ca^{++} concentrations of 0.05–0.3 mM, cortical adenyl cyclase activity did not differ significantly from the control values and at 1.0 mM was reduced only to 81.3 ±2.0%. In contrast, medullary and papillary enzyme activity were significantly inhibited throughout the concentration range tested. The papillary enzyme system was more sensitive to Ca^{++} inhibition than the medullary system. The curvilinear character of the inhibition curves suggests that about 60% of medullary adenyl cyclase and 20% of the papillary enzyme were resistant to Ca^{++} inhibition. Thus at 1.0 mM Ca^{++}, the activities were 56.1 ±6.3% and 16.3 ±4.2% of the control values in medulla and papilla, respectively. Since Ca^{++} inhibition is exerted on basal enzyme activities, these findings suggest that the core enzymes in cortex, medulla, and papilla either differ in structure or in accessibility of the Ca^{++}-sensitive site to the Ca^{++} in the external media. The inhibitory effect of Ca^{++} on adenyl cyclase systems has been well-documented in many mammalian tissues (see Ramwell and Shaw [14] for a summary of these findings).

In view of the relative insensitivity of cortical adenyl cyclase to vasopressin, the remainder of our studies made use only of the medullary and papillary segments. The effect of PGE_1 on medullary and papillary adenyl cyclase activities was assessed at concentration of 10^{-8} to 10^{-5} moles/liter. Enzyme activity in the control- and PGE_1-treated preparations did not differ significantly in either renal segment over this entire concentration range. Accordingly, no evidence was obtained of a direct effect of PGE_1 on the core enzyme or on the associated receptors.

To define the modifying effects of external Ca^{++} on the enzymatic response to vasopressin, we chose a concentration of 0.1 mM $CaCl_2$. At this concentration, Ca^{++} inhibited basal adenyl cyclase by 10%, assuring that the inhibitory effect was present but not at a level that would preclude a response to the hormone. As shown in Fig. 3, 0.1 mM Ca^{++} reduced basal activity by approximately 10% in both medullary and papillary preparations. Addition of the hormone (final concentrations = 0.5, 5.0, or 50.0 mU/ml) resulted in proportionate increases in adenyl cyclase activity with or without added Ca^{++}. The pattern of proportionate increases was present in both medulla and papilla. The depression in adenyl cyclase activity produced by 0.1 mM $CaCl_2$ was statistically significant ($P < 0.05$), at all concentrations of vasopressin in both renal segments. These results are consistent with the view that at low concentrations, Ca^{++} reduced total enzyme activity but that the remainder or uninhibited population responded normally to the agonist. The character of the effect is that of a noncompetitive inhibitor.

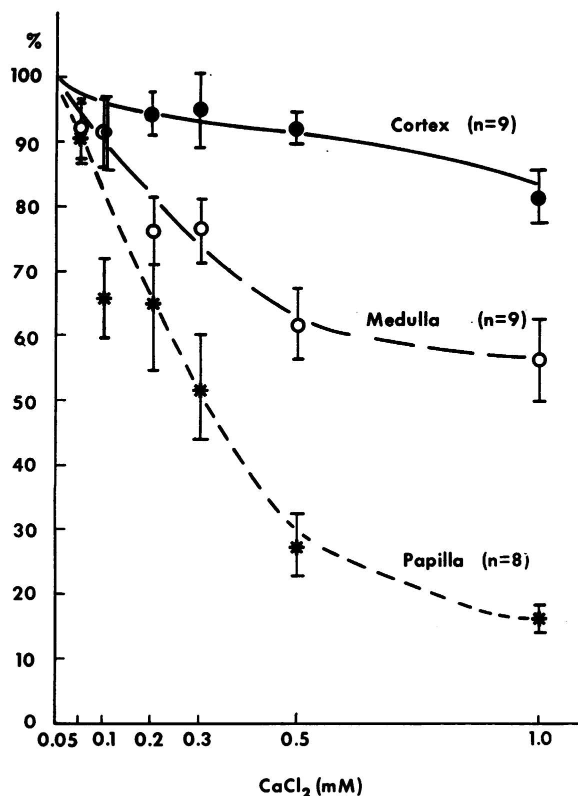

FIGURE 2 Dose-response of renal adenyl cyclase activity to $CaCl_2$. The conventions used in this Figure are described in the legend of Fig. 1. Adenyl cyclase activity is expressed as per cent of the activity in the basal medium which contained no added Ca^{++}. In these preparations, basal adenyl cyclase activity was 279 ±25, 653 ±67, and 993 ±100 $\mu\mu$moles/mg protein per 5 min in cortex, medulla, and papilla, respectively.

The pattern of response to PGE_1 differed from that seen with Ca^{++}. At 10^{-7} M, PGE_1 had no effect on basal enzyme activity; in paired studies the activity was 103 ±3.5% of the control value in medulla and 99.9 ±2.3% in papilla. These are shown as intercept values in Fig. 3. At each dosage level, however, the stimulatory effect of vasopressin was significantly reduced in both medulla and papilla ($P < 0.05$). In papillary segments, the response to vasopressin was almost flat over the full concentration range (i.e., 0.5–50.0 mU/ml) and the maximum per cent inhibition by PGE_1 was seen at a vasopressin concentration of 5.0 mU/ml. The pattern was somewhat different in medulla in which there was a minimal response to 0.5 mU/ml of vasopressin but a proportionate increase in adenyl cyclase activity in the control and PGE_1-treated segments at concentrations of 5.0 and 50.0 mU/ml. In the medulla, therefore, the relative degree of inhibition was greatest at a peptide concentration of 0.5 mU/ml. In both the medulla and papilla, however, these results suggest that PGE_1 impaired the activation of the enzyme by the hormone-receptor complex but did not inhibit the enzyme directly.

Sodium fluoride activation of adenyl cyclase activity exceeds that of the known physiological agents (e.g., epinephrine, glucagon, ACTH), does not require the presence of any regulatory intermediates and preempts the response mechanism so that no further effect is achieved by the subsequent addition of a regulatory hormone (15). These results imply direct activation of the enzyme proper and that NaF may be an index of the total quantity of enzyme available for stimulation. The results we obtained with 0.1 mM Ca^{++} and 10^{-7} M PGE_1 suggested that Ca^{++} acted on the enzyme and PGE_1 on the interaction between enzyme and receptor. These

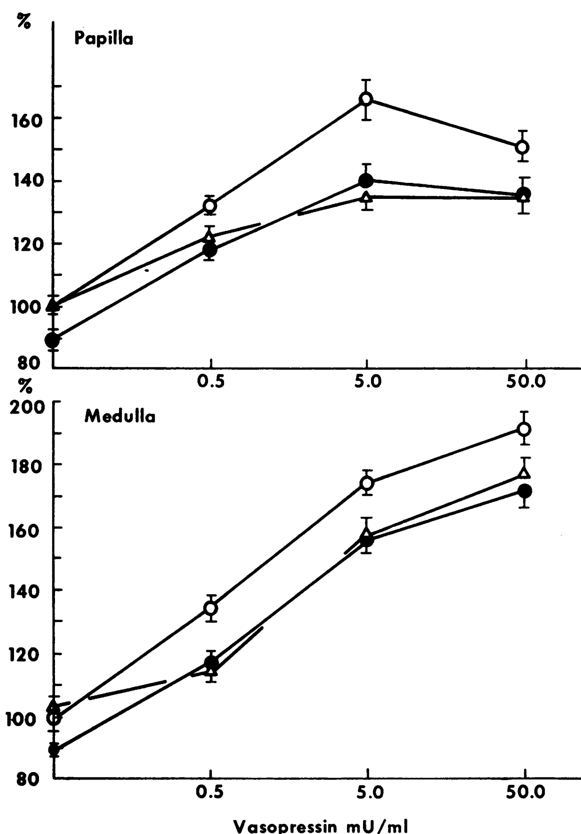

FIGURE 3 The effects of $CaCl_2$ and PGE_1 on the dose-response of renal adenyl cyclase activity to vasopressin. Adenyl cyclase activity is expressed as per cent of the activity in the basal medium which contained no added vasopressin, $CaCl_2$ or PGE_1. The response to vasopressin without added $CaCl_2$ or PGE_1 is denoted by open circles (—○—), with $CaCl_2$ (0.1 mM) by solid circles (—●—) and with PGE_1 (10^{-7} M) by open triangles (—△—). The upper half of the Figure shows the response of papillary homogenates and the lower half that of medullary homogenates. Basal adenyl cyclase activity, in these preparations, was 351 ±29 and 893 ±41 $\mu\mu$moles/mg protein per 5 min in medulla and papilla, respectively. Each point is the mean of 14–22 determinations.

inferences prompted us to determine the effects of Ca^{++} and PGE_1 on NaF activation of the adenyl cyclase enzyme system in crude homogenates of the renal medulla. The results in Fig. 4 indicate that 10 mM NaF stimulated activity by 381 ±23% and that 10^{-7} M PGE_1 had no significant effect on this response (i.e., $PGE_1 = 404$ ±25%). In the presence of 0.1 mM $CaCl_2$, however, NaF stimulation was reduced to 316 ±31% and the difference from the Ca^{++}free value was statistically significant. On a paired basis the ratio of activity with NaF and Ca^{++} to that of NaF alone was 0.79 ±0.06 ($P < 0.005$). At this concentration, Ca^{++} reduced total fluoride-sensitive enzyme activity by an amount similar to the reduction in basal activity which, as noted above, was about 10% (cf Figs. 2–4).

Fried et al. (16) recently reported the synthesis of a series of analogues of the prostaglandins. One of these analogues, Id, antagonized PGE_1 activity in a number of intestinal tract preparations.

The effect of the analogue, Id, was tested on vasopressin stimulation of medullary adenyl cyclase activity. In paired portions (seven experiments) addition of vasopressin at a concentration of 0.5 mU/ml increased the relative adenyl cyclase activity to 140 ±7%. Simultaneous addition of PGE_1 at a concentration of 10^{-7} M reduced the vasopressin-induced increase to 125 ±7%. A similar effect was exerted by Id; at a concentration of 10^{-7} M, the vasopressin-induced increase in adenyl cyclase activity was 128 ±7% of the untreated control. These results indicate that PGE_1 and Id inhibited vasopressin activation of the enzyme to an equivalent extent and were significant at the 5% confidence limit. To test the potency of Id at higher concentrations, studies were carried out at a concentration of 10^{-4} M. The analogue at a concentration of 10^{-4} M alone had no effect on basal enzyme activity nor did the combination of PGE_1 (10^{-7} M) and Id (10^{-4} M) have a significant effect (Fig. 5). The relative activities were 102 ±2% for Id and 101 ±3% for PGE_1 + Id. PGE_1 (10^{-7} M) had no effect on basal activity and depressed vasopressin (0.5 mU/ml) stimulation of activity, significantly. Thus, adenyl cyclase activity was 134 ±3% of basal levels with vasopressin alone and 117 ±3% with added PGE_1; a 50% inhibition of hormonal stimulation ($P < 0.001$). The analogue inhibited vasopressin activation to a slightly greater degree than PGE_1; the activity was 112 ±3% of the basal level ($P < 0.001$). In this system, therefore, Id at high concentration behaved as a prostaglandin-like agent, in antagonizing the action of vasopressin. This finding raises the issue of the specificity of the PGE_1 effect, which might be a common property of any long chain, unsaturated fatty acid. To test this possibility, we used equimolar concentrations (10^{-7} M) of 11-eicosanoic acid, as a potential antagonist

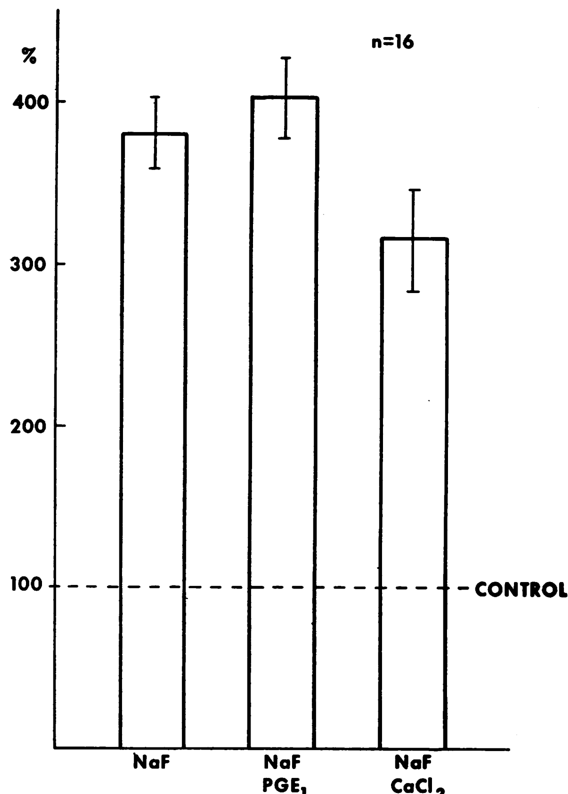

FIGURE 4 The effects of PGE_1 and $CaCl_2$ on the response of renal medullary adenyl cyclase activity to NaF. Adenyl cyclase activity is expressed as per cent of the activity in the basal medium which contained no added NaF, PGE_1, or $CaCl_2$. Basal adenyl cyclase activity was 215 ±23 μμmoles/mg protein per 5 min, in these preparations. The concentration of the reagents in the enzyme assays was NaF = 10 mM, $PGE_1 = 10^{-7}$M, and $CaCl_2$ = 0.1 mM. Basal activity in indicated by the dashed line. The mean of the relative activity is given by the height of the bars and ±1 SEM by the lengths of the vertical lines.

of the action of vasopressin. This fatty acid was chosen as a reference, "nonspecific" compound since it is closely related to the precursor of PGE_1, 8, 11, 14-eicosatrienoic acid (14). The results summarized in Table I indicate that 11-eicosanoic acid did not inhibit the response to 0.5 mU/ml of vasopressin. The ability of PGE_1 to antagonize vasopressin stimulation of adenyl cyclase activity, therefore, probably is not a result of a nonspecific fatty acid effect.

DISCUSSION

Evidence of heterogeneity in receptor–adenyl cyclase complexes, in the sense of hormone–specific responsiveness has been increasingly apparent in recent studies. The existence of segregated adenyl cyclase systems in toad bladder epithelium was inferred from the differential effects of Ca^{++} on the hydroosmotic and Na^{+} trans-

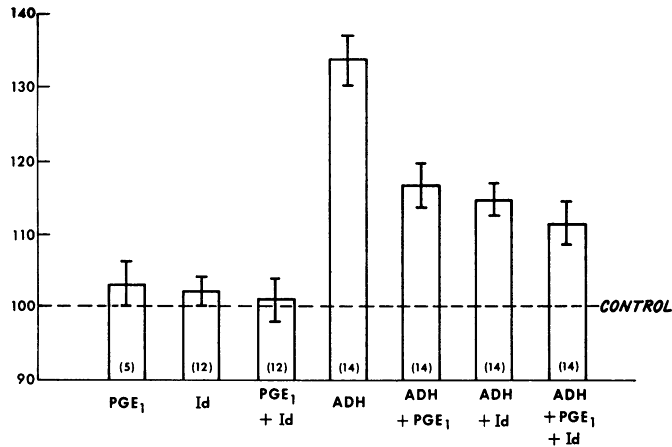

FIGURE 5 The effects of PGE_1 and Id on the response of renal medullary adenyl cyclase activity to vasopressin. The conventions used in this Figure are given in the legend of Fig. 4. Basal adenyl cyclase activity was 235 ±6 μμmoles/mg protein per 5 min. The concentrations of the reagents in the enzyme assays were vasopressin = 0.5 mU/ml, $PGE_1 = 10^{-7}$ M, Id = 10^{-4} M. The number of determinations in each group of assays is shown in parentheses.

port responses to vasopressin (5). At concentrations of 10 mM, Ca^{++} blocked only the hydroosmotic effect of vasopressin and had no inhibiting action on the response to cyclic AMP (3–6). The findings of Bourguet and Maetz (17) of differential effects of oxytocin and vasopressin on osmotic water flow and Na^+ transport across the frog bladder support this inference. Examples of hormone–specific adenyl cyclase systems in direct assays have been described in mammalian heart and liver (18, 19).

Chase, Aurbach, and Melson (2, 20) discovered distinctive adenyl cyclase systems in broken cell and isolated tubule preparations of the rat kidney. They found that vasopressin stimulated the medullary system to a greater extent than the cortical system. In contrast, parathyroid hormone activated the cortical system to a greater extent than the medullary system. As shown in Fig. 1, the specific activities of the adenyl cyclase systems in the hamster kidney were in the order papilla > medulla > cortex and the absolute increase, at each dose level, in response to vasopressin was greater in the papilla and medulla than in the cortex. In different groups of rats, assayed at separate times of the years, the absolute basal enzyme levels in any one segment of the kidney varied widely, although within each group the reproducibility was high (cf. Fig. 1 and Table I).

TABLE I
*The Effect of 11-Eicosanoic Acid on Vasopressin-Stimulation of Medullary Adenyl Cyclase**

	Control	ADH/control	ADH + EA/ control
	μμmoles/mg protein per 5 min	*Ratios*	
Mean ±SEM	318 ±36	1.44 ±0.06	1.47 ±0.06

* ADH denotes the addition of 0.5 mU/ml of vasopressin to the incubation medium and EA denotes the simultaneous addition of 10^{-7} M eicosanoic acid. Mean ±SEM. N = 8 simultaneous control, ADH and ADH + EA experiments.

In previous studies, Ca^{++} inhibited adenyl cyclase activity in cell fractions although Ca^{++} excitation has been reported in liver plasma membrane fractions (14). Our findings suggest that the inhibitory effect is a result of an action on the core enzyme since, even at minimal levels of inhibition (i.e. 10–20%), the per cent inhibition remained relatively constant at all levels of stimulation with vasopressin. An equivalent relative decrease in activity was also found under conditions of maximal stimulation with NaF (Fig. 4). The differential effects of Ca^{++} on the various segments of the kidney reinforce the concept that the adenyl cyclase systems in the inner and outer segments of the kidney are distinctive, presumably because of differences in molecular architecture. The striking inhibitory effects of Ca^{++}, at concentrations of 1 mM and less in these broken cell preparations, may reflect a different site of action than in the intact toad bladder. In the latter system, Ca^{++} concentrations in excess of 5 mM were required to inhibit the hydroosmotic effects of low concentrations of vasopressin and inhibition was completely reversed at high concentrations of vasopressin (3, 5, 6).

PGE_1 has been found to have a variety of effects on cyclic AMP formation, depending on the target tissue and the hormone involved in the regulation of that

target. The diversity of PGE_1 effects have been described extensively by Ramwell and Shaw (14). With respect to the regulatory properties of vasopressin, two systems have been exploited, the toad bladder and mammalian kidney. In the toad bladder, PGE_1 inhibited the hydroosmotic effects of both vasopressin and theophylline: Orloff, Handler, and Bergstrom (8) and Eggena, Schwartz, and Walter (21) concluded that PGE_1 inhibited the enzyme noncompetitively in the toad bladder epithelial cell system. Although the response to vasopressin in the isolated rabbit-collecting tubule is also inhibited by PGE_1, the theophylline effect was enhanced considerably (9). Grantham and Orloff (9) suggested that PGE_1 and vasopressin compete for a common receptor in the rabbit tubule adenyl cyclase system. Butcher and Sutherland[3] were cited as finding that PGE_1 reduced the vasopressin-dependent increase in cyclic AMP in the rat kidney. It is apparent that there is a confusing multiplicity in the actions of PGE_1, which may behave as an agonist, antagonist, or synergist, depending on the system (14). It may also inhibit competitively in one system and noncompetitively in another. An explanation of these diverse effects can be constructed by reference to the presumed three component system of: (*a*) core enzyme, (*b*) coupling site or region, and (*c*) the hormone-specific receptor. If the coupling region is hydrophobic in character, the prostaglandins could have affinity for this region. The character of its effects would then depend on interactions with both the core enzyme and the receptor and would allow for considerable diversity in effects. Our results indicate no stimulation or inhibition of the core enzyme but significant inhibition by FGE_1 of the response of the enzyme to vasopressin, especially at low peptide concentrations, in the medullary and papillary crude homogenates. In the medullary homogenates. Id simulated the PGE_1 effect. Thus Id was not a PGE_1 antagonist as observed by Fried et al. (16) in smooth muscle and by Kuehl, Humes, Tarnoff, Cirillo, and Ham (22) in ovaries.

To our knowledge, there have been no previous reports of PGE_1 inhibition of hormone-dependent stimulation of an adenyl cyclase system in cell-free preparations. Bär et al. (10), for example, found no effect of PGE_1 on vasopressin stimulation of adenyl cyclase activity in the 600 $\times$ g fraction of toad bladder epithelium. Our use of unpurified crude homogenates may account for preservation of the sensitivity to PGE_1. However, in view of the high concentrations of vasopressin required to activate these homogenates and difficulties of preserving sensitivity to PGE_1, the broken-cell preparations may well be defective in crucial molecular components. Thus extrapolation of these results to intact cell systems must be viewed with caution.

[3] Butcher, R. W., and E. W. Sutherland. Cited as a personal communication by Grantham and Orloff (9).

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REFERENCES

1. Orloff, J., and J. S. Handler. 1964. The cellular mode of action of antidiuretic hormone. *Amer. J. Med.* **36:** 686.
2. Chase, L. R., and G. D. Aurbach. 1968. Renal adenyl cyclase: anatomically separate sites for parathyroid hormone and vasopressin. *Science (Washington).* **159:** 545.
3. Bentley, P. J. 1959. The effects of ionic changes on water transfer across the isolated urinary bladder of the isolated toad, *Bufo marinus. J. Endocrinol.* **18:** 327.
4. Bentley, P. J. 1960. The effects of vasopressin on the short-circuit current across the wall of the isolated bladder of the toad, *Bufo marinus. J. Endocrinol.* **21:** 161.
5. Petersen, M. J., and I. S. Edelman. 1964. Calcium inhibition of the action of vasopressin on the urinary bladder of the toad. *J. Clin. Invest.* **43:** 583.
6. Argy, W. P., Jr., J. S. Handler, and J. Orloff. 1967. Ca^{++} and Mg^{++} effects on toad bladder response to cyclic AMP, theophylline, and ADH analogues. *Amer. J. Physiol.* **213:** 803.
7. Whittembury, G., N. Sugino, and A. K. Solomon. 1960. Effect of antidiuretic hormone and calcium on the equivalent pore radius of kidney slices from *Necturus. Nature (London).* **187:** 699.
8. Orloff, J., J. S. Handler, and S. Bergstrom. 1965. Effect of prostaglandin (PGE_1) on the permeability response of toad bladder to vasopressin, theophylline and adenosine 3′,5′-monophosphate. *Nature (London).* **205:** 397.
9. 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.
10. Bär, H.-P., O. Hechter, I. L. Schwartz, and R. Walter. 1970. Neurohypophyseal hormone-sensitive adenyl cyclase of toad urinary bladder. *Proc. Nat. Acad. Sci. U. S. A.* **67:** 7.
11. Krishna, G., B. Weiss, and B. B. Brodie. 1968. A simple, sensitive method for the assay of adenyl cyclase. *J. Pharmacol. Exp. Ther.* **163:** 379.
12. Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solution in a liquid scintillation counter. *Anal. Biochem.* **1:** 279.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 265.
14. Ramwell, P. W., and J. E. Shaw. 1970. Biological significance of the prostaglandins. *Recent Progr. Hormone Res.* **26:** 139.
15. Rodbell, M., A. B. Jones, G. E. Chiappe de Cingolani, and L. Birnbaumer. 1968. The actions of insulin and catabolic hormones on the plasma membrane of the fat cells. *Recent Progr. Hormone Res.* **24:** 215.

16. Fried, J., T. S. Santhanakrishnan, J. Himizu, C. H. Lin, S. H. Ford, B. Rubin, and E. O. Grigas. 1969. Prostaglandin antagonists: Synthesis and smooth muscle activity. *Nature* (*London*). **223**: 208.
17. Bourguet, J., and J. Maetz. 1961. Arguments en faveur de l'indépendance des mécanismes d'action de divers peptides neurohypophysaires sur le flux osmotique d'eau et sur le transport actif de sodium au sein d'un même récepteur; études sur la vessie et la peau de rana esculenta. *Biochim. Biophys. Acta.* **52**: 552.
18. Levey, G. S., and S. E. Epstein. 1969. Myocardial adenyl cyclase: Activation by thyroid hormones and evidence for two adenyl cyclase systems. *J. Clin. Invest.* **48**: 1663.
19. Reik, L., G. L. Petzold, J. A. Higgins, P. Greengard, and R. J. Barrnett. 1970. Hormone-sensitive adenyl cyclase: cytochemical localization in rat liver. *Science* (*Washington*). **168**: 382.
20. Melson, G. L., L. R. Chase, and G. D. Aurbach. 1970. Parathyroid hormone-sensitive adenyl cyclase in isolated renal tubules. *Endocrinology.* **86**: 511.
21. Eggena, P., I. L. Schwartz, and R. Walter. 1970. Threshold and receptor reserve in the action of neurohypophyseal peptides. *J. Gen. Physiol.* **56**: 250.
22. Kuehl, F. A., Jr., J. L. Humes, J. Tarnoff, V. J. Cirillo, and E. A. Ham. 1970. Prostaglandin receptor site: Evidence for an essential role in the action of luteinizing hormone. *Science* (*Washington*). **169**: 883.