

# Effects of Vasopressin and Prostaglandin E<sub>1</sub> on the Adenyl Cyclase—Cyclic 3',5'-Adenosine Monophosphate System of the Renal Medulla of the Rat

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**ABSTRACT** Vasopressin increased adenyl cyclase activity in homogenates of both inner and outer renal medulla of the rat. It also increased the concentration of cyclic 3',5'-adenosine monophosphate (AMP) in slices of both inner and outer medulla but not in renal cortex. In the inner medulla, a concentration of prostaglandin E<sub>1</sub> (PGE<sub>1</sub>), which was ineffective by itself significantly reduced the stimulation of adenyl cyclase activity and cyclic AMP concentration induced by vasopressin. These results are consistent with the hypothesis that PGE<sub>1</sub> can compete with vasopressin for adenyl cyclase-binding sites. However, the findings in the outer medulla suggest the situation is more complex. Although 10<sup>-8</sup> M PGE<sub>1</sub> had no effect by itself and inhibited the vasopressin-induced elevation of cyclic AMP, larger amounts of PGE<sub>1</sub> increased both adenyl cyclase activity and cyclic AMP levels. The maximum effect on the latter parameter was at least 6 times as great as that of maximum amounts of vasopressin.

## INTRODUCTION

It has been proposed that cyclic 3',5'-adenosine monophosphate (cyclic AMP)<sup>1</sup> is the intracellular mediator of the physiological effects of vasopressin. This suggestion, first made by Orloff and Handler (1), has received considerable experimental support. Vasopressin raises the concentration of cyclic AMP in the toad bladder (2) and increases the activity of adenyl cyclase in homogenates of the medulla of the rat kidney (3).

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<sup>1</sup>Abbreviations used in this paper: cyclic AMP, cyclic 3',5'-adenosine monophosphate; PGE<sub>1</sub>, prostaglandin E<sub>1</sub>.

Furthermore cyclic AMP, like vasopressin, increases water permeability and active sodium transport in the urinary bladder of the toad (1) and increases water permeability of the isolated perfused collecting duct of the rabbit (4).

Grantham and Orloff (5) observed that prostaglandin E<sub>1</sub> (PGE<sub>1</sub>), by itself, increases the permeability of perfused collecting ducts to water but inhibits the effects of submaximal amounts of vasopressin. Although the effects of theophylline and PGE<sub>1</sub> are additive, PGE<sub>1</sub> does not influence the cyclic AMP-induced increases in water permeability of the collecting duct. To explain these observations, Grantham and Orloff proposed that PGE<sub>1</sub> stimulated adenyl cyclase activity and increased production of cyclic AMP. Furthermore, by competing with vasopressin for receptors which influence adenyl cyclase, but being a less potent activator than vasopressin, PGE<sub>1</sub> decreased vasopressin-mediated increases in cyclic AMP production. This finding and this hypothesis were in general agreement with Bergstrom's proposal that prostaglandins might act as modulators of the adenyl cyclase reaction (6). The present investigation was undertaken to evaluate further this concept by measuring the effects of vasopressin and PGE<sub>1</sub>, singly and in combination, on the concentration of cyclic AMP and on adenyl cyclase activity in the renal medulla.

## METHODS

Male Sprague-Dawley rats weighing 140–250 g were sacrificed by decapitation. Tissue slices weighing 10–75 mg were prepared from outer red medulla, inner white medulla, or cortex using a Stadie-Riggs microtome. Slices were incubated for 15 min at 37°C in 2 ml Krebs-Ringer bicarbonate buffer containing 0.5 mg/ml glucose, 2 mg/ml albu-

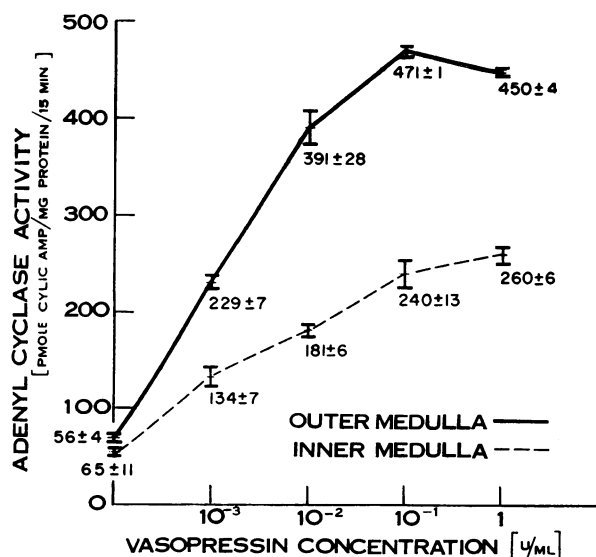


FIGURE 1 Represents a dose-response relationship between concentration of vasopressin on the ordinate and adenylyl cyclase activity on the abscissa. The outer medulla is the solid line, and the broken line is the inner medulla.

min, and 10 mM theophylline. The gas phase was 95% O<sub>2</sub> and 5% CO<sub>2</sub>. PGE<sub>1</sub>, vasopressin, or both were added to the appropriate flasks. 1 mg of PGE<sub>1</sub> was dissolved in 0.1 ml of absolute ethanol. To this was added 0.9 ml Na<sub>2</sub>CO<sub>3</sub> (0.2 mg/ml, pH 7). This stock solution was diluted with Krebs-Ringer bicarbonate buffer to give the appropriate concentration in the flask. The final concentration of ethanol never exceeded 0.1% (v/v). This diluent had no effect on cyclic AMP concentration in medulla, control 5.5 ± 0.5 (SE) nmoles/g wet tissue and diluent 5.8 ± 1.3. The final concentration of ethanol in the incubation flask varied between 0.1 and 0.001% (v/v). Bovine arginine-vasopressin in aqueous solution was used. The potency units referred to in the text are international pressor units (USP). A molar solution of vasopressin would be the equivalent of 433.6 U/ml. The concentration of the test substance is indicated in the Results section. The incubation was terminated by homogenizing the slices in cold 5% trichloroacetic acid. Cyclic AMP was extracted by the method of Krishna, Weiss, and Brodie (7) and assayed by the method of

Kaneko and Field (8). When adenylyl cyclase activity was assayed, the inner white or outer red medulla was separated and homogenized separately in 2 M sucrose-0.05 M Tris buffer, pH 7.4. After centrifugation at 4°C at 2000 *g* for 15 min the precipitate was resuspended in an equal volume of 0.05 M Tris and centrifuged again at the same speed and for the same period of time. Adenylyl cyclase activity of the final precipitate was measured by the method of Chase and Aurbach (3). The protein concentration of the preparation was determined by the Lowry, Rosebrough, Farr, and Randall technique (9).

## RESULTS

**Adenylyl cyclase activity.** Vasopressin increased adenylyl cyclase activity in both inner and outer medulla. Fig. 1 demonstrates the dose-response relationships between vasopressin concentration and adenylyl cyclase activity in both the inner and outer medulla. In both significant increases were produced by 10<sup>-3</sup> U/ml and 10<sup>-1</sup> U/ml gave a maximal response.

PGE<sub>1</sub> in concentrations as high as 10<sup>-4</sup> M had no effect on adenylyl cyclase activity in inner medulla but caused a significant increase in activity in the outer medulla (Table I). In the inner medulla, the addition of 10<sup>-4</sup> M PGE<sub>1</sub>, which by itself had no effect on adenylyl cyclase activity, together with a submaximal concentration of 10<sup>-3</sup> U/ml vasopressin, resulted in less of an increase in adenylyl cyclase activity than was observed with that amount of vasopressin alone (Table I). The mean difference in 22 paired incubations between vasopressin alone and the combination was 14.0 ± 5.0 (SE) pmoles cyclic AMP/mg protein per 15 min (*P* < 0.01). Also noted in Table I is the fact that 10<sup>-4</sup> M PGE<sub>1</sub> had no effect on concentrations of vasopressin as high as 5 × 10<sup>2</sup> U/ml. 10<sup>-4</sup> M PGE<sub>1</sub> had no apparent effect on vasopressin, 10<sup>-3</sup> U/ml, in the outer medulla. In a separate group of experiments in the outer medulla, control adenylyl cyclase activity was 24.8 ± 3.9 SE. Vasopressin 10<sup>-3</sup> U/ml increased this value to 46.6 ± 4.4 (*P* < 0.01), but 10<sup>-8</sup> M PGE<sub>1</sub> had no measurable effect 25.4 ± 4.1. The adenylyl cyclase activity with the com-

TABLE I  
Effects of Vasopressin and PGE<sub>1</sub> on Adenylyl Cyclase Activity (picomole Cyclic AMP/milligram Protein per 15 min)

| Media   | Inner medulla | Outer medulla |
|---|---------------|---------------|
| Control   | 83 ± 7 (21)   | 71 ± 2 (10)   |
| PGE <sub>1</sub> , 10 <sup>-4</sup> M   | 87 ± 6 (21)   | 104 ± 7 (10)  |
| Vasopressin, 10 <sup>-3</sup> U/ml  | 134 ± 7 (22)* | 229 ± 7 (10)  |
| Vasopressin, 10 <sup>-3</sup> U/ml, and PGE <sub>1</sub> 10 <sup>-4</sup> M       | 120 ± 7 (22)* | 254 ± 18 (10) |
| Vasopressin, 5 × 10 <sup>-2</sup> U/ml  | 323 ± 9 (13)  |               |
| Vasopressin, 5 × 10 <sup>-2</sup> U/ml, and PGE <sub>1</sub> , 10 <sup>-4</sup> M | 327 ± 10 (13) |               |

Values are the mean ± SE. The number of experiments are represented in the parentheses.

\* The mean difference of 14 ± 5 (*P* < 0.01).

bination of vasopressin  $10^{-8}$  U/ml and  $10^{-8}$  M  $PGE_1$ ,  $53.9 \pm 3.6$ , was not measurably different than vasopressin alone.

**Cyclic AMP concentration.** Vasopressin increased cyclic AMP in both inner and outer medulla, and there was a dose-response relationship between the amount of vasopressin added and cyclic AMP concentration in both (Figs. 2 and 3). As with adenylyl cyclase activity, vasopressin  $10^{-8}$  U/ml appeared to have submaximal effect in both inner and outer medulla. In the inner medulla  $PGE_1$  in concentrations as high as  $10^{-4}$  M had no measurable effect on cyclic AMP concentration (Table II). In the outer medulla  $PGE_1$  increased cyclic AMP concentration, and as is apparent in Fig. 4 there was a dose-response relationship between the amount of  $PGE_1$  added and the measured concentration of cyclic AMP.  $10^{-7}$  M  $PGE_1$  was the lowest concentration noted to effect tissue cyclic AMP concentration. The highest concentration of  $PGE_1$  tested ( $10^{-8}$  M) raised tissue cyclic AMP concentration to  $131.0 \pm 10.8$ , a value over 6-fold the maximum amount measured with vasopressin in the outer medulla.

In the inner medulla  $10^{-6}$  M  $PGE_1$ , which alone had no effect on cyclic AMP concentration, decreased the effect of submaximal vasopressin,  $10^{-8}$  U/ml, to increase tissue cyclic AMP concentration, but had no effect on concentrations of vasopressin as high as  $5 \times 10^{-2}$  U/ml (Table III). The combination of  $10^{-6}$  M  $PGE_1$ , which by itself increased cyclic AMP concentration in the outer medulla, and vasopressin  $10^{-8}$  U/ml increased cyclic AMP concentration more than vasopressin alone. However,  $10^{-8}$  M  $PGE_1$ , which by itself is ineffective, had an inhibitory effect on vasopressin,  $10^{-8}$  U/ml. (Table III).

In contrast with the effects of vasopressin in the renal medulla, this hormone had no effect on the renal cortex where the mean control cyclic AMP concentra-

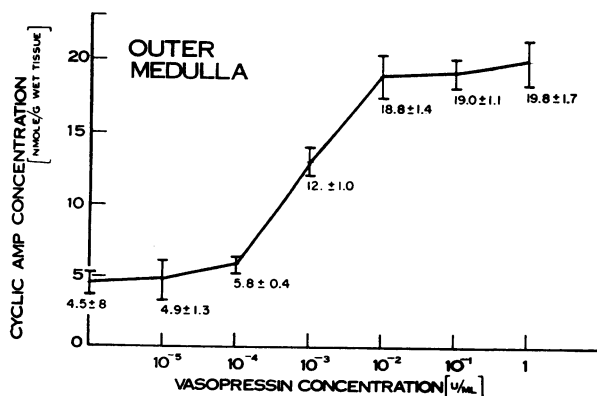


FIGURE 2 The dose-response curve of vasopressin concentration (ordinate) and cyclic AMP concentration (abscissa) in the outer medulla.

TABLE II  
Effect of  $PGE_1$  on Cyclic AMP Concentration (nanomoles/gram wet tissue) in Inner Medulla

| Media                 | Inner medulla       |
|-----------------------|---------------------|
| Control               | $2.2 \pm 0.3$ (16)* |
| $PGE_1$ , $10^{-6}$ M | $2.1 \pm 0.2$ (16)  |
| Control               | $3.5 \pm 0.7$ (10)* |
| $PGE_1$ , $10^{-4}$ M | $3.6 \pm 0.3$ (12)  |

Values are the mean  $\pm$ SE. The numbers of experiments are represented in the parentheses.

\* Control values are different because experiments were done on separate days.

tion of  $2.2 \pm 0.3$  was not significantly altered by vasopressin  $10^{-8}$  U/ml ( $2.6 \pm 0.4$ ).

## DISCUSSION

Handler, Butcher, Sutherland, and Orloff reported that vasopressin increased cyclic AMP concentration in the anuran bladder (2). Previous results in the kidney, however, have been variable. Brown, Clarke, Roux, and Sherman reported that vasopressin increased cyclic AMP concentration in the particulate fraction of a homogenate of the dog kidney (10). They noted, however, that the cortex was more sensitive to stimulation by vasopressin than the medulla. Senft, Hoffmann, Munske, and Schultz documented an increase in cortical and outer medullary cyclic AMP with dehydration, but the concentration in

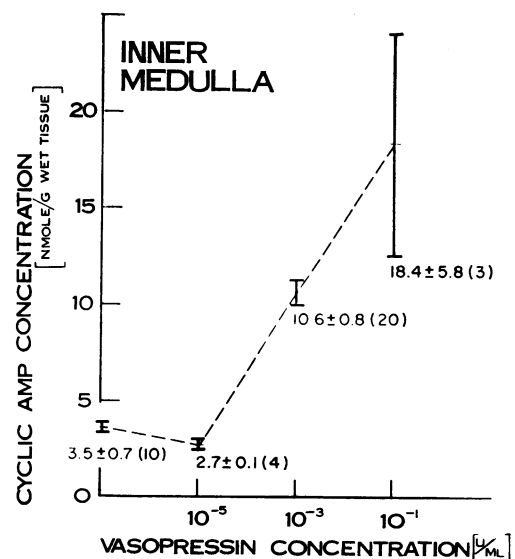


FIGURE 3 The dose-response relationship between vasopressin concentration (ordinate) and cyclic AMP concentration (abscissa) in the inner medulla.

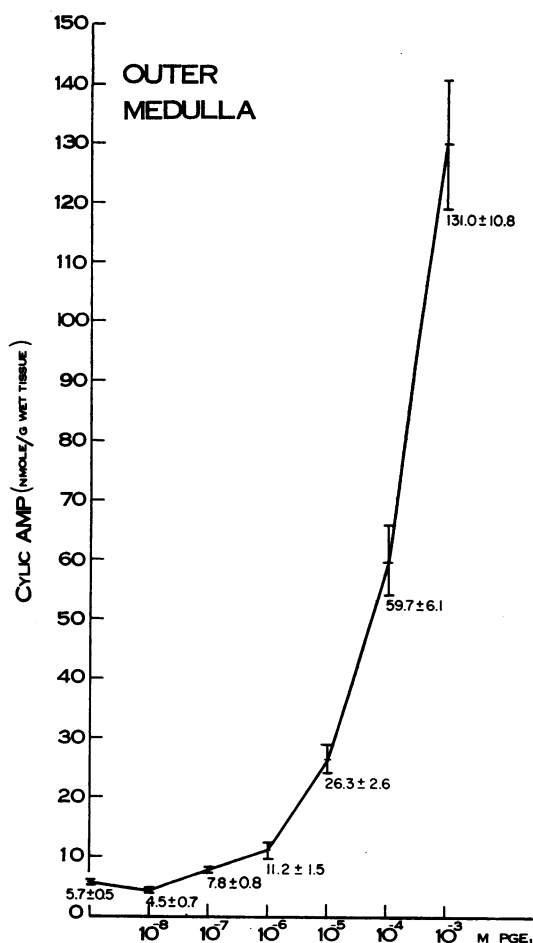


FIGURE 4 Represents the dose-response relationship between  $\text{PGE}_1$  concentration on the ordinate and cyclic AMP concentration on the abscissa.

the inner medulla was below the sensitivity of their method (11). Chase and Aurbach studied hormonal effects on adenylyl cyclase activity in cortex and medulla in vitro (3). The medulla was much more responsive to vasopressin while the cortex was much more sensitive to parathyroid hormone.

In the present experiments vasopressin significantly increased adenylyl cyclase activity and tissue concentration of cyclic AMP in inner and outer medulla but had no effect on cyclic AMP concentration in cortex. These findings further support the concept of Orloff and Handler that the physiological actions of vasopressin on responsive epithelial structures are mediated by cyclic AMP (1).

The prostaglandins influence the adenylyl cyclase-cyclic AMP system in several tissues in various ways.  $\text{PGE}_1$  increased tissue cyclic AMP concentration in pituitary (12) and thyroid (13) but inhibited the effects of epinephrine on cyclic AMP concentration and free fatty acid mobilization from adipose tissue (14).  $\text{PGE}_1$  increases the permeability of the isolated perfused collecting duct to water (5). The  $\text{PGE}_1$ -induced increases in adenylyl cyclase activity and tissue cyclic AMP concentration in the outer medulla are consistent with this hydro-osmotic effect of  $\text{PGE}_1$  being mediated by the adenylyl cyclase-cyclic AMP system. In the isolated collecting duct,  $\text{PGE}_1$  decreases the effect of vasopressin to augment water permeability (5). The inhibition of vasopressin-induced increases in adenylyl cyclase activity and cyclic AMP concentration in the inner medulla suggests that  $\text{PGE}_1$  inhibits the physiological effect of vasopressin by competing with vasopressin for adenylyl cyclase re-

TABLE III  
Effects of Vasopressin and  $\text{PGE}_1$  on Cyclic AMP Concentration (nanomoles/gram wet tissue)

| Media  | Inner medulla    | Outer medulla    |
|--|------------------|------------------|
| Control  | 3.5 ± 0.7 (10)   | 3.8 ± 0.5 (13)   |
| $\text{PGE}_1$ , $10^{-8}$ M   |                  | 4.2 ± 0.8 (13)   |
| $\text{PGE}_1$ , $10^{-6}$ M   | 3.6 ± 0.3 (12)   | 13.0 ± 0.7 (5)   |
| Vasopressin, $10^{-3}$ U/ml  | 10.6 ± 0.8 (14)  | 11.0 ± 1.3 (22)* |
| Vasopressin, $10^{-3}$ U/ml, and $\text{PGE}_1$ , $10^{-8}$ M          |                  | 7.0 ± 1.2 (22)*  |
| Vasopressin, $10^{-3}$ U/ml, and $\text{PGE}_1$ , $10^{-6}$ M          | 7.3 ± 0.9 (14)†  | 15.8 ± 2.5 (6)§  |
| Vasopressin, $5 \times 10^{-3}$ U/ml                                   | 36.8 ± 11.8 (12) |                  |
| Vasopressin, $5 \times 10^{-3}$ U/ml, and $\text{PGE}_1$ , $10^{-6}$ M | 39.1 ± 5.2 (12)  |                  |

Values are the mean ± SE. The number of experiments are represented in the parentheses.

\* The increases from the control are  $\Delta 7.2 \pm 0.8$  and  $\Delta 4.8 \pm 1.4$ , and the difference is significant  $P < 0.01$ .

†  $P < 0.01$  when compared with vasopressin alone.

§  $P < 0.05$  when compared with vasopressin alone.

ceptors. In addition, the inhibition of vasopressin-induced increases in cyclic AMP concentration by an amount of  $10^{-8}$ M PGE<sub>1</sub>, which by itself had no effect on cyclic AMP concentration, is consistent with vasopressin-PGE<sub>1</sub> competition for adenylyl cyclase receptors. It should be emphasized that the PGE<sub>1</sub> inhibition of vasopressin-induced increases in adenylyl cyclase activity and cyclic AMP concentration was not complete. The amount of increase in cyclic AMP concentration caused by vasopressin even in the presence of PGE<sub>1</sub>, may be sufficient to exert maximal physiological effect. Conversely, the PGE<sub>1</sub> inhibition of the hydro-osmotic effect of vasopressin is not complete (5). Providing these in vitro data have physiological significance all of these findings then support the Grantham and Orloff hypothesis with respect to both the mechanism of action of PGE<sub>1</sub> itself on the kidney and the means by which it interferes with the action of vasopressin. However, Grantham and Orloff also suggested PGE<sub>1</sub> was a less potent activator of adenylyl cyclase than vasopressin (5). The observation that in outer medulla PGE<sub>1</sub> caused as much as a 6-fold greater increase in cyclic AMP concentration than did maximal amounts of vasopressin does not indicate that PGE<sub>1</sub> is less effective in stimulating adenylyl cyclase than is vasopressin. In addition the combination of PGE<sub>1</sub> in amounts which had an effect on cyclic AMP concentration with vasopressin, actually caused a greater increase in cyclic AMP concentration than did either agent alone. These findings suggest that in outer medulla PGE<sub>1</sub> is either a more effective activator of adenylyl cyclase or there are at least two species of adenylyl cyclase receptors in the outer medulla. If the latter is true then at least one species of receptor might be responsive to PGE<sub>1</sub> but not to vasopressin, and the additive effects of vasopressin and PGE<sub>1</sub> in the outer medulla might not be inconsistent with the Grantham-Orloff hypothesis (5).

#### ACKNOWLEDGMENTS

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