Renal Adenyl Cyclase: Anatomically Separate Sites for Parathyroid Hormone and Vasopressin

Abstract. *Adenyl cyclase from plasma membrane fractions of rat renal cortex or medulla was assayed by measuring conversion of adenosine triphosphate labeled at the α-phosphate with ^{32}P to cyclic 3′,5′-adenosine monophosphate labeled with ^{32}P. Parathyroid hormone activated the enzyme primarily in cortex; vasopressin acted primarily in medulla. These experiments support the conclusion that cyclic adenosine monophosphate mediates the action of parathyroid hormone on the kidney and show that parathyroid hormone and vasopressin stimulate adenyl cyclase at anatomically separable areas within the kidney.*

Earlier reports showed that vasopressin activates adenyl cyclase in the kidney (*1, 2*) and increases urinary excretion of cyclic 3′,5′-adenosine monophosphate (3′,5′-AMP) (*3*). Recently, we found that parathyroid hormone also exerts a major physiological influence on the urinary excretion of cyclic 3′,5′-AMP through a direct action on the kidney (*4*). We proposed this action of parathyroid hormone might best be explained by a similar stimulation of renal adenyl cyclase. This hypothesis has been substantiated by measuring adenyl cyclase in particulate fractions from homogenates of rat kidney. The purpose of this report is to present these findings and to show that the renal adenyl cyclase sensitive to parathyroid hormone is anatomically distinct from that sensitive to vasopressin.

Kidneys from rats killed by decapitation were removed immediately and divided with scissors into cortical and medullary portions; the zone of demarcation between the two segments was discarded. Plasma membrane fractions from renal cortex and medulla were prepared by homogenizing 500 mg of tissue in eight volumes of 50 m*M* tris-HCl buffer (*p*H 7.4) and centrifuging the homogenate at 2200*g* for 10 minutes. The particulate fraction was suspended in 4 ml of tris-HCl, centrifuged again, and resuspended in 1 to 2 ml of buffer. Adenyl cyclase in homogenates of rat kidney was assayed by measuring the conversion of adenosine triphosphate (ATP) labeled at the α-phosphate with ^{32}P (*5*) to cyclic 3′,5′-AMP labeled with ^{32}P. A similar assay has been described (*6*) with the use of ATP labeled with ^{14}C as the substrate for adenyl cyclase. The final reaction mixture for the assay of adenyl cyclase contained 5 m*M* $MgCl_2$, 10 m*M* theophylline, 0.015 percent bovine plasma albumin, 1.23 m*M* labeled ATP (2.5 to 3.0 × 10^{13} count/min per mole), and membrane fraction equivalent to 400 to 800 μg of protein in 0.065 ml of 50 m*M* tris-HCl, *p*H 7.4. The *p*H and concentrations of ATP and magnesium were found to be optimal for this system. Incubations were carried out at 37°C for 15 seconds to 10 minutes and stopped by the addition of 100 μl of carrier solution (*7*) and immediate boiling for 3 minutes; 0.4 ml of water were then added and each reaction mixture centrifuged at 1000*g* for 10 minutes.

The labeled cyclic 3′,5′-AMP thus produced was separated (*8*) from other substances labeled with ^{32}P as follows: The supernatant solution from each reaction mixture was applied to a 0.5- by 3-cm column of Dowex-50-X8 (100 to 200 mesh) in the hydrogen form and the column was eluted with water. Cyclic AMP appeared in the second fraction (fourth through sixth milliliter) eluted from the column and to this fraction were added 0.2 ml of 5 percent zinc sulfate and 0.2 ml of 0.3*N* barium hydroxide; after centrifugation the additions of zinc sulfate and barium hydroxide were repeated without disturbing the first precipitate. Virtually all the radiophosphorus in the supernatant fluid represented 3′,5′-AMP labeled with ^{32}P (*9*). The tritiated cyclic 3′,5′-AMP added in the carrier solution was used to calculate recovery of 3′,5′-AMP through the separation procedures. Approximately 30 percent of the added tritium marker was recovered in the sample assayed simultaneously for ^{32}P and tritium by liquid scintillation detection.

Adenyl cyclase was found primarily in the plasma membrane fraction from homogenates of renal cortex and medulla. The reaction rate was linear with respect to time when measurements of the cyclic 3′,5′-AMP produced were made every 15 seconds for 1 minute. Concentration of this nucleotide became maximal in 2 to 5 minutes and declined thereafter even though theophylline, an inhibitor of phosphodiesterase (*10*), was included in the reaction medium. Addition of purified parathyroid hormone (*11, 12*) to homogenates from renal cortex caused a marked increase in adenyl cyclase activity (Fig. 1a). Significant stimulation was found with concentrations of hormone as low as 4 μg/ml. Maximal stimulation by parathyroid hormone was reached within 5 minutes and represented 30 percent of total available adenyl cyclase, determined by measuring activity in a parallel reaction containing 10 m*M* sodium fluoride. Parathyroid hormone did not stimulate additional activity when added to reactions containing sodium fluoride. Greater than 95 percent of 90 or 750 mμ*M* tritiated 3′,5′-AMP added to standard reaction mixtures was recovered unchanged after 10 minutes of incubation with or without hormone, which showed that cyclic nucleotide phosphodiesterase was completely inhibited by theophylline. The hormone-induced rise in cyclic AMP must therefore result from stimulation of adenyl cyclase and not from inhibition of the phosphodiesterase.

Purified vasopressin (*13*) stimulated adenyl cyclase prepared from homog-

Table 1. Effect of parathyroid hormone and vasopressin on adenyl cyclase in rat renal cortex and medulla. Adenyl cyclase was assayed by measuring the conversion of ATP labeled at the α-phosphate with ^{32}P to cyclic 3′, 5′-AMP labeled with ^{32}P. Each reaction contained 500 μg of protein representing the 2200*g* fraction from a homogenate of renal cortex or medulla. Peptides were added at the concentrations shown. Other reactants were as described in the text. Reactions were for 2 minutes at 37°C. Lysine vasopressin was not tested in experiment 2. Each result represents the mean of duplicate determinations. Measurements varied by no greater than 10 percent from the mean.

Hormone added	Cyclic 3′, 5′-AMP formed (μμmole per milligram of protein)			
	Experiment 1		Experiment 2	
	Cortex	Medulla	Cortex	Medulla
None	20.8	24.7	10.0	14.1
Parathyroid hormone (23 μg/ml)	83.9	33.7	41.6	21.0
Arginine vasopressin (3.5 μg/ml)	45.1	75.6	15.6	42.6
Lysine vasopressin (5.1 μg/ml)	41.9	75.5	N.T.	N.T.

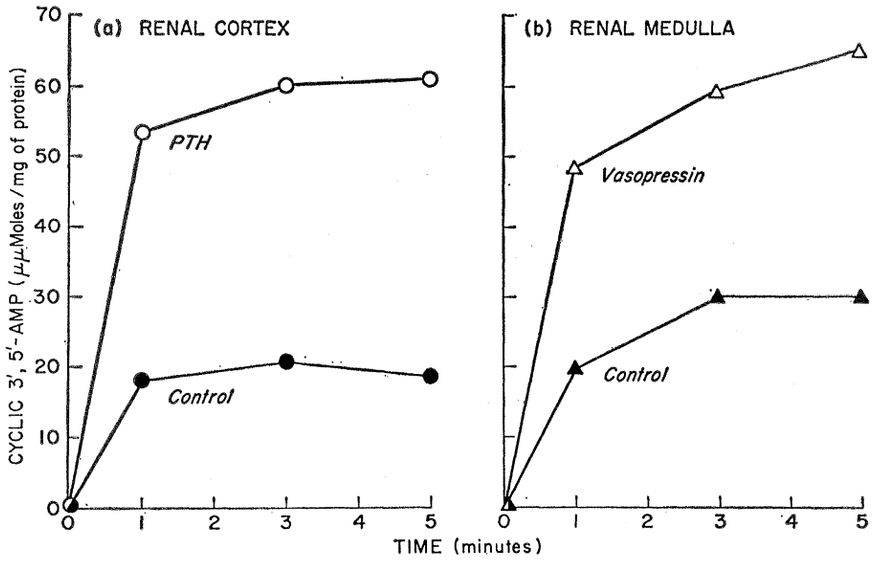

Fig. 1. (a) Effect of parathyroid hormone on adenyl cyclase in rat renal cortex. Adenyl cyclase was assayed by measuring the conversion of ATP labeled at the α-phosphate with ^{32}P to cyclic 3′,5′-AMP labeled with ^{32}P. Each reaction contained 810 μg of protein representing the 2200g fraction from a homogenate of renal cortex; when used, purified parathyroid hormone was added to give a concentration of 23 μg/ml. Other reactants were as described in the text. Control contained no added parathyroid hormone. Reactions were incubated at 37°C for the times shown. (b) Effect of arginine vasopressin on adenyl cyclase in rat renal medulla. Each reaction contained 460 μg of protein representing the 2200g fraction from a homogenate of renal medulla; when used, purified arginine vasopressin was added to give a concentration of 3.5 μg/ml. Other reactants and conditions were identical to (a). Control contained no added vasopressin. Each point represents the mean of duplicate determinations. Individual results varied by no greater than 10 percent from the mean.

enates of renal medulla (Fig. 1b). Activation by vasopressin approached a maximum within 5 minutes and involved approximately 25 percent of the total enzyme available. Significant stimulation occurred with concentrations of hormone as low as 10 milliunit/ml. Vasopressin did not stimulate additional activity in reactions containing sodium fluoride.

Further studies were carried out with particulate fractions from cortex and medulla prepared in parallel from the same rat kidney. The plasma membrane fraction was incubated with vasopressin or parathyroid hormone (Table 1). Parathyroid hormone caused marked stimulation of adenyl cyclase in cortex but only slight stimulation in medulla, whereas the opposite result was obtained with vasopressin. There was no detectable difference between the effects of arginine and lysine vasopressin on cortex or medulla.

The finding that parathyroid hormone directly stimulates renal adenyl cyclase in vitro confirms our previous conclusion derived from in vivo studies that measured the rate of urinary excretion of cyclic 3′,5′-AMP by the rat (*4*). The activation of adenyl cyclase in vitro occurs within 1 minute and is consonant with the rapid increase in urinary cyclic AMP found after parathyroid hormone was administered intravenously.

The activation of adenyl cyclase by vasopressin in homogenates of rat kidney is in accord with the previous conclusion (*1*, *2*) that similar activation occurs in homogenates of dog kidney. However, the previous conclusion was based on experimental results showing stimulation of adenyl cyclase by vasopressin in reactions containing sodium fluoride, whereas our experiments show no further stimulation of enzyme activity by vasopressin under these conditions. Current results show that arginine and lysine vasopressin act in a similar manner and cause greater enzyme activation in renal medulla than in renal cortex; this finding also differs from results described in the earlier reports.

Although parathyroid hormone and vasopressin both activate adenyl cyclase in the kidney, our experiments show that the receptor sites for each hormone are located in anatomically separable areas. The localization of parathyroid hormone–sensitive adenyl cyclase to renal cortex and vasopressin-sensitive adenyl cyclase to renal medulla is consonant with reports that cellular transfer of phosphate (*14*) and calcium (*15*) occurs primarily in the proximal portions of the nephron where parathyroid hormone seems to act, and that sodium transport and water permeability are induced by vasopressin primarily in the collecting tubule (*16*). It is also believed that vasopressin acts on the distal convoluted tubule (*17*) in rats; the stimulation of adenyl cyclase in cortex in response to vasopressin may represent this action on the distal tubule. However, incomplete separation of cortex and medulla by the gross dissection used may account for the apparent overlapping effect of vasopressin on renal cortex.

It is likely that the cyclic AMP produced in kidney through activation of adenyl cyclase by vasopressin accounts for the physiological actions of this hormone, since cyclic AMP itself mimics the effects of vasopressin in vitro (*18*). The function of cyclic AMP in the mechanism of action of parathyroid hormone is not known. As postulated elsewhere (*4*), the phosphaturic response to parathyroid hormone may well be mediated by an enzyme that is activated by cyclic 3′,5′-AMP within the renal cell. The rise in blood calcium caused by pharmacological effects of theophylline (*19*) has led to speculation that the cyclic nucleotide might be a mediator in the hypercalcemic response to parathyroid hormone. Specific tests on adenyl cyclase in bone tissue will be required to substantiate this hypothesis.

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7. The carrier solution contained 40 m*M* ATP and 13 m*M* tritiated cyclic 3′,5′-AMP (2×10^7 count/min per mmole) in 50 m*M* tris-HCl buffer, *p*H 7.4.
8. We are indebted to Dr. Gopal Krishna, NIH, for suggesting the use of this separation method which he devised.
9. Identity of the ^{32}P-labeled product was confirmed by thin-layer chromatography on cellulose (Brinkmann Instruments, Inc., CEL-300) with the use of a solvent system containing a mixture of *n*-butanol, acetone, acetic acid, 5 percent ammonia, and water (7:5:3:3:2). Incubation of the ^{32}P-labeled

cyclic 3′,5′-nucleotide product with a specific phosphodiesterase yielded 5′-AMP labeled with ^{32}P which was then precipitable with the addition of zinc sulfate and barium hydroxide.
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12. Purified parathyroid hormone assayed at 2000 to 3000 unit/mg.
13. Purified arginine vasopressin (400 unit/mg) was the gift of Dr. M. Peterson. Purified lysine vasopressin (260 unit/mg) prepared by Drs. R. Guillemin and A. V. Schally was obtained from the Endocrinology Study Section, National Institutes of Health, Bethesda, Maryland.
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Fish: Serologic Evidence of Infection with Human Pathogens

Abstract. *Specific antibodies to several bacteria pathogenic to humans were detected in the serums of white perch from surface waters adjacent to heavily populated areas on Chesapeake Bay. White perch from surface waters adjacent to sparsely populated areas were free of such antibodies. We suggest that fish may become actively infected with human pathogens by exposure to contaminated water and may constitute a hazard to public health.*

Many individuals and even communities discharge their excrement into convenient rivers, lakes, or estuaries in spite of the fact that such material frequently contains bacteria capable of causing human disease. It is generally assumed that surface waters so contaminated are naturally purified by dilution, aeration, exposure to sunlight, and antibacterial action of the indigenous microflora. Unfortunately little consideration has been given to the possibility that fish exposed to such contaminated water may become actively infected with pathogenic bacteria and pose a threat to public health: directly through their handling and ingestion, and indirectly by releasing infectious bacteria into waters that may be considered safe according to usual standards of analysis.

Pathogenic bacteria associated with the digestive tracts of man and other mammals have been isolated from many varieties of fish. It has been reported that these bacteria are short-lived contaminants of the surface and gut of the fish, that they derive from human fecal pollution of the aquatic environment, and that they are unable actively to infect the fish (*1*); however, there is evidence that at least some of these bacteria can establish active infection in fish and persist for several weeks or longer (*2*).

Our purpose was to determine whether fish, from surface waters subject to human fecal contamination, had evidence of active infection with pathogenic bacteria associated with such contamination, since actively infected fish could serve as long-term active carriers of human disease as well as short-term passive vectors. We made a serologic survey of serums from white perch, *Roccus americanus*, for antibodies to some of the human pathogens known to be introduced into the aquatic environment chiefly by excrement from man and other mammals. The survey was based on the assumption that antibody in white perch, to a particular human pathogen, would be prima facie evidence that the fish had been actively infected with the pathogen or some closely related species at some time, since fish are known to produce highly specific antibody in response to antigenic stimuli (*3*). The assumption that active infection of the fish is required in order to achieve an antigenic mass large enough to induce production of detectable antibody seems reasonable to us in view of available evidence that experimental injections of large amounts of antigens are required for such production in various fishes (*4*). We should point out that the antibody response of fish to ingested dead bacteria is unknown; it may possibly explain our results.

White perch were selected because they rarely leave the river in which they hatch (*5*); therefore, if they carried evidence of infection, the condition of the river in which they were caught might indicate the source of infecting bacteria. Fish ranging from 10 to 30 cm were netted at random during late summer in rivers flowing into Chesapeake Bay through heavily populated areas and likely to be contaminated frequently, and in sparsely populated rivers of the eastern shore of the bay that are probably rarely contaminated. Each fish was bled by cardiac puncture with a hypodermic syringe, and the

Table 1. Serologic survey of white perch for precipitins against human pathogens. Numbers of fish appear in parentheses.

Source of fish	Fish (No.) with precipitins against pathogen:								
	Past. pestis	*Past. pstb.*	*Salm. para.* A	*Shig. flex.*	*Prot. vulg.*	*Ps. aerug.*	*E. coli* B	*Aero. hydro.*	*Aero. shig.*
				Heavily populated areas					
Potomac River (63)	4	4	1	1	1	1	1	1	
Patuxent River (38)	1	1	1		1	1			1
Severn River (10)						2		1	
Magothy River (25)				1	2	5		*	*
Patapsco River (13)						1			
Back River (26)	3	3		3	3	2	3		
Middle River (16)				1	1	1			
Tolchester Beach (15)			2	*	2	2	2	*	*
				Sparsely populated areas					
Chester River (8)									
Choptank River (25)									
Nanticoke River (7)									
Wicomico River (17)									
				Percentages positive					
	3.0	3.0	1.5	2.3	3.8	5.7	2.3	0.9	0.5

* Not tested.