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Research Article

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Beta adrenergic activity increased cyclic AMP concentration in the renal cortex, a finding consistent with the hypothesis that beta-adrenergic stimulation augments renin synthesis by increasing cyclic AMP generation.

Beta adrenergic stimulation, like vasopressin, increased cyclic AMP concentration in the renal medulla. This suggests that beta adrenergic stimulation causes antidiuresis by augmenting cyclic AMP generation in the renal medulla.

Alpha adrenergic activity inhibited the effect of vasopressin to stimulate cyclic AMP generation. These results support the hypothesis that the diuretic effect of alpha adrenergic stimulation is mediated by inhibition of the effect of vasopressin to increase cyclic AMP generation.

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ABSTRACT Catecholamines have several physiological effects on the kidney. These include: (*a*) stimulation of renin synthesis in the cortex; (*b*) antidiuresis by beta adrenergic agents; and (*c*) diuresis by alpha adrenergic stimulation. The role of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) in the renal actions of catecholamines was evaluated by measuring the effects of several adrenergic agents on cyclic AMP concentration in the dog kidney.

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INTRODUCTION

Catecholamines are thought to effect, either directly or by interaction with other hormones, the generation of cyclic 3′,5′-adenosine monophosphate (cyclic AMP)[1] in several tissues (1, 2). In the kidney, it has been suggested that catecholamines stimulate renin secretion (3–5). Michelakis, Caudle, and Liddle (6) demonstrated that both exogenous cyclic AMP and catecholamines, when added to renal cortical slices in vitro, stimulate renin synthesis. They suggested that catecholamines augment renin synthesis by stimulating cyclic AMP generation in the renal cortex. Additionally, it has been shown that beta adrenergic stimuli induce an antidiuretic effect by unknown mechanisms (7–9), and that alpha-adrenergic stimuli cause a diuresis by inhibiting the effect of vasopressin to increase membrane permeability (8–10). In support of this latter concept Handler, Bensinger, and Orloff (11) demonstrated that alpha adrenergic agents inhibit the hydro-osmotic effect of vasopressin, but not of exogenous cyclic AMP. On the basis of these observations, they proposed that alpha adrenergic activity inhibits the effect of vasopressin to stimulate cyclic AMP generation.

In this study these hypotheses were evaluated by investigating the effect of catecholamines alone and in combination with other hormones on cyclic AMP concentration in dog kidney.

METHODS

Mongrel dogs, weighing 10–25 kg, were anesthetized with Nembutal (Abbott Laboratories, North Chicago, Ill.), and the kidneys were removed rapidly and kept in ice-cold Krebs-Ringer bicarbonate buffer during the following procedures. The cortex, the red outer medulla, and the white inner medulla were studied separately.

Cyclic AMP concentration. The kidney tissues were sliced to a thickness of less than 0.5 mm. Each slice was then divided into the appropriate number of smaller slices with a weight of 40–120 mg. The slices were incubated as appropriate pairs for 15 min at 37°C in Krebs-Ringer bicarbonate buffer containing 10^{-2} M theophylline, and gassed with 5% CO_2 and 95% O_2. The study substances were added to the incubation media as indicated in the Results section. After the incubation, the slices were homogenized in 5% trichloro-

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[1] *Abbreviations used in this paper:* 5′-AMP, 5′-adenosine monophosphate; cyclic AMP, cyclic 3′,5′-adenosine monophosphate; PGE_1, prostaglandin E-1.

acetic acid with glass homogenizer, and centrifuged at 700 *g* for 15 min.

Cyclic AMP was assayed using a modification of Gilman's method (12). In summary, the supernates of the homogenized tissue were mixed with the equal volume of 1 N sodium acetate, pH 12, which brought the final pH of the mixture to pH 5. Then, 0.5 pmoles of cyclic AMP-^{3}H (specific activity 14.2 Ci/mmole), a mixture of cyclic AMP-binding protein and protein kinase inhibitor was added in proper dilution. They were then incubated at 4°C in a refrigerator for 60 min. After the incubation, the free unbound cyclic AMP was separated from the protein-bound cyclic AMP uisng dextran-coated charcoal as described by Herbert, Lau, Gettlieb, and Bleicher (13). The samples were counted in a liquid scintillator. All assays were performed in triplicate. The standard curve in the range of 1.5–100 pmoles of cyclic AMP was simultaneously obtained in each series of assay. In this range, the standard cyclic AMP curve was linear, and the assayed tissue cyclic AMP were always in that range.

Cyclic AMP-binding protein was prepared as described by Miyamoto, Kuo, and Greengard (14). Bovine heart muscle was used and was purified to the steps of acid precipitation and ammonium sulfate fractionation. Each batch of cyclic AMP-binding protein was titrated to have the proper dilution to bind 20–30% of cyclic AMP-^{3}H in the assay. Protein kinase inhibitor was prepared as described by Gilman (12), and its activity titrated each time to determine the maximal inhibitory effect.

The recovery rate of cyclic AMP after the entire tissue extraction procedures, measured with cyclic AMP-^{3}H as a tracer, was 76.85% with a standard deviation of 1.90%. These data indicate a constant recovery rate.

Cyclic nucleotide phosphodiesterase. Cyclic AMP phosphodiesterase was prepared as described by Cheung (15). Kidney slices of cortex, and inner medulla were prepared, homogenized in 5 vol of glass-distilled water, and centrifuged at 30,000 *g* for 30 min. The supernatant fluid was dialyzed overnight against 200–400 vol of 0.02 M Tris-HCl, pH 7.4. Phosphodiesterase activity was assayed by incubating the enzyme in 0.1 ml 0.05 M Tris-HCl, pH 7.4, containing 10^{-8} moles of cyclic AMP, 10^{-11} moles of cyclic AMP-^{3}H, and 1.8 mM $MgCl_2$, at 37°C for 30 min. The reaction was stopped by placing the tubes in a boiling water bath for 3 min. Cyclic AMP was extracted by precipitating nucleotides other than cyclic AMP with 0.2 ml each of 5% zinc sulfate and 0.3 N barium hydroxide, followed by Dowex 50w-x8 resin (100–200 mesh; Dow Chemical Co., Midland, Mich.) column chromatography, as described by Krisna et al. (16). Tritium radioactivity in the cyclic AMP fraction was counted in the liquid scintillator, which showed the amounts of cyclic AMP remaining, and from it the amount of cyclic AMP converted to 5'-adenosine monophosphate (5'-AMP) by phosphodiesterase was calculated. Protein concentration of the phosphodiesterase enzyme preparation was measured using Lowry's method. Enzyme activity was then expressed as picomoles of cyclic AMP hydrolyzed per milligram protein.

RESULTS

Cyclic AMP assay method. The validity of the cyclic AMP assay method was evaluated. As shown in Table I, 25 pmoles of cyclic AMP displaced 32% of bound cyclic AMP-^{3}H. Other nucleotides, however, had no measurable effect on cyclic AMP binding. The tested nucleotides were 5'-adenosine monophosphate, adenosine 5'-diphosphate, adenosine 5'-triphosphate, guanosine 5'-triphosphate, uridine 5'-triphosphate, and cytidine 5'-triphosphate. The ranges of concentration of the nucleotides tested were as high as 100–2000 times the cyclic AMP concentration. They were also much higher than usual concentrations of those nucleotides in renal tissue (17). In addition, 50 pmoles of pure cyclic AMP and the tissue homogenates of renal cortex (containing 8 pmoles of cyclic AMP) displaced cyclic AMP-^{3}H binding to the protein, 82% and 43%, respectively. When the same amount of cyclic AMP or the tissue homogenates were incubated with cyclic AMP-phosphodiesterase for 30 min before cyclic AMP assay, the displacement of cyclic AMP binding to the protein was completely abolished. However, boiling the phosphodiesterase before incubation with the standards did not affect the displacement of cyclic AMP-^{3}H binding (82% and 43%, respectively). These results indicate that specificity for cyclic AMP is a feature of the method.

TABLE I

Comparative Effects of Various Nucleotides on the Displacement of Cyclic AMP-^{3}H Binding to Cyclic AMP-Binding Protein

Nucleotides	pmoles/tube	Radioactivity bound to protein	Displacement
		cpm	%
Control	0	371 ±11*	0
Cyclic AMP	25	254 ±13	32
5'-AMP	2,500	362 ±19	2
5'-ADP	10,000	370 ±7	0
5'-ATP	50,000	387 ±13	0
5'-GTP	50,000	391 ±10	0
5'-UTP	50,000	369 ±14	1
5'-CTP	50,000	352 ±6	5

5'-ADP, adenosine 5'-diphosphate; 5'-ATP, adenosine 5'-triphosphate; 5'-GTP, guanosine 5'-triphosphate; 5'-UTP, uridine 5'-triphosphate; 5'-CTP, cytidine 5'-triphosphate.
* The numbers are means ±SEM.

Effects of catecholamines on cyclic AMP concentrations. Epinephrine 10^{-6} M, which has both alpha and beta adrenergic activity, increased cyclic AMP concentrations in renal cortex, and both the outer red medulla and the inner white medulla: from the control of 0.47 ±SE 0.06 nmoles of cyclic AMP/g of wet tissue to 1.52 ±0.13 in the cortex; from 0.78 ±0.10 to 3.08 ± 0.51 in the outer medulla; and from 1.41 ±0.10 to 3.65 ± 0.42 in the inner medulla, $P < 0.001$ for each area. Norepinephrine 10^{-6} M also increased the value in the inner medulla to 3.54 ±0.16, $P < 0.001$, Table II.

However, when the beta adrenergic effect of epinephrine was inhibited by the addition of propranolol 10^{-4} M (beta adrenergic blockade), presumably leaving only the alpha adrenergic effect, the stimulatory effect of epinephrine on cyclic AMP generation was abolished, and cyclic AMP concentration was not measurably increased; 0.56 ±0.10 in the cortex, 1.22 ±0.27 in the

TABLE II

Effects of Catecholamines on Cyclic AMP Concentrations in Dog Kidneys

Hormones	Adrenergic effects	Cortex	Outer medulla	Inner medulla
		*nmoles cyclic AMP/g wet tissue**		
Control		0.47 ±0.06 (18)‡	0.78 ±0.10 (22)	1.41 ±0.10 (36)
Epinephrine	a + b	1.52 ±0.13 (7) $P < 0.001$	3.08 ±0.51 (5) $P < 0.001$	3.65 ±0.42 (5) $P < 0.001$
Epinephrine + propranolol	a only	0.56 ±0.10 (7) $P > 0.05$	1.22 ±0.27 (5) $P > 0.05$	1.80 ±0.47 (5) $P > 0.05$
Epinephrine + phentolamine	b only	1.99 ±0.18 (7) $P < 0.001$	3.91 ±0.26 (5) $P < 0.001$	3.53 ±0.73 (4) $P < 0.001$
Isoproterenol	b only	1.05 ±0.14 (8) $P < 0.001$	2.79 ±0.27 (8) $P < 0.001$	3.77 ±0.33 (8) $P < 0.001$
Isoproterenol + propranolol	None	0.42 ±0.06 (8) $P > 0.05$	0.84 ±0.13 (8) $P > 0.05$	1.90 ±0.16 (8) $P > 0.05$
Norepinephrine	a + b			3.54 ±0.16 (5) $P < 0.001$

Catecholamines are at the concentration of 10^{-6} M, and adrenergic blockades are 10^{-4} M.

* The numbers are means ±SEM.

‡ The numbers of observations are in the parentheses.

outer medulla, and 1.80 ±0.47 in the inner medulla, when compared with the controls. On the contrary, when the alpha adrenergic effect was inhibited by the addition of phentolamine 10^{-4} M (alpha adrenergic blockade), leaving only the beta adrenergic effect, cyclic AMP concentration was increased in all three parts of the kidney as high as with epinephrine alone: 1.99 ±0.18 in cortex, 3.91 ±0.26 in outer medulla, and 3.53 ±0.73 in inner medulla, $P < 0.001$ for each area as compared to the controls. The beta adrenergic stimulator, isoproterenol 10^{-6} M also increased cyclic AMP concentration to 1.05 ±0.14 in the cortex, 2.79 ±0.27 in the outer medulla, and 3.77 ±0.33 in the inner medulla, $P < 0.001$ for each as compared to the controls. The addition of the beta adrenergic blockade, propranolol, also abolished the effect of isoproterenol to increase cyclic AMP concentration 0.42 ±0.06 in the cortex, 0.84 ±0.13 in the outer medulla, and 1.90 ±0.16 in the inner medulla, values which were not significantly different from the control. These results indicate that alpha adrenergic stimulus has no effect on cyclic AMP concentration, but beta adrenergic agents stimulate cyclic AMP generation in all three parts of the kidney.

Effects of catecholamines on cyclic AMP phosphodiesterase activity. As shown in Table III, epinephrine 10^{-6} M (both alpha and beta adrenergic activity) had no measurable effect on phosphodiesterase activity in either the cortex or the inner medulla; the control of 542 ±SE 4 pmoles of cyclic AMP converted to 5′-AMP/mg of protein per min, and 548 ±2 with the addition of epinephrine in the cortex; and the control of 1756 ±9, and 1765 ±7 with addition of epinephrine in the inner medulla. Neither did the addition of either propranolol 10^{-4} M (beta adrenergic blockade) or phentolamine 10^{-4} M (alpha adrenergic blockade) with epinephrine have any effect on phosphodiesterase activity. The 544 ±2 and 1,763 ±10 in cortex and inner medulla with the addition of propranolol and epinephrine (alpha adrenergic effect only); and the 531 ±6 and 1,726 ±17 with the addition of phentolamine and epinephrine (beta adrenergic effect only), were not measurably different than controls.

Theophylline 10^{-2} M inhibited phosphodiesterase activity to 335 ±2 from the control of 542 ±2 in the cortex, and to 762 ±17 from the control of 1756 ±9 in the inner medulla, $P < 0.001$ each. However, the values with the

TABLE III

Effect of Catecholamines and their Interactions with Theophylline on Cyclic AMP-Phosphodiesterase

	Cortex	Medulla
	*pmoles cyclic AMP hydrolyzed/mg protein/min**	
Control	542 ±4 (4)‡	1756 ±9 (6)
Epinephrine 10^{-6} M	548 ±2 (4)	1765 ±7 (6)
Epinephrine 10^{-6} M + propranolol 10^{-4} M	544 ±2 (4)	1763 ±10 (6)
Epinephrine 10^{-6} M + phentolamine 10^{-4} M	531 ±6 (4)	1726 ±17 (6)
Theophylline 10^{-2} M	335 ±2 (4)	762 ±17 (6)
Theophylline 10^{-2} M + epinephrine 10^{-6} M	311 ±11 (4)	790 ±37 (6)
Theophylline 10^{-2} M + epinephrine 10^{-6} M + propranolol 10^{-4} M	342 ±14 (4)	852 ±55 (6)

* Mean ±SEM.

‡ Within the parentheses are the numbers of observations.

TABLE IV

Effects of Alpha-Adrenergic Agent on Vasopressin in Dog Renal Medulla

Hormones	Inner medulla
	*nmoles cyclic AMP/g wet tissue**
Control	1.42 ±0.12 (31)§
Vasopressin 10^{-3} U/ml	2.35 ±0.14 (31)
Vasopressin 10^{-3} U/ml + epinephrine 10^{-6} M + propranolol 10^{-4} M	1.98 ±0.15 (31)
Mean difference between vasopressin alone, and vasopressin + epinephrine + propranolol	−0.37 ±0.14 (31)
Control	0.63 ±0.14 (6)
Vasopressin 10^{-3} U/ml	1.53 ±0.20 (6)
Vasopressin 10^{-3} U/ml + propranolol 10^{-4} M	1.56 ±0.20 (6)

* Mean ±SE.

‡ Within the parenthesis are the numbers of observations.

addition of epinephrine 10^{-6} M alone (311 ±11 in the cortex, and 790 ±37 in the inner medulla, and with the combination of the epinephrine and propranolol 10^{-4} M (342 ±14 in the cortex, and 852 ±55 in the inner medulla) to theophylline 10^{-2} M, were not different from theophylline alone, Table III. These results indicate that catecholamines have no effect on cyclic AMP phosphodiesterase. Neither do they interact with theophylline to inhibit phosphodiesterase activity.

Interaction of catecholamines and vasopressin on cyclic AMP concentration. A submaximal dose of vasopressin (Pitressin; Parke, Davis & Co., Detroit, Mich.) 10^{-3} U/ml (18) increased cyclic AMP concentration from the control of 1.42 ±0.12 to 2.35 ±0.14 in the inner medulla, $P < 0.001$, Table IV. An alpha adrenergic stimulus obtained by the combination of epinephrine 10^{-6} M and propranolol 10^{-4} M, decreased vasopressin effect to 1.98 ±0.15, ($P < 0.05$). In a separate series, the effect of propranolol 10^{-4} M per se on vasopressin was evaluated. As shown in Table IV, vasopressin 10^{-3} U/ml increased cyclic AMP concentration in the inner medulla from the control of 0.63 ±0.14 to 1.53 ±0.20 ($P < 0.001$). The addition of propranolol 10^{-4} M alone to vasopressin had the value of 1.56 ±0.20 which is not measurably different from that of vasopressin alone. These data suggest that the inhibitory effect on vasopressin is not from propranolol, but rather from the alpha adrenergic activity of epinephrine.

Effects of alpha adrenergic stimuli on other hormones. As shown in Table V, parathyroid hormone 1 U/ml increased cyclic AMP concentration in the cortex from the control of 0.36 ±0.02 to 0.97 ±0.13, $P < 0.01$. The addition of an alpha adrenergic effect by the combination of epinephrine 10^{-6} M and propranolol 10^{-4} M along with parathyroid hormone also increased the value to 0.87 ± 0.09, not measurably different from the value of parathyroid hormone alone.

Prostaglandin E-1 (PGE_1) 10^{-6} M increased cyclic AMP concentration in the inner medulla, from the control of 1.38 ±0.15 to 2.56 ±0.39, $P < 0.001$. The addition of an alpha adrenergic stimulus to prostaglandin E-1 did not measurably change the effect of PGE_1 alone,

TABLE V

Effects of Alpha-Adrenergic Stimulus on Parathyroid Hormone and PGE_1 in Dog Kidneys

Hormones	Cortex	Inner medulla
	*nmoles cyclic AMP/g wet tissue**	
Control	0.36 ±0.02 (7)‡	
Parathyroid hormone 1 U/ml	0.97 ±0.13 (7)	
Parathyroid hormone 1 U/ml + epinephrine 10^{-6} M + propranolol 10^{-4} M	0.87 ±0.09 (7)	
Control		1.38 ±0.15 (8)
PGE_1 10^{-6} M		2.56 ±0.39 (8)
PGE_1 10^{-6} M + epinephrine 10^{-6} M + propranolol 10^{-4} M		2.60 ±0.19 (8)

* Mean ±SE.

‡ The numbers within the parentheses are the number of observations.

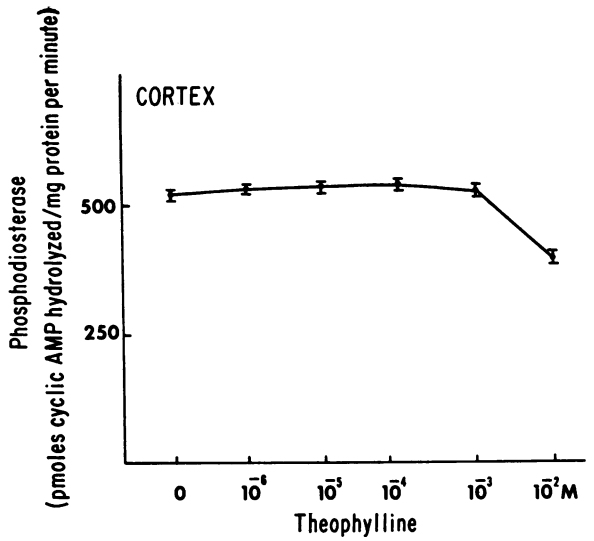

FIGURE 1 The activity of phosphodiesterase in picomoles of cyclic AMP converted to 5'-AMP per milligram of protein per minute is plotted on the vertical axis and the concentration of theophylline is on the horizontal. The points represent the means over the vertical bars ±1 SEM.

2.60 ±0.19. It demonstrates the absence of an inhibitory effect of the alpha adrenergic stimulus on either parathyroid hormone or prostaglandin E-1, further emphasizing the specificity of the inhibitory effect of the alpha adrenergic stimulus on vasopressin-induced increases in tissue cyclic AMP concentration.

This alpha adrenergic effect was produced with the combination of epinephrine (containing both alpha and beta adrenergic stimuli) and propranolol (beta adrenergic blockade), instead of norepinephrine, because as shown in Table II, norepinephrine increased cyclic AMP concentration. This is increase in cyclic AMP concentration induced by norepinephrine could be due to the fact that it also exerts a beta adrenergic effect in this system.

Effects of theophylline. As shown in Figure 1, theophylline 10^{-2} M was the lowest concentration to inhibit cyclic AMP phosphodiesterase activity significantly:

TABLE VI

Effects of Theophylline 10^{-2}M on Cyclic AMP Concentration in Dog Renal Cortex

Hormones and/or theophylline	Cyclic AMP
	*nmoles/g tissue**
Control without theophylline	0.82 ±0.07 (4)‡
Theophylline	1.67 ±0.33 (4) $P < 0.05$§
Epinephrine 10^{-6} M without theophylline	1.21 ±0.16 (5)
Epinephrine 10^{-6} M with theophylline	2.67 ±0.35 (5) $P < 0.01$§

* The numbers are means ±SE.
‡ The numbers within the parentheses are the numbers of studies.
§ *P* values are in comparison between control vs. theophylline, and epinephrine vs. epinephrine + theophylline.

from the control of 512 ±SE 10 pmoles of cyclic AMP hydrolysis/mg of protein per min, to 385 ±2 by theophylline 10^{-2} M in the cortex, $P < 0.001$. Theophylline 10^{-2} M was also the lowest concentration to increase cyclic AMP concentration, Table VI.

The effect of theophylline 10^{-2} M in the cortical tissue slices was studied in the absence or presence of epinephrine. Theophylline 10^{-2} M by itself increased cyclic AMP concentration from the base line of 0.82 ±0.07 nmoles of cyclic AMP/g of wet tissue, to 1.67 ±0.33 ($P < 0.01$). Epinephrine 10^{-6} M increased cyclic AMP concentration to 1.21 ±0.16 in the absence of theophylline, and the addition of theophylline 10^{-2} M to the same amount of epinephrine increased the value further to 2.67 ±0.35 ($P < 0.01$).

DISCUSSION

The principle of Gilman's new method of cyclic AMP assay is the competition for binding to a protein between a known constant amount of cyclic AMP-^{3}H and the unknown amount of nonradioactive cyclic AMP in the study specimens (12). As shown in Table I, numerous other nucleotides do not affect the displacement of cyclic AMP-^{3}H binding to the protein, supporting the previously reported specificity of the assay method (12). The complete abolishment of the displacement of cyclic AMP-^{3}H binding, when the nonradioactive cyclic AMP was converted to 5'-AMP by phosphodiesterase before the assay, demonstrates its specificity and validity of cyclic AMP assay method. Furthermore the low standard deviation of the recovery rate makes the assay reproducible.

It has been suggested that catecholamines initiate their biological effects in various tissues by increasing the intracellular concentration of cyclic AMP (1, 2). One physiological effect of catecholamines on the kidney is to increase renin synthesis (3–5). Michelakis et al. (6) proposed that catecholamine stimulates renin synthesis, mediated by cyclic AMP. This hypothesis was on the basis of their observation that catecholamine in the incubation media increased renin synthesis in tissue slices of dog renal cortex; and exogenous cyclic AMP also increased renin synthesis. Our observation of the induction of an increase in cyclic AMP concentration in the renal cortex by beta adrenergic stimulation is consistent with the Michelakis hypothesis.

Beta adrenergic stimuli have an antidiuretic effect similar to vasopressin both in intact and denervated kidneys (7). These results show that beta adrenergic agents, like vasopressin, increase cyclic AMP concentration in the outer and the inner medullae. This finding is consistent with the hypothesis that beta adrenergic–induced antidiuresis is mediated by increasing cyclic AMP concentration in the renal medulla.

Conversely, alpha adrenergic agents have been demon-

strated to have a diuretic effect in mammalian kidneys (8–10), and to decrease water permeability in the isolated toad bladder (11). Handler, Bensinger, and Orloff, and Liberman, Klein, and Kleeman (10, 11) proposed that this diuretic effect of alpha adrenergic agents is mediated by decreasing the ability of vasopressin to stimulate cyclic AMP generation. The observation that alpha adrenergic stimulus inhibits the effect of vasopressin to increase cyclic AMP concentration is consistent with that hypothesis.

Parathyroid hormone and PGE_1 are two other hormones known to increase cyclic AMP concentrations in the cortex and the medulla each (18, 19). However, alpha adrenergic stimulus had no inhibition on either parathyroid hormone or PGE_1. These results suggest specificity of the inhibitory effect of alpha adrenergic agents on vasopressin.

None of the agents tested except theophylline had any demonstrable effect on cyclic AMP phosphodiesterase activity, indicating that the increases of cyclic AMP concentrations by catecholamines are due primarily to an increase in the rate of cyclic AMP generation.

Cyclic AMP concentrations in the tissue slices were studied in the presence of 10^{-2} M theophylline unless specified, to inhibit phosphodiesterase activity. That concentration was the lowest to increase cyclic AMP concentration. Theophylline increases cyclic AMP concentrations either in the presence or absence of catecholamines, but theophylline probably does not induce any qualitative changes in the effect of catecholamines on cyclic AMP concentration.

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REFERENCES

1. Sutherland, E. W., and G. A. Robison. 1966. Metabolic effects of catecholamines. *Pharmacol. Rev.* **18**: 145.
2. Sutherland, E. W., G. A. Robison, and R. W. Butcher. 1968. Some aspects of the biological role of cyclic AMP. *Circulation.* **37**: 279.
3. Gordon, R. D., O. Kuchel, G. W. Liddle, and D. P. Island. 1967. Role of the sympathetic nervous system in regulating renin and aldosterone production in man. *J. Clin. Invest.* **46**: 599.
4. Vander, A. J. 1965. Effects of catecholamines and the renal nerves on renin secretion in anesthetized dogs. *Amer. J. Physiol.* **209**: 659.
5. Wathen, R. L., W. S. Kingsbury, D. A. Stouter, E. G. Schneider, and H. H. Rostorfer. 1965. Effects of infusion of catecholamines and angiotensin II on renin release in anesthetized dogs. *Amer. J. Physiol.* **209**: 1012.
6. Michelakis, A. M., J. Caudle, and G. W. Liddle. 1969. In vitro stimulation of renin production by epinephrine, norepinephrine, and cyclic AMP. *Proc. Soc. Exp. Biol. Med.* **130**: 748.
7. Blotting, R., J. B. Farmer, and M. F. Lockett. 1961. The effect of subcutaneous adrenaline and isoprenaline on the excretion of electrolytes by rats. *Arch. Intern. Physiol. Biochem.* **69**: 203.
8. Baldwin, E. S., E. A. Gombos, and H. Chasis. 1963. Changes in sodium and water excretion induced by epinephrine and l-norepinephrine in normotensive and hypertensive subjects. *J. Lab. Clin. Med.* **61**: 832.
9. Fisher, D. A. 1968. Norepinephrine inhibition of vasopressin antidiuresis. *J. Clin. Invest.* **47**: 540.
10. Liberman, B., L. A. Klein, and C. R. Kleeman. 1970. Effect of adrenergic blocking agents on the vasopressin inhibiting action of norepinephrine. *Proc. Soc. Exp. Biol. Med.* **133**: 131.
11. Handler, J. S., R. Bensinger, and J. Orloff. 1968. Effect of adrenergic agents on toad bladder response to ADH, cyclic AMP, and theophylline. *Amer. J. Physiol.* **215**: 1024.
12. Gilman, A. G. 1970. A protein binding assay for adenosine 3′,5′-cyclic monophosphate. *Proc. Nat. Acad. Sci. U.S.A.* **67**: 305.
13. Herbert, V., K. S. Lau, C. W. Gettlieb, and S. J. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* **25**: 1375.
14. Miyamoto, E., J. F. Kuo, and P. Greengard. 1969. Cyclic nucleotide-dependent protein kinases. *J. Biol. Chem.* **244**: 6395.
15. Cheung, W. Y. 1967. Properties of cyclic 3′,5′-nucleotide phosphodiesterase from rat brain. *Biochemistry.* **6**: 1079.
16. Krishna, G., B. Weiss, and B. B. Brodie. 1968. A simple sensitive method for the assay of adenyl cyclase. *J. Pharmacol. Exper. Ther.* **163**: 379.
17. Nagata, N., and H. Rasmussen. 1970. Renal gluconeogenesis: effects of Ca^{++} and H^+. *Biochim. Biophys. Acta.* **215**: 1.
18. Beck, N. P., J. B. Field, and B. B. Davis. 1970. Effect of prostaglandin E_1, chlorpropamide, and vasopressin on cyclic AMP in renal medulla of rats. *Clin. Res.* **18**: 494.
19. Beck, N. P., F. DeRubertis, M. F. Michelis, R. D. Fusco, J. B. Field, and B. B. Davis. 1970. Prostaglandin E_1 inhibition of parathyroid hormone-induced increases in renal cortical adenyl cyclase activity, cyclic adenosine monophosphate concentration, P incorporation into phospholipid and in phosphate excretion. *J. Lab. Clin. Med.* **76**: 1005.