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Research Article

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Plasma cyclic AMP rose in response to infusions of the β -adrenergic agent, isoproterenol, or in response to infusions of either epinephrine or norepinephrine alone or in combination with the α -adrenergic-blocking agent, phentolamine. Although urinary cyclic AMP also rose, the percentage increase was less than that observed in the plasma. These treatments caused no increase in plasma cyclic GMP.

Plasma cyclic GMP rose in response to infusions of α -adrenergic agents, viz., epinephrine or norepinephrine infused together with the β -blocking agent, propranolol. These treatments caused no increase in plasma cyclic AMP.

These observations are consistent with the current concept that the actions of β -adrenergic agents are mediated by increases in cyclic AMP formation in target tissues. Such a mediating role has not been established for cyclic GMP, but the data suggest the possibility that cyclic GMP metabolism is responsive either to α -adrenergic stimulation or to parasympathetic stimulation which occurs as a reflexive consequence of the pressor effect of α -adrenergic agents.

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Effects of Catecholamines and Adrenergic-Blocking Agents on Plasma and Urinary Cyclic Nucleotides in Man

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ABSTRACT Studies were performed in healthy volunteers to determine the effects of catecholamines and adrenergic-blocking agents on plasma and urinary levels of adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP).

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A preliminary report of these studies was presented at the Annual Meeting of the American Federation for Clinical Research, 1970 (1).

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INTRODUCTION

Epinephrine was the first of several hormones that have now been demonstrated to effect their physiologic actions through stimulation of the formation of an intracellular "second messenger," adenosine 3',5'-monophosphate (cyclic AMP)¹ (2). Recently, some of these hormones (most notably glucagon and parathyroid hormone) have been shown to raise extracellular cyclic AMP levels as well (3-6). However, the possible effects of catecholamines on extracellular cyclic AMP have yet to be described in detail.

Interest in extracellular cyclic AMP derives largely from the fact that the concentration of cyclic AMP in extracellular fluids appears to be a convenient index of the effects of various hormones on target tissue levels of the nucleotide.

In the present study, various catecholamines and adrenergic-blocking agents were administered to human subjects, under otherwise basal conditions, to determine their effects on the levels of cyclic AMP and cyclic GMP in plasma and urine. As a means of studying the relationship between plasma and urinary cyclic nucleotides and to obtain an idea of whether they were derived from renal tissue (nephrogenous) or extrarenal tissue, simultaneous measurements were made of renal clearance of cyclic AMP, cyclic GMP, and inulin.

In the course of these studies, it became apparent that a clear distinction could be made between α - and β -adrenergic agents on the basis of the cyclic nucleotide that the agents affected. This distinction was facilitated by employing selective α - or β -adrenergic-block agents along with the agents epinephrine and norepinephrine, each of which by itself has both α - and β -adrenergic activities.

¹ Abbreviations used in this paper: cyclic AMP, adenosine 3',5'-monophosphate; cyclic GMP, guanosine 3',5'-monophosphate.

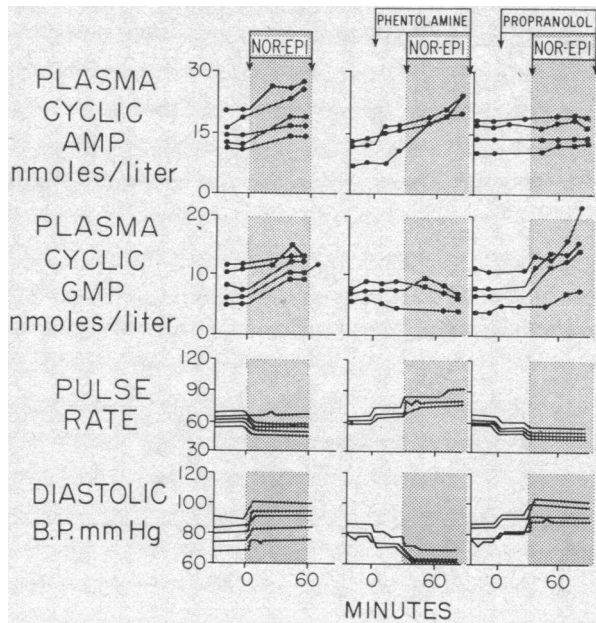


FIGURE 1 The contrasting effects of infusions of norepinephrine, norepinephrine-plus-phenolamine, and norepinephrine-plus-propranolol on plasma levels of cyclic AMP and cyclic GMP, pulse rate, and diastolic blood pressure. Norepinephrine was infused at a rate of $6.0 \mu\text{g}/\text{min}$ for 60 min into healthy, normal, well-hydrated male volunteers. When either of the blocking drugs was infused, an initial dose of 5.0 mg was given intravenously followed by infusions of either 0.5 mg/min of phentolamine or 0.08 mg/min of propranolol. After 45 min of blocking agent, norepinephrine was infused simultaneously for 60 min (indicated by the shaded area).

METHODS

Materials. Epinephrine (lot HJ 110) was purchased from Parke, Davis & Co. (Detroit, Mich.) Levarterenol bitartrate (Levophed) and isoproterenol hydrochloride (Isuprel) were obtained from Winthrop Laboratories (New York), propranolol hydrochloride (Inderal injectable) from Ayerst Laboratories (New York), lyophilized phentolamine mesylate (Regitine) from Ciba Pharmaceutical Company (Summit, N. J.), inulin from Arnar-Stone Laboratories, Inc. (Mount Prospect, Ill.), ascorbic acid solution (Cenolate) from Abbott Laboratories (North Chicago, Ill.), cyclic AMP- ^3H (2.35 Ci/mmole) from Schwarz Bio Research Inc. (Orangeburg, N. Y.), and cyclic GMP- ^3H (2.6 Ci/mmole) from Calbiochem (Los Angeles, Calif.).

Subjects and procedures. Healthy, normotensive males in excellent physical condition between the ages of 21 and 32 were selected as subjects for these studies. All volunteers had fasting blood-glucose levels of less than 100 mg/100 ml. They were fasted overnight before the catecholamine infusions, and on the morning of study were hydrated with 0.45% sodium chloride intravenously and water orally to achieve a urine flow of 10–15 ml/min. Pulse and blood pressure were checked every 5 min. Blood specimens were obtained every 15 min through a catheter in the antecubital vein of the arm opposite the infusion site. Voided urine specimens were collected every 15 min with the patients

recumbent. A priming dose of 50 mg of inulin/kg was followed by an infusion of inulin calculated to produce a plasma concentration of 0.3 mg/ml. 1 hr was allowed for equilibration before the beginning of the first control period. Epinephrine, norepinephrine, and isoproterenol were diluted in isotonic saline containing ascorbic acid (2.5 mg/ml) to protect against oxidation and administered by a constant flow infusion pump. The subjects were infused with $6.0 \mu\text{g}/\text{min}$ of epinephrine or norepinephrine. Isoproterenol infusion rates, although constant in any one study, ranged in various studies, from 0.6 to $6.0 \mu\text{g}/\text{min}$. When either propranolol or phentolamine was used, an initial dose of 5 mg of either drug was given intravenously followed by a constant infusion of propranolol 0.08 mg/min or phentolamine 0.5 mg/min. The blocking agent was infused alone for 45 min at which time an epinephrine or norepinephrine infusion was added for 60 min. The subsequent handling of plasma and urine samples as well as the measurement of cyclic AMP, cyclic GMP, and inulin have been described in detail in previous publications (4, 6, 7).

RESULTS

Effects of α -adrenergic agents on plasma cyclic GMP. Norepinephrine, a mixed adrenergic agent, was given in combination with propranolol, a β -adrenergic-blocking agent, in order to achieve a relatively pure α -adrenergic effect. Cyclic GMP rose in plasma; the diastolic blood pressures increased, and the cardiac rates slowed in all cases (Fig. 1).

Epinephrine, which also has mixed α - and β -adrenergic activities, was also given in combination with pro-

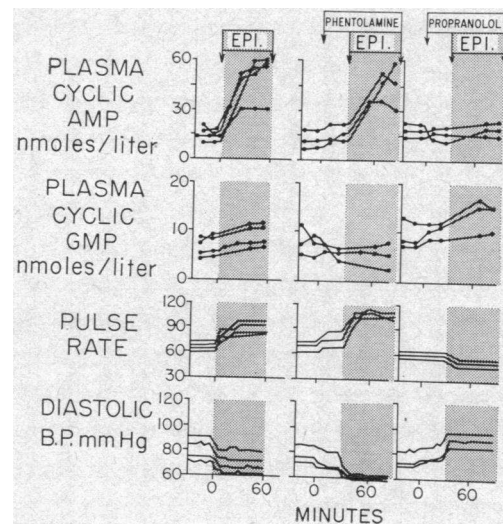


FIGURE 2 The contrasting effects of infusions of epinephrine, epinephrine-plus-phenolamine, and epinephrine-plus-propranolol on plasma levels of cyclic AMP and cyclic GMP, pulse rate, and diastolic blood pressure. Epinephrine was infused at a rate of $6.0 \mu\text{g}/\text{min}$ for 60 min. Conditions otherwise were identical with those stated in Fig. 1. In one subject, plasma levels of propranolol were measured and found to be between 30 and 45 ng/ml during the epinephrine infusion.

pranolol in order to study its effects as an α -adrenergic agent. This combination of drugs also caused increases in plasma cyclic GMP, increases in diastolic blood pressure, and slowing of the cardiac rate (Fig. 2).

Urinary cyclic GMP tended to change in the same direction as plasma cyclic GMP but the changes were not sufficiently uniform to justify meaningful interpretation.

Lack of effect of α -adrenergic agents on plasma cyclic AMP. In contrast to cyclic GMP, cyclic AMP levels in plasma did not change significantly during the infusion of norepinephrine-plus-propranolol, or of epinephrine-plus-propranolol (Figs. 1 and 2).

Under most circumstances normal subjects excrete more cyclic AMP in their urine than can be accounted for by glomerular filtration of plasma cyclic AMP. This

excess has been referred to as "nephrogenous" cyclic AMP (6). As shown in Table I, nephrogenous cyclic AMP in several normal subjects under control conditions amounted to between 1 and 4 nmoles/min (49 ± 3 (SEM) % of their total urinary cyclic AMP).

Urinary cyclic AMP rose slightly during infusions of norepinephrine-plus-propranolol or of epinephrine-plus-propranolol despite concomitant slight decreases in glomerular filtration rate as measured by inulin clearance (Table I). Thus, there was a consistent increase in the ratio of cyclic AMP clearance: inulin clearance, which can be interpreted as evidence of increased excretion of nephrogenous cyclic AMP in response to α -adrenergic agents (Table I).

Effects of β -adrenergic agents on cyclic AMP. Isopro-

TABLE I
The Effects of Catecholamines on Apparent Nephrogenous Cyclic AMP

Experiment	Subject	Plasma cyclic AMP	Total urinary cyclic AMP	Inulin clearance	Cyclic AMP clearance	Cyclic AMP/ Cinulin	Apparent filtered cyclic AMP	Apparent nephrogenous cyclic AMP
		<i>nmoles/liter</i>	<i>nmoles/min</i>	<i>ml/min</i>	<i>ml/min</i>		<i>nmoles/min</i>	<i>nmoles/min</i>
1 Control period	J. C.	14.6	3.19	111	218	1.96	1.62	1.57
Epinephrine, 6 μ g/min*		31.8	3.91	117	123	1.05	3.72	0.19
2 Control period	J. C.	21.8	5.83	147	267	1.82	3.20	2.63
Epinephrine, 6 μ g/min		56.4	8.50	145	150	1.04	8.18	0.32
3 Control period	K. S.	15.0	3.55	122	236	1.93	1.83	1.72
Epinephrine, 6 μ g/min		60.2	7.63	125	127	1.02	7.53	0.10
4 Control period	T. C.	18.2	2.51	88	138	1.57	1.60	0.91
Epinephrine, 6 μ g/min plus propranolol, 80 μ g/min		19.8	3.10	71	157	2.21	1.41	1.69
5 Control period	J. C.	19.7	4.76	117	243	2.08	2.30	1.46
Epinephrine, 6 μ g/min plus propranolol, 80 μ g/min		19.8	6.28	109	317	2.91	2.16	4.12
6 Control period	K. S.	15.6	3.40	108	245	2.27	1.68	1.72
Epinephrine, 6 μ g/min plus propranolol, 80 μ g/min		17.1	4.25	73	249	3.41	1.25	3.00
7 Control period	F. D.	11.2	2.80	126	250	1.98	1.41	1.39
Norepinephrine, 6 μ g/min		14.7	3.07	122	209	1.71	1.79	1.28
8 Control period	K. S.	14.0	3.12	113	223	1.97	1.58	1.54
Norepinephrine, 6 μ g/min		15.6	2.81	125	180	1.44	1.95	0.86
9 Control period	W. P.	21.6	3.44	107	159	1.49	2.31	1.13
Norepinephrine, 9 μ g/min		26.7	4.06	104	152	1.46	2.78	1.28
10 Control period	H. H.	16.3	6.03	108	370	3.42	1.76	4.27
Norepinephrine, 6 μ g/min plus propranolol, 80 μ g/min		17.4	7.00	107	402	3.76	1.86	5.14
11 Control period	D. L.	10.6	4.12	139	389	2.80	1.47	2.65
Norepinephrine, 6 μ g/min plus propranolol, 80 μ g/min		13.4	4.53	117	338	2.89	1.57	2.96
12 Control period	G. A.	14.0	3.00	94	214	2.28	1.32	1.68
Norepinephrine, 6 μ g/min plus propranolol, 80 μ g/min		13.9	3.82	64	275	4.30	0.89	2.93
13 Control period	K. S.	12.8	3.87	139	302	2.17	1.78	2.09
Isoproterenol, 0.6 μ g/min		22.1	4.23	122	191	1.57	2.70	1.53
14 Control period	K. S.	16.3	3.43	136	210	1.54	2.22	1.21
Isoproterenol, 1.2 μ g/min		32.3	3.52	112	110	0.98	3.62	-0.10
15 Control period	K. S.	14.4	3.97	138	274	1.98	1.99	1.98
Isoproterenol, 2.0 μ g/min		38.6	4.44	114	115	1.01	4.40	0.04
16 Control period	K. S.	14.9	4.29	103	288	2.80	1.53	2.76
Isoproterenol, 3.0 μ g/min		43.6	5.92	113	136	1.20	4.93	0.99

* Plasma and urine levels of cyclic AMP were at or near plateau values during the final 30 min of catecholamine infusion (Figs. 1-3). The values shown here represent average values taken from this period and from the 30 min control period before drug infusion.

terenol, a relatively selective β -adrenergic agent, induced dose-related increases in plasma cyclic AMP (Fig. 3). Little change was observed in urinary cyclic AMP when doses of 3 $\mu\text{g}/\text{min}$ or less were given (Table I), and slight decreases were observed in inulin clearance. The subject could not void regularly during infusion of 6 $\mu\text{g}/\text{min}$. These doses of isoproterenol were sufficient to increase the cardiac rate by 15–60 beats/min with concomitant widening of the pulse pressure. Systolic pressures rose by as much as 20 mm Hg while diastolic pressures fell by 20–35 mm Hg (Fig. 3).

Norepinephrine or epinephrine (agents producing mixed α - and β -effects) were given in combination with phentolamine, an α -adrenergic-blocking agent, in order to achieve relatively pure β -adrenergic effects. The results were similar to those observed with isoproterenol. Plasma cyclic AMP levels increased by as much as threefold with norepinephrine-plus-phentolamine (Fig. 1) and by as much as fivefold with epinephrine-plus-phentolamine (Fig. 2).

Marked decreases in urine volume during the infusions of norepinephrine-plus-phentolamine or of epinephrine-plus-phentolamine precluded meaningful studies of renal clearance of cyclic nucleotides in most of these experiments. In those studies in which adequate data were available, there was little or no increase in urinary cyclic AMP. During infusions of isoproterenol, apparent nephrogenous cyclic AMP in the urine fell strikingly (Table I).

Lack of effect of β -adrenergic agents on cyclic GMP. In contrast to cyclic AMP, cyclic GMP levels in plasma did not change significantly during the infusion of isoproterenol, norepinephrine-plus-phentolamine, or epinephrine-plus-phentolamine (Figs. 1–3).

Effects of mixed adrenergic agents on cyclic nucleotides. Norepinephrine has both α - and β -adrenergic activity, although it is in general relatively more potent as an α - than as a β -agent. When administered in the absence of adrenergic blocking agents (Fig. 1), norepinephrine caused modest increases in plasma levels of both cyclic GMP and cyclic AMP. Diastolic blood pressures increased and the cardiac rates slowed. Urinary cyclic AMP rose little if at all, and the clearance ratio (cyclic AMP: inulin) decreased slightly (Table I). There was no significant change in inulin clearance.

Epinephrine too is a mixed adrenergic agent, but it is relatively more potent as a β - than as an α -agent. When administered in the absence of adrenergic-blocking agents, epinephrine caused small but consistent increases in plasma cyclic GMP (Fig. 2). Epinephrine infusions brought about two- to fourfold increases in plasma cyclic AMP and lesser increases in urinary cyclic AMP (Fig. 2 and Table I). There was no significant change in inulin clearance. Comparison of cyclic AMP and

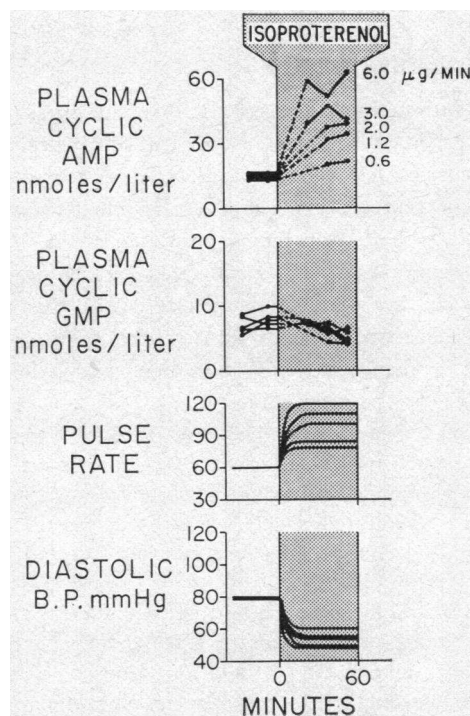


FIGURE 3 The dose-response relationship between infused isoproterenol and plasma levels of cyclic AMP and cyclic GMP, pulse, and diastolic blood pressure in a fasting, well-hydrated normal subject. The doses of isoproterenol in micrograms per minute are indicated beside the plasma cyclic AMP curve. Pulse rate rose in direct proportion and diastolic blood pressure fell in inverse proportion to the amount of isoproterenol infused. Time is expressed in relation to the beginning of the isoproterenol infusion.

inulin clearance rates indicated that, as was the case with isoproterenol, epinephrine caused a marked decrease in the amount of apparent nephrogenous cyclic AMP in the urine and that the increase in total urinary cyclic AMP that occurred during the infusion was due to the filtration of increased levels of plasma cyclic AMP (Table I).

Both epinephrine and norepinephrine tended to increase cyclic GMP in urine, but the changes were not consistent enough to allow firm conclusions to be drawn.

Effects of phentolamine and propranolol on cyclic nucleotides. Phentolamine, an α -adrenergic-blocking agent, caused small but consistent increases in pulse rate and decreases in diastolic blood pressure. At the same time there were small but consistent increases in plasma cyclic AMP but no consistent changes in plasma cyclic GMP. Propranolol alone, while tending to decrease the pulse rate and increase the diastolic blood pressure, caused no consistent changes in plasma cyclic AMP or cyclic GMP (Figs. 1 and 2).

DISCUSSION

Since β -adrenergic agents are known to stimulate cyclic AMP formation in several tissues (myocardium, fat, liver, muscle, etc.) (8-11), it might have been anticipated that a portion of the cyclic AMP thus formed might escape into the extracellular space and lead to increases in plasma cyclic AMP. It remained for the present investigation, however, to demonstrate that this actually occurred in man during treatment with doses of isoproterenol, epinephrine, and norepinephrine which are within the range used clinically.

The fact that the β -adrenergic-blocking agent, propranolol, abolishes the effect of catecholamines on plasma cyclic AMP while abolishing the cardiovascular effects of catecholamines is consistent with the conventional view that the β -adrenergic effects of catecholamines are mediated by cyclic AMP.

It should not be assumed that extracellular cyclic AMP is important as a mediator of catecholamine action, for it has been shown that 30-fold elevations of plasma cyclic AMP occurring in response to glucagon are without any clear-cut effect on pulse rate or pulse pressure (4). From studies with exogenous cyclic AMP, one might infer that circulating cyclic AMP would have to be at least 1000 times normal in order to exert physiological effects comparable with those that can be seen after the administration of hormones (12, 13). The major actions of cyclic AMP are thought to occur within cells. Hormonal stimulation of adenylate cyclase is an efficient means of elevating intracellular cyclic AMP; passage of cyclic AMP from extracellular fluid into the cell is an inefficient means of elevating intracellular cyclic AMP. The possibility that extracellular cyclic nucleotides can, at or near their sites of release, reach concentrations that are high enough to affect nearby cells cannot be ruled out, however.

Because epinephrine and norepinephrine have mixed α - and β -adrenergic activities, studies of their actions have contained many paradoxes. By using these agents in combination with either α - or β -adrenergic-blocking drugs, one can observe either a relatively pure β - or a relatively pure α -effect, respectively. Under these conditions, the physiologic responses were consistent, and there was qualitatively specific correlation between the type of adrenergic stimulus that was employed and the kind of cyclic nucleotide response that was produced. Thus, plasma cyclic AMP consistently rose in response to β -adrenergic agents but not in response to α -adrenergic agents, while the reverse was true for plasma cyclic GMP.

Studies in a variety of tissues have shown that there was no increase in intracellular cyclic AMP in response

to α -adrenergic stimulation. On the contrary, α -adrenergic agents sometimes lower cyclic AMP levels which have been elevated by other agents (9).

The meaning of the rise in cyclic GMP in response to α -adrenergic agents is not clear. Since α -adrenergic agents cause vasoconstriction, they lead to reflex activation of the parasympathetic nervous system with resultant bradycardia. It is conceivable that the rise in plasma cyclic GMP observed during treatment with α -adrenergic agents is in reality an indirect response to cholinergic activity, or perhaps other factors, rather than a direct response to α -adrenergic activity. George, Polson, O'Toole, and Goldberg (14) have recently shown that acetylcholine causes an increase in the level of cyclic GMP (and a fall in the level of cyclic AMP) in the isolated, perfused rat heart and Kuo et al. (15) have made similar observations in heart and brain slices. In the absence of other lines of evidence, it is premature to conclude that cyclic GMP is involved in the actions of α -adrenergic agents. Clarification of the relationship between cyclic GMP and cholinergic agents also will require further study.

Changes in urinary cyclic AMP that were observed during treatment with catecholamines should be interpreted conservatively at this time. Contributing to the complexity of interpreting changes in urinary cyclic AMP is the fact that part of this nucleotide in the urine is derived from plasma and part from the kidney tissue (6). Even though urinary cyclic AMP rose in response to β -adrenergic agents, this increase was less than proportional to the simultaneous increase in plasma cyclic AMP. The apparent renal clearance of cyclic AMP fell to levels closely approximating the renal clearance of inulin. It has been shown previously that parathyroid hormone leads to increased excretion of nephrogenous cyclic AMP (3, 5). Ancillary studies have indicated that β -adrenergic agents do not prevent the expected increase in the excretion of cyclic AMP during a concomitant infusion of parathyroid hormone. Although it appears that the excretion of nephrogenous cyclic AMP is diminished by β -adrenergic agents, the mechanism of this effect is obscure at the present time. The mechanism of the effect of α -adrenergic agents in increasing apparent nephrogenous cyclic AMP is likewise obscure.

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