# Sources of Cyclic Nucleotides in Plasma

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ABSTRACT In order to determine the sites of net production and removal of the cyclic nucleotides in plasma, various blood vessels were catheterized in 17 anesthetized dogs and arterial and venous concentrations of adenosine 3′,5′-monophosphate (cAMP) and guanosine 3′,5′-monophosphate (cGMP) were measured by radioimmunoassay.

Aortic cAMP was 30±2 nM (mean±SE) and cGMP was 13±1 nM. There were no significant differences for either cyclic nucleotide between the concentration in the aorta and that in the inferior vena cava, coronary sinus, hepatic vein, and femoral vein. The concentration of cAMP in renal venous plasma was 25% lower than in aortic plasma, and renal venous cGMP was 51% lower than in the aorta. The pulmonary arterial concentrations of cAMP and cGMP were slightly lower than in the aorta. The concentration of cGMP in the superior mesenteric vein plasma was 83% greater than in aortic plasma; the concentration of cAMP in this vessel was only 16% greater than that in the aorta. Superior vena cava concentrations of both cyclic nucleotides were slightly greater than arterial concentrations.

The results suggest that : (*a*) the kidneys are a major site of removal of both cyclic nucleotides from plasma. (*b*) The lungs may be a site of net addition of both cyclic nucleotides to plasma. (*c*) The small intestine is a site of net production of both cyclic nucleotides, particularly cGMP. (*d*) The liver probably removes cyclic nucleotides from plasma. (*e*) Since no other organs or regions studied added detectable net amounts of cyclic nucleotides to plasma, and since the turnover of these compounds in plasma is known to be rapid, the production of plasma cyclic nucleotides under basal conditions may well be the result of small net contributions from many tissues or bidirectional fluxes between tissues and plasma, or both.

## INTRODUCTION

It has been demonstrated that the turnover of adenosine 3′,5′-monophosphate (cAMP)[1] and guanosine 3′,5′-monophosphate (cGMP) in human plasma is rapid (1), and that certain hormones can greatly alter the concentration of these cyclic nucleotides in plasma and urine (2–8). For example, cAMP levels in plasma and urine can be increased by the administration of parathyroid hormone (PTH) (3), glucagon (4), or epinephrine (4–6), and infusions of calcium (2) and norepinephrine (5, 6) are each capable of elevating plasma and urinary cGMP. It is therefore possible that determinations of the level of these cyclic nucleotides in plasma and urine could be of considerable value in the investigation and diagnosis of human disease states. Indeed, it has already been found that certain patients with hyperparathyroidism excrete increased amounts of cAMP in the urine (7, 8), and that patients with pseudohypoparathyroidism fail to exhibit a normal rise in urinary excretion of cAMP after PTH administration (3).

It is desirable to identify the sites of production and removal of the plasma cyclic nucleotides, for such information should facilitate the interpretation of the changes in concentration of plasma cyclic nucleotides

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[1] *Abbreviations used in this paper:* c, cyclic; IVC, inferior vena cava; PTH, parathyroid hormone; SVC, superior vena cava.

that occur after stimulation with certain hormones and in certain disease states. Furthermore, since data obtained in humans indicates that urinary cyclic nucleotides are derived largely from plasma, understanding of factors regulating plasma concentrations of the nucleotides is also required for interpretation of urinary concentrations. In an attempt to identify organs or tissues that contribute to the regulation of plasma concentrations of cAMP and cGMP, we have measured plasma arteriovenous concentration differences for these nucleotides across specific organs and anatomic regions in anesthetized dogs (9).

## METHODS

Iodinated derivatives of the cyclic nucleotides and the antibodies used in the radioimmunoassay were prepared as described elsewhere (10). cAMP, cGMP, and beef heart 3',5'-cyclic nucleotide phosphodiesterase were purchased from Sigma Chemical Co., St. Louis, Mo. Rabbit gamma globulin was obtained from Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill. ACTH (Cortrosyn) was obtained from Organon, Inc., West Orange, N. J. Polyethylene catheters were purchased from U. S. Catheter & Instrument Corp., Glens Falls, N. Y.

*Preparation of animals.* Adult male mongrel dogs weighing between 18 and 25 kg were starved overnight and anesthetized with sodium pentobarbital, 27.5 mg/kg body wt, injected intravenously. Respiration was maintained by ventilation with room air through a cuffed endotracheal tube by a Harvard respirator pump (Harvard Apparatus Co., Inc., Millis, Mass.) During the course of the experiments the animals were infused continuously with a 0.9% (wt/vol) sodium chloride solution at a rate of 5 ml/min.

Mean intra-arterial blood pressure was monitored by a Moss manometer connected to a catheter inserted into the abdominal aorta. Mean arterial blood pressure in the animals used in these experiments remained stable in the range of 110–140 mm Hg. At the start and conclusion of each experiment, samples of arterial blood were drawn into a heparinized air-tight syringe for subsequent determination of the pH; values ranged between 7.30 and 7.50.

Three different groups of experiments were performed. In the first, incisions were made to expose the femoral vessels and the external jugular veins bilaterally. A catheter was introduced into the femoral artery on the right side, and its tip was positioned in the abdominal aorta for measurement of the blood pressure, as described above. In addition, the samples of arterial blood were drawn through this catheter. Next, the abdominal cavity was opened by a midline incision. A catheter was inserted into the right femoral vein, and the tip was advanced to a position in the inferior vena cava (IVC) about 4–5 cm above the level of the diaphragm. The superior mesenteric vein was identified, and a 19-gauge Intracath (Jelco Laboratories, Raritan, N. J.) was inserted into the vessel and secured to its ventral endothelial surface so that the flow of blood through the vein was not impeded. Another catheter was passed via the right external jugular vein through the right atrium and into the hepatic vein. Finally another catheter was passed via the left external jugular vein into the superior vena cava (SVC) to a position 4–5 cm from its entry into the right atrium. Thus, blood could be obtained from the aorta, from the IVC before its entry into the right atrium, from the SVC before its entry into the right atrium, from the superior mesenteric vein, and from the hepatic vein.

In the second group of experiments the thorax was entered via a midline sternotomy, and the pericardium was incised. A catheter was advanced from the right external jugular vein through the right atrium and right ventricle into the pulmonary artery. Another catheter was placed in the SVC through the right external jugular vein. Arterial blood was obtained from the aortic blood pressure line as described above. Through a femoral cut-down on the left side, a catheter was inserted into the femoral vein and advanced distally. The left femoral artery was left undisturbed so that the left femoral venous blood would represent the effluent of a normally perfused limb. In these experiments blood was drawn from the aorta, from the pulmonary artery, from the SVC, from the femoral vein, and, by repeated puncture with a 22-gauge needle, from the coronary sinus.

In the final group of experiments, one catheter was placed in the abdominal aorta, as above, and another was inserted into the left femoral vein and positioned 3–4 cm into the right renal vein, under direct vision with the abdomen open.

In all experiments, 1 h was allowed for the physiologic status to stabilize between the time of positioning of the last catheter and the collection of the first sample. At the conclusion of the experiment the correct position of all catheters was confirmed by dissection.

In one animal, the left phrenicoabdominal vein, into which the left adrenal vein empties, was catheterized. Samples of this venous plasma and arterial plasma were withdrawn and analyzed for cAMP and cGMP concentrations, both before and after the systemic intravenous injection of 0.05 mg (5 U) of Cortrosyn.

*Collection of blood samples.* Blood was aspirated simultaneously from all vessels into disposable plastic syringes at a rate of 6 ml/30 s; one sample of blood was taken from each vessel, except in the case of arterial blood, where two samples were obtained. These drawings were repeated three or four times at 15–20-min intervals. Samples of blood (4–6 ml) were rapidly transferred to heparinized centrifuge tubes and mixed well. After centrifugation at 1,500 *g* for 3–5 min, 1.5 ml of plasma was aspirated immediately and was added to an equal volume of 10% trichloroacetic acid (TCA) for deproteinization.

It was determined in preliminary studies that there was no marked difference between concentrations of cyclic nucleotides in peripheral venous plasma of anesthetized dogs and those of unanesthetized dogs.

It was noted that when arterial plasma from several dogs was incubated in vitro at 25°C, the half-time for disappearance of both cAMP and cGMP ranged from 25 to 135 min. Incubation of samples of renal vein plasma and superior mesenteric vein plasma obtained at the same time yielded parallel disappearance curves, demonstrating that concentration differences between the vessels could not be accounted for by different rates of metabolism. These rates of degradation are comparable to those found in human plasma (1) and much lower than those described for rat plasma (11). It can be calculated that even at the most rapid rate of degradation observed, less than 20% of the cyclic nucleotide in the sample would be destroyed before deproteinization with TCA.

In order to assess the possible contributions of the formed elements of blood to the metabolism of cyclic nucleotides in plasma, several experiments were performed.

TABLE I

*Effect of Phosphodiesterase and Recovery of Added Cyclic Nucleotides in Samples of Plasma as Measured by Radioimmunoassays for cAMP and cGMP*

| Sample* | Volume | cAMP measured | | | | | cGMP measured | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample alone | | Sample + 2 pmol/tube cAMP | | Recovery | Sample alone | | Sample + 1 pmol/tube cGMP | | Recovery |
| | 1 | −PDE | +PDE‡ | −PDE | +PDE | | −PDE | +PDE | −PDE | +PDE | |
| | *µl* | *pmol/tube* | | | | % | *pmol/tube* | | | | % |
| 1 | 100 | 1.96 | <0.125 | 3.7 | 0.15 | 87 | 1.13 | <0.03 | 2.3 | <0.03 | 117 |
| 2 | 100 | 2.41 | 0.14 | 4.6 | 0.13 | 110 | 1.41 | 0.06 | 2.3 | 0.0 | 89 |
| 3 | 100 | 1.47 | <0.125 | 3.3 | <0.125 | 92 | 2.27 | 0.04 | 3.2 | 0.05 | 93 |

PDE, cyclic 3′,5′-nucleotide phosphodiesterase.

* Each sample of plasma was divided into two aliquots. One was processed with no addition; the other was added to an equal volume of TCA (10 g/100 ml) which contained cGMP (10 pmol/ml) and cAMP (20 pmol/ml), and subsequently processed routinely for measurement of cyclic nucleotide content.

‡ Treatment of the samples with phosphodiesterase was performed as follows: to 100 µl of the plasma extract was added 5 µl of 0.1 M $MgCl_2$ and 2 µl of beef heart phosphodiesterase (2 U/ml) and the volume brought to 300 µl with sodium acetate buffer. After incubation at 37°C for 60 min the samples were boiled for 3 min, and the mixture was used directly in the radioimmunoassay.

First, concentrations of both nucleotides in samples of arterial whole blood and in plasma derived from these samples of whole blood were measured. Plasma concentrations of both nucleotides exceeded those in whole blood, indicating that the levels in the intracellular volume were lower than in plasma. From the observed hematocrit, it was calculated that concentrations in the cellular volume were 25–35% of plasma concentrations for cAMP and 65–75% of plasma concentration for cGMP. We do not know which of the formed elements contains the majority of the intracellular cyclic nucleotides in whole blood.

Second, experiments were performed in which $^3$H-labeled cyclic nucleotides were added to a large aliquot of arterial blood incubated in vitro at 25°C. The concentration of each $^3$H-labeled cyclic nucleotide in whole blood was about one-half that found in plasma. Degradation curves of the labeled cyclic nucleotides in whole blood and in plasma were parallel, implying that there was little net uptake of the nucleotides from plasma by blood cells. The concentrations of both unlabeled cyclic nucleotides in plasma decreased with time, although somewhat less rapidly than the disappearance of the tracers. The decrease in specific activities indicated that one or more of the formed elements are capable of contributing small amounts of both nucleotides to plasma; the magnitude of this process was greater for cAMP than for cGMP.

*Preparation of samples for assay of cyclic nucleotides.* The protein precipitates of the mixture of plasma and TCA were sedimented by centrifugation at 2,000 *g* for 20 min. 1.5 ml of the resulting supernate was aspirated and washed four times with 5 vol of diethyl ether saturated with water. The aqueous phase was evaporated to dryness at 60°C under a stream of air. The dry residue was resuspended in 750 µl of sodium acetate buffer (0.05 M, pH 6.2) and aliquots of this solution were assayed for cAMP and cGMP by the radioimmunoassay procedures described by Steiner, Parker, and Kipnis (10), and Wehmann, Blonde and Steiner (12).

The validity of the radioimmunoassays for measuring the cyclic nucleotides in TCA extracted samples of canine plasma was ascertained by two procedures. First, the recovery of cyclic nucleotides added to plasma before deproteinization with TCA was found to be 87–117% for each compound (Table I). Second, the effect of treatment of the plasma extract with phosphodiesterase was investigated. Table I shows that for each cyclic nucleotide, more than 90% of the immunoreactive material in the assay was destroyed by this treatment.

*Statistical analysis.* Plasma concentrations values for each vessel from all collection periods were compared with the corresponding aortic plasma concentration by *t* test of paired observations (13).

## RESULTS

Concentrations of cyclic nucleotides in aortic plasma were compared with the concentrations in plasma obtained from the other blood vessels. The absolute values of all the samples are given in Tables II–VI.

The concentration of cAMP in aortic plasma from 16

TABLE II

*Concentration of cAMP in Plasma from Aorta, Femoral Vein, Coronary Sinus, Pulmonary Artery, and SVC*

| Dog | Aorta | Femoral vein | Coronary sinus | Pulmonary artery | SVC |
|---|---|---|---|---|---|
| | | | *nM* | | |
| 1 | 24.1±3.4* | 32.6±1.8 | 26.9±2.8 | 21.4±1.6 | 24.2±1.3 |
| 2 | 40.3±1.0 | 34.4±3.8 | 35.0±3.2 | 31.0±3.4 | 30.4±3.0 |
| 3 | 20.8±0.6 | 24.1±1.6 | 22.1±0.9 | 21.6±2.1 | 24.1±0.8 |
| 4 | 23.0±1.3 | 24.9±2.5 | 21.8±1.6 | 17.7±1.1 | 31.8±3.5 |
| Mean‡ | 27.0±4.5 | 29.0±2.6 | 26.4±3.1 | 22.9±2.8 | 29.9±3.7 |

* For each vessel, the mean and SEM of four determinations is given.

‡ This figure represents the mean and SEM of all 16 determinations.

TABLE III

*Concentration of cGMP in Plasma from Aorta, Femoral Vein, Coronary Sinus, Pulmonary Artery, and SVC*

| Dog | Aorta | Femoral vein | Coronary sinus | Pulmonary artery | SVC |
|---|---|---|---|---|---|
| | | | *nM* | | |
| 1 | 8.8±0.2 | 8.6±0.5 | 8.6±0.7 | 8.0±0.2 | 7.0±0.8 |
| 2 | 16.4±0.5 | 17.2±1.2 | 15.7±0.9 | 14.7±0.6 | 17.3±1.1 |
| 3 | 13.4±0.4 | 13.8±0.6 | 13.8±0.6 | 12.8±0.6 | 14.4±0.8 |
| 4 | 10.2±0.3 | 8.8±0.2 | 10.1±0.3 | 9.2±0.5 | 11.2±0.3 |
| Mean | 12.2±1.7 | 12.1±2.0 | 12.0±1.6 | 11.2±1.6 | 12.5±2.2 |

Data are presented as in Table II.

dogs was 30±2 nM (mean±SE) with values ranging from 21 to 48. The level of cGMP from 17 dogs was about one half this value, 13±1 nM, with values ranging from 8.5 to 18. The concentrations in individual dogs were quite stable throughout the several collection periods, since the average coefficient of variation of the means for individual experiments, (SD ÷ mean) × 100%, was 9% for cAMP and 7% for cGMP.

The possibility that the level of one nucleotide might be related to that of the other was investigated by an unweighted linear regression analysis. When the mean aortic concentration of cAMP was plotted as a function of the mean aortic concentration of cGMP, the correlation coefficient derived was equal to 0.28, indicating that there was no strong correlation between the levels in the aortic plasma for the group of dogs.

For each collection period in the various groups, the concentration of cyclic nucleotide in the plasma of each vessel was expressed as a percent of the corresponding concentration in aortic plasma of that dog. The mean relative concentrations in each vessel are illustrated in Figs. 1 and 2.

Concentrations of cAMP were not significantly different in plasma from the aorta, hepatic vein, femoral vein, coronary sinus, and IVC (Fig. 1). The concentration of cAMP in the renal vein plasma was 25% lower than that from the aorta ($P < 0.001$), while that in the pulmonary artery was 13% lower than in the aorta ($P < 0.01$). SVC cAMP was 10% greater than in the aorta ($P < 0.01$). Data for SVC were pooled from both groups of experiments. Superior mesenteric vein plasma cAMP was 16% greater than the aortic concentration ($P < 0.001$). These results suggest that the kidneys remove cAMP from the circulating plasma, while the lungs and intestine contribute cAMP to the plasma. It appears furthermore, that some cAMP is added to blood by organs whose effluent blood returns to the SVC. It should be pointed out that while statistically significant by our method of analysis, such small differences as 15% or less are close to the limits of detectability by the radioimmunoassays. Clinical usefulness of measuring such differences would therefore be slight.

Renal venous plasma cGMP concentration was 51% lower than arterial ($P < 0.001$), a greater net differ-

TABLE IV

*Concentration of cAMP in Plasma from Aorta, Hepatic Vein, IVC, SVC, and Superior Mesenteric Vein*

| Dog | Aorta | Hepatic vein | IVC | SVC | Superior mesenteric vein |
|---|---|---|---|---|---|
| | | | *nM* | | |
| 5 | 32.5±0.9* | 34.1±0.8 | 31.2±0.7 | 36.5±1.1 | 34.4±1.8 |
| 6 | 27.4±2.3 | 22.7±1.1 | 24.9±2.6 | 30.4±2.6 | 31.6±3.7 |
| 7 | 32.4±1.8 | 27.3±4.1 | 30.4±2.5 | 28.7±1.5 | 35.6±0.9 |
| 8 | 21.9±0.9 | 25.5±1.4 | 25.5±1.9 | 24.8±0.7 | 29.7±3.0 |
| 9 | 47.6±0.9 | 49.2±1.7 | 51.1±3.2 | 54.2±4.0 | 58.1±4.1 |
| 10 | 34.0±0.6 | 31.7±3.2 | 37.2±3.9 | 39.8±3.0 | 39.1±3.0 |
| Mean‡ | 32.6±3.5 | 31.8±3.9 | 33.4±4.0 | 35.7±4.3 | 38.1±4.2 |

* For each vessel, the mean and SEM of three determinations is given.
‡ This figure represents the mean and SEM of all 18 determinations.

TABLE V

*Concentration of cGMP in Plasma from Aorta, Hepatic Vein, IVC, SVC, and Superior Mesenteric Vein*

| Dog | Aorta | Hepatic vein | IVC | SVC | Superior mesenteric vein |
|---|---|---|---|---|---|
| | | | *nM* | | |
| 5 | 10.5±0.9 | 11.9±0.3 | 9.4±0.3 | 11.9±0.3 | 15.4±1.4 |
| 6 | 9.8±0.4 | 11.2±1.3 | 10.8±0.5 | 10.8±0.6 | 21.1±1.4 |
| 7 | 15.5±0.9 | 11.4±0.9 | 16.1±1.3 | 16.3±0.8 | 31.8±2.2 |
| 8 | 16.2±0.4 | 17.5±0.3 | 13.9±1.2 | 18.9±1.1 | 30.8±1.4 |
| 9 | 14.3±0.4 | 14.1±0.9 | 16.2±0.3 | 16.6±0.5 | 21.7±3.1 |
| 10 | 11.3±0.9 | 9.8±0.7 | 13.5±1.8 | 14.4±1.7 | 21.5±1.3 |
| Mean | 12.9±1.1 | 12.6±1.1 | 13.3±1.1 | 14.8±1.1 | 23.7±2.6 |

Data are presented as in Table IV.

TABLE VI

*Concentrations of cGMP and cAMP in Plasma from Aorta and Renal Vein*

| Dog | cGMP | | cAMP | |
|---|---|---|---|---|
| | Aorta | Renal vein | Aorta | Renal vein |
| | | *nM* | | |
| 11 | 17.2±1.1* | 5.9±0.5 | 33.1±0.9 | 25.6±2.1 |
| 12 | 8.5±0.2 | 3.4±0.1 | 31.7±0.5 | 25.0±0.8 |
| 13 | 11.2±0.3 | 4.8±0.1 | 28.9±2.1 | 21.0±1.4 |
| 14 | 15.6±1.3 | 11.1±0.6 | 37.8±4.0 | 32.7±1.1 |
| 15 | 17.7±0.7 | 9.6±0.5 | —‡ | — |
| 16 | 14.0±0.5 | 6.4±0.2 | 21.2±1.8 | 11.6±1.2 |
| 17 | 10.7±0.3 | 5.5±0.2 | 31.3±2.2 | 23.3±1.8 |
| Mean§ | 13.6±1.3 | 6.7±1.0 | 30.7±2.2 | 23.2±2.8 |

* Each value is the mean and SEM of three or four determinations.
‡ No data for cAMP were obtained in exp 15.
§ The mean and SEM for all determinations for all dogs is given.

ence than for cAMP (Fig. 2). The pulmonary artery cGMP concentration was slightly lower (8%) than the aortic cGMP concentration ($P < 0.002$). The only vessels sampled in which there was significantly increased concentration of cGMP were the SVC, in which the cGMP content was 9% greater than in the aorta ($P < 0.002$), and the superior mesenteric vein, in which the cGMP content was 83% greater than in aorta ($P < 0.001$). Portal vein samples taken in three additional dogs also contained higher concentrations of cGMP (56%, 92%, and 63%, respectively) than in aorta. Despite this, the hepatic vein concentration was equal to arterial concentration, indicating that the liver removed cGMP from the plasma.

In an attempt to ascertain the utility of selective catheterization combined with pharmacologic stimulation, the concentrations of cAMP and cGMP were measured in the effluent plasma from the left phrenicoabdominal vein, into which the left adrenal vein empties. Fig. 3 illustrates that the basal concentration of cAMP in arterial plasma was about 30 nM, while that in the venous effluent from the adrenal gland was significantly greater, about 120 nM. Intravenous injection of Cortrosyn into a systemic vein produced no effect on arterial plasma cAMP concentration; however, a rapid rise in the concentration of cAMP in the adrenal venous effluent to levels of 600–900 nM was observed. No difference in the concentration of cGMP in these vessels was observed before or after the injection of Cortrosyn.

## DISCUSSION

Little information is available concerning sources of plasma cyclic nucleotides under basal conditions. The liver is a source of the glucagon-mediated rise in plasma cAMP (4, 14), and it seems likely that the kidney is responsible for the elevated concentrations of cAMP in both plasma and urine that occur after administration of parathyroid hormone (2). However, it is not clear to what extent either the liver or the kidney contributes to plasma cAMP in the basal state.

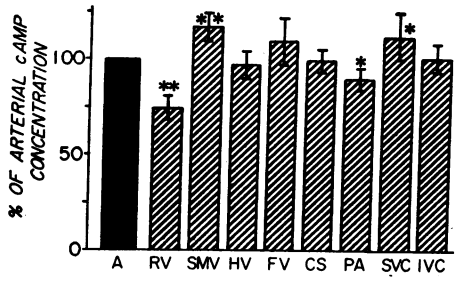


FIGURE 1 cAMP levels in plasma from the blood vessels sampled. The aortic plasma level (A) is shown as 100%. The level in each sample was expressed as a percent of the level in the sample of aortic plasma obtained simultaneously. The vertical line represents one SEM. RV, renal vein; SMV, superior mesenteric vein; HV, hepatic vein; FV, femoral vein; CS, coronary sinus; PA, pulmonary artery. Data for SVC was pooled from both groups of experiments in which this vessel was catheterized. $*P < 0.01$; $**P < 0.001$, for difference from the aorta.

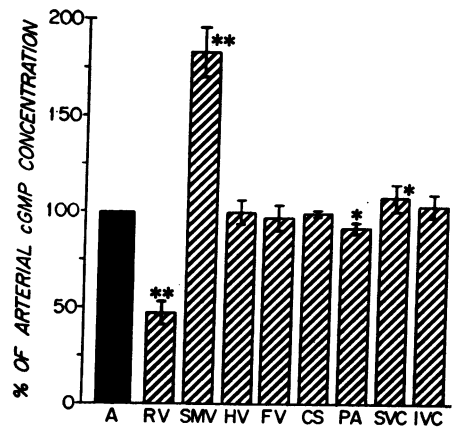


FIGURE 2 cGMP levels in plasma from the blood vessels sampled. The data are presented as in Fig. 1. $*P < 0.002$; $**P < 0.001$ for difference from aorta.

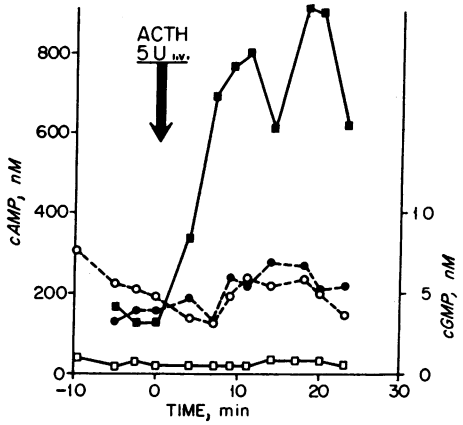


FIGURE 3 Effect of infusion of ACTH (Cortrosyn) on concentrations of cAMP and cGMP in arterial plasma and adrenal gland effluent plasma. Catheterization of the dog's left phrenicoabdominal vein was performed under direct vision with the abdomen open. 5 U (0.05 mg) of Cortrosyn, a synthetic analogue of ACTH, was administered by intravenous injection in 10 ml of 0.9% sodium chloride over 2 min. Arterial blood samples were obtained through a catheter placed in the abdominal aorta, while simultaneous collections of 5–6-ml samples of effluent plasma from the adrenal gland were made. Time is shown on the abscissa and the concentrations of cAMP and cGMP are given on the left and right ordinate, respectively.

□—□, arterial plasma cAMP concentration; ■—■, phrenicoabdominal vein plasma cAMP concentration; ○—○, arterial plasma cGMP concentration; ●—●, phrenicoabdominal vein plasma cGMP concentration.

Even less is known regarding the sources of cGMP in plasma. A two-fold increase in its concentration has been produced in a few human subjects to whom norepinephrine was administered (5, 6), and infusions of calcium have caused a 2–3-fold increase in urinary and plasma cGMP (2, 5) but the tissues responsible for these effects have not been identified.

In the present study, we have attempted to obtain information concerning the sources of plasma cyclic nucleotides and the sites of their elimination. A large net arteriovenous difference across the kidney was noted for both cyclic nucleotides. This could be due to excretion of the nucleotides in the urine by glomerular filtration or tubular secretion, or by degradation of these compounds in the kidney, or both. Since the kidney clears plasma cyclic nucleotides by glomerular filtration in man, a net decrease in plasma concentration across the dog kidney was not unexpected.

The superior mesenteric venous plasma concentration of cGMP exceeded that of arterial plasma, suggesting that the small intestine is a source of some of the cGMP in portal vein plasma. Theoretically, an enterohepatic circulation of cGMP could also account for the elevated level of cGMP in the mesenteric circulation. This seems unlikely, however, since our preliminary data indicate that the concentration of these nucleotides in bile is not markedly greater than in aortic plasma. Our evidence that the small intestine is probably an important source of plasma cGMP is of interest in view of the finding of relatively high concentrations of cGMP in the small intestine of the rat (15). Although portal vein plasma cGMP was also greater than in arterial plasma, the hepatic vein cGMP concentration did not exceed that in aorta. This indicates that the liver eliminates some of the plasma cGMP added by the small intestine.

Several or many tissues might have positive net arteriovenous differences too small to be reliably detected. Such small net increases might be sufficient to account for much of the production of cyclic nucleotides in plasma and to offset net elimination by the kidneys, especially if these differences occur across regions with large blood flows. It is of interest that the lungs may account for production of plasma cyclic nucleotides at a rate approximately equal to that of their elimination by the kidneys, since the pulmonary arterial concentration was about 10% greater than the aortic concentration of both cAMP and cGMP and the blood flow through the pulmonary circuit is about five times that of renal blood flow. The possibility that other regions may contribute importantly to the circulating levels of the cyclic nucleotides cannot be excluded. One organ or tissue within a region might add cyclic nucleotides while another eliminates these compounds, so that their concentrations in the mixed venous effluent plasma of the region would not differ from those in arterial plasma. Adipose tissue, skeletal muscle, and bone in the lower limb comprise one such possible combination of tissues.

Finally, one or more organs of tissues may exist in which the rate of uptake of cyclic nucleotide is counterbalanced by its secretion. Thus, there would be no discernable net arteriovenous difference across the organ, despite a large flux of cyclic nucleotide between tissue and plasma. Such a phenomenon could be identified by infusing tracer doses of $^{3}H$-labeled cyclic nucleotides, and measuring the specific radioactivity of the nucleotides in the afferent and efferent plasma of an organ, and we have applied this technique in studies of the kidney's arteriovenous concentration difference, as reported in an accompanying paper (16).

The absence of major arteriovenous differences for cAMP and cGMP in many of the regions studied does not imply that measurements of cyclic nucleotides in plasma could not be of value. For example, certain disease states could be associated with marked alterations in the concentrations of these compounds in systemic plasma. Furthermore, even if the concentration in peripheral plasma remained within a normal range, the cyclic nucleotide content of plasma in specific vessels might be altered in the basal state or after the infusion of appropriate agents, and this could be detected by selective catheterization techniques. For example, in this study, we catheterized the phrenicoabdominal vein, into which the adrenal vein empties in the dog, and found that basal concentrations of cAMP in this vessel were four times greater than the arterial concentration. After the administration of ACTH, there was an additional five-fold increase in the level of cAMP in this vein, even though there was not detectable change in cAMP concentration in the aorta. No change in blood flow was observed, and there was no difference between arterial and venous concentrations of cGMP before or after stimulation with ACTH. These findings suggest that selective catheterization techniques combined with hormonal stimulation might be of value in the study and diagnosis of human disease states.

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## REFERENCES


1. Broadus, A. E., N. I. Kaminsky, J. G. Hardman, E. W. Sutherland, and G. W. Liddle. 1970. Kinetic parameters and renal clearances of plasma adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in man. *J. Clin. Invest.* **49**: 2222.
2. Kaminsky, N. I., A. E. Broadus, J. G. Hardman, P. J. Jones, Jr., J. H. Ball, E. W. Sutherland, and G. W. Liddle. 1970. Effects of parathyroid hormone on plasma and urinary adenosine 3',5'-monophosphate in man. *J. Clin. Invest.* **49**: 2387.
3. Chase, L. R., G. L. Melson, and G. D. Aurbach. 1969. Pseudohypoparathyroidism: defective excretion of 3',5'-AMP in response to parathyroid hormone. *J. Clin. Invest.* **48**: 1832.
4. Broadus, A. E.., N. I. Kaminsky, R. C. Northcutt, J. G. Hardman, E. W. Sutherland, and G. W. Liddle. 1970. Effects of glucagon on adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in human plasma and urine. *J. Clin. Invest.* **49**: 2237.
5. Kaminsky, N. I., J. M. Ball, A. E. Broadus, J. G. Hardman, E. W. Sutherland, and G. W. Liddle. 1970. Hormonal effects on extracellular cyclic nucleotides in man. *Trans. Assoc. Am. Physicians Phila.* **83**: 235.
6. Ball, J. H., N. I. Kaminsky, J. G. Hardman, A. E. Broadus, E. W. Sutherland, and G. W. Liddle. 1972. Effects of catecholamines and adrenergic-blocking agents on plasma and urinary cyclic nucleotides in man. *J. Clin. Invest.* **51**: 2124.
7. Taylor, A. L., B. B. Davis, L. G. Pawlson, J. B. Josimovich, and D. H. Mintz. 1970. Factors influencing the urinary excretion of 3',5'-adenosine monophosphate in humans. *J. Clin. Endocrinol. Metab.* **30**: 316.
8. Murad, F., and C. Y. C. Pak. 1972. Urinary excretion of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate. *N. Engl. J. Med.* **286**: 1382.
9. Zierler, K. L. 1961. Theory of the use of arteriovenous concentration differences for measuring metabolism in steady and non-steady states. *J. Clin. Invest.* **40**: 2111.
10. Steiner, A. L., C. W. Parker, and D. M. Kipnis. 1972. Radio-immunoassay for cyclic nucleotides. I. Preparation of antibodies and iodinated cyclic nucleotides. *J. Biol. Chem.* **247**: 1106.
11. Broadus, A. E., J. G. Hardman, N. I. Kaminsky, J. H. Ball, E. W. Sutherland, and G. W. Liddle. 1971. Extracellular cyclic nucleotides. *Ann. N. Y. Acad. Sci.* **185**: 50.
12. Wehmann, R. E., L. Blonde, and A. L. Steiner. 1972. Simultaneous radioimmunoassay for the measurement of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate. *Endocrinology.* **90**: 330.
13. Snedecor, G. W. 1956. Statistical Methods. Iowa State University Press, Ames, Iowa. 33.
14. Liljenquist, J. E., J. D. Bomboy, B. C. Sinclair-Smith, S. B. Lewis, P. W. Felts, W. W. Lacy, O. B. Crofford, and G. W. Liddle. 1972. Net splanchnic cyclic AMP (cAMP) production in normal and diabetic men: effect of glucagon. *J. Clin. Invest.* **51**: 58*a*. (Abstr.)
15. Ishikawa, E., S. Ishikawa, J. W. Davis, and E. W. Sutherland. 1969. Determination of guanosine 3',5'-monophosphate in tissues and of guanyl cyclase in rat intestine. *J. Biol. Chem.* **244**: 6371.
16. Blonde, L., R. E. Wehmann, and A. L. Steiner. 1974. Plasma clearance rates and renal clearance of $^{3}$H-labeled cyclic AMP and $^{3}$H-labeled cyclic GMP in the dog. *J. Clin. Invest.* **53**: 163.