Plasma Clearance Rates and Renal Clearance of ^{3}H-labeled Cyclic AMP and ^{3}H-labeled Cyclic GMP in the Dog

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ABSTRACT Previously, in an attempt to understand the mechanisms involved in the regulation of plasma cyclic nucleotides, we measured concentrations of adenosine 3′,5′-monophosphate (cAMP) and guanosine 3′,5′-monophosphate (cGMP) in plasma from selected blood vessels of anesthetized dogs. The observation that the renal venous plasma concentrations of both cyclic nucleotides were less than arterial concentrations suggested that the kidney might be an important site for the elimination of these compounds from plasma and prompted further investigation of the renal handling of these compounds.

Tracer doses of either [^{3}H]cAMP or [^{3}H]cGMP were administered to anesthetized dogs by constant intravenous infusion, and metabolic clearance rates were determined. Concentrations of endogenous cyclic nucleotide and of cyclic nucleotide radioactivity were measured in aortic and renal venous plasma as well as in urine. Renal venous plasma [^{3}H]cGMP was 39% and [^{3}H]cAMP was 65% of the concentration in arterial plasma. Endogenous cyclic nucleotide levels showed a similar relationship. The plasma clearance rates (PCR) were 271±27 ml/min (mean±SE) for cGMP and 261±17 for cAMP. The total kidney clearance (calculated as the renal plasma flow × renal cyclic nucleotide extraction ratio) accounted for 52±4% and 30±2% of the PCR for cGMP and cAMP, respectively. Only about two-thirds of the total kidney clearance of each cyclic nucleotide could be accounted for by urinary excretion, the remainder presumably being the result of renal metabolism.

The urinary clearances of ^{3}H-labeled cGMP (40.9±4.2 ml/min) and endogenous cGMP (45.0±2.3 ml/min) were not significantly different from each other. Both were approximately 50% greater than the glomerular filtration rate, which was 27.1±2.0 ml/min, indicating that a significant amount of urinary cGMP is derived from plasma by tubular secretion.

In contrast, the urinary clearances of ^{3}H-labeled cAMP (23.7±1.9 ml/min) and endogenous cAMP (27.2±2.6 ml/min) were nearly equal both to each other and to the glomerular filtration rate, which was 24.6±1.7 ml/min. Thus, in the dog, glomerular filtration of plasma cAMP appears to be responsible for most of the cAMP found in urine. Renal production of cAMP, which in humans contributes from a third to a half of the urinary cAMP, was quantitatively of minor importance in the dog.

Thus, under the conditions of these experiments in dogs, renal elimination appears to be responsible for half of the PCR of cGMP and about a third of the PCR of cAMP. About a third of the renal elimination of both cyclic nucleotides appears to be due to metabolic degradation within the kidney, and the balance is due to excretion in the urine.

INTRODUCTION

The presence of adenosine 3′,5′-monophosphate (cAMP) and guanosine 3′,5′-monophosphate (cGMP) in mammalian plasma and urine, as well as in other extracellular fluids, has been established for several years (1–5). Little is yet known about their functional significance in these fluids, but considerable insight into

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the kinetics of plasma and urinary cyclic nucleotides has been gained by Broadus, Kaminsky, Hardman, Sutherland, and Liddle (6). They demonstrated that both cAMP and cGMP in human plasma have a rapid turnover rate. In their experiments, urinary excretion accounted for only 15–20% of the elimination of each nucleotide from plasma, with the majority of the removal presumably being accomplished by uptake and metabolism by unidentified tissues.

In an accompanying paper (7), we report plasma arteriovenous cyclic nucleotide concentration differences across a number of organs and regions in anesthetized dogs. Renal venous plasma cAMP was observed to be 25%, and cGMP 51%, lower than their respective concentrations in arterial plasma. The considerable renal extraction of both nucleotides has prompted further investigation of the renal handling of these compounds. Infusions of ^{3}H-labeled cyclic nucleotides were used to evaluate the extent to which clearance by renal extraction contributes to the total metabolic clearance rate of each nucleotide. In addition, clearance by urinary excretion was compared with clearance due to renal extraction. Finally, the urinary clearances of the ^{3}H-labeled cyclic nucleotide and the endogenous cyclic nucleotide were compared with each other and with the glomerular filtration rate.

METHODS

Materials. Cyclic nucleotides were purchased from Sigma Chemical Co., St. Louis, Mo. Radioimmunoassay materials were prepared as described elsewhere (8). Beef heart 3′,5′-cyclic nucleotide phosphodiesterase (Lot 120 C-7760) was purchased from Sigma Chemical Co. Bovine parathyroid injection, U.S.P., was obtained from Eli Lilly & Co., Indianapolis, Ind. (Control 4RT778).

[8-^{3}H]-cGMP (Lot #552100, specific activity 24.6 Ci/mmol) was purchased from ICN Corp., Chemical & Radioisotopes Div., Irvine, Cal. [8-^{3}H]cGMP (Lot XR-2010, specific activity 20.8 Ci/mmol) was purchased from Schwarz Bio Research Inc., Orangeburg, N. Y. Uniformly labeled [^{14}C]cAMP (Lot 607-127, specific activity 53 mCi/mmol, ammonium form) was obtained from New England Nuclear, Boston, Mass. [^{3}H]toluene and [^{14}C]-toluene were purchased from Amersham/Searle Corp., Arlington Heights, Ill.

Dowex-50 (AG 50W, hydrogen form, 100–200 mesh, control #10028) was obtained from Bio-Rad Laboratories, Richmond, Calif. Cellulose thin layer chromatography plates (Avicel, 250 μ thickness, Lot LSSK) were purchased from Analtech, Inc., Newark, Del.

Preparation of animals. Adult male mongrel dogs weighing between 15 and 22 kg were anesthetized with sodium pentobarbital. The abdomen was entered via a midline incision. Catheters were advanced from the femoral artery into the abdominal aorta, and from the femoral vein through the inferior vena cava into the right renal vein. Another catheter was secured in the right ureter. A jugular vein cutdown allowed placement of a catheter for the infusion.

A priming dose of 0.35 g para-aminohippurate (PAH),[1] 1.05 g creatinine, and 0.04 mCi of either ^{3}H-labeled cAMP or ^{3}H-labeled cGMP was then administered intravenously in a volume of 20 ml 0.9% sodium chloride. This was followed by a constant infusion of 0.9% sodium chloride containing 0.60 g/liter PAH; 1.80 g/liter creatinine, and 0.05–0.09 mCi/liter of one of the ^{3}H-labeled cyclic nucleotides with a Harvard infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.) set to deliver 10 ml/min.

A 45-min stabilization period was followed by three 20-min clearance periods, during which urine was collected quantitatively from the right ureter and stored on ice. Samples (12 ml) of arterial and renal venous blood were obtained over a 30-s period at the 5th and 15th min of each clearance period. After cessation of the radioactive infusion, samples of arterial blood were drawn at frequent intervals to determine the disappearance rate of ^{3}H-labeled cyclic nucleotide.

In some of the experiments, samples of tissues and whole organs were removed at the conclusion of the experiment in order to determine their content of tritium. In these experiments, after the infusion of ^{3}H-labeled cyclic nucleotide was terminated, 0.9% sodium chloride was infused for 90 min. By this time, radioactivity in urine and plasma had fallen to less than 10% of the values observed during the infusion of tracer. Samples of tissue weighing about 300 mg were excised and immediately frozen between aluminum clamps precooled in liquid nitrogen. These samples were stored in liquid nitrogen for subsequent determination of the content of ^{3}H-labeled cyclic nucleotide. The remainder of the kidneys, lungs, and liver were also removed and kept at room temperature for about 30 min until homogenization for analysis of total tritium content.

Preparation of samples of plasma and urine. Freshly drawn blood was immediately transferred to heparinized centrifuge tubes and mixed well. After centrifugation of blood for 3–5 min at 1,500 *g*, 2.0 ml of plasma were added to 2.0 ml of 10% trichloroacetic acid (TCA) and prepared for analysis of unlabeled cyclic nucleotides, as described in our companion paper (7). In preliminary studies, when urine was added directly into the radioimmunoassay, it was observed that in urine from an occasional dog there had occurred some degradation of endogenous cAMP and cGMP. Therefore, in order to minimize the possibility of error due to this degradation, samples of urine were treated in a manner identical to that used for plasma. Recovery of added cyclic nucleotides with this procedure was found to be 90–105%. The content of cAMP and cGMP in the prepared extracts of plasma and urine were determined by the radioimmunoassay techniques developed by Steiner, Parker, and Kipnis (8).

The fraction of plasma and urine radioactivity existing in the form of cAMP or cGMP was isolated by the following procedures: 2 ml of plasma or urine was deproteinized by addition of 2.0 ml of 0.6 N perchloric acid, followed by centrifugation at 2,000 g for 15 min. In those experiments where [^{3}H]cAMP had been the tracer infused, 1.0 ml of the perchloric acid supernate from plasma or urine was added to a test tube containing 100 μl of 0.3 N perchloric acid that contained [^{14}C]cAMP (1,000–1,500 dpm) for determination of the recovery. 1.0 ml of this mixture was applied to a 0.5 × 4.0-cm column of Dowex-50 (100–200 mesh, hydrogen form) and eluted with distilled water.

[1] *Abbreviations used in this paper:* c, cyclic; PAH, para-aminohippurate; PCR, plasma clearance rate; TKC, total kidney clearance.

Under these conditions, the perchloric acid appeared in the first 2 ml and the succeeding fractions were neutral. Preliminary fluorometric studies with unlabeled nucleotides showed that adenosine was retained on the column, ATP and ADP came through in the first 3 ml, cAMP appeared from the 4th through the 9th ml, and 5'-AMP was eluted from the column in a broad band after cAMP. Therefore, the 4th through 9th ml were collected, dried under a stream of air, and the residue was resuspended in 500 μl of distilled water. A 250-μl portion of each plasma sample and a 25-μl portion of each urine sample were removed for liquid scintillation counting, and the amount of [^{3}H]cAMP in the original extracts was determined by correction for the recovery of added [^{14}C]cAMP. This recovery averaged 85±2% (mean±SE) for plasma and 78±3% for urine.

The procedure utilized to isolate [^{3}H]cGMP from other radioactivity in plasma and urine differed from that used for [^{3}H]cAMP. Because the 0.5 × 4.0-cm Dowex 50 column yielded inadequate separation of cGMP from 5'-GMP, a larger (1.0 × 7.5-cm) column was used. Furthermore, recovery was determined by fluorometric measurement of unlabeled cGMP added to each sample. Therefore, in the final procedure, 100 μl 10^{-2} M cGMP was added to 1.5 ml of the perchloric acid extract of plasma or urine. 1.5 ml of this mixture was applied to the 1.0 × 7.5-cm column of Dowex-50 and eluted with distilled water. The first 5-ml fraction containing nucleoside di- and triphosphates was discarded, and the succeeding 5 ml was collected, dried under air, and resuspended in 750 μl of distilled water. 400 μl of the resuspended plasma extract or 50 μl of the urine extract were added to liquid scintillation vials for counting, while 100 μl of the remainder of the extract was diluted with 2.5 ml of distilled water, and the optical density read at 254 nm. The concentration of [^{3}H]cGMP in the sample was corrected for the recovery of the added unlabeled cGMP, which was 74±2% for plasma and 78 ±2% for urine.

The radioactivity in both plasma and urine remained predominantly in the form of cylic nucleotide, even 2 h after the infusion of the tritium-labeled cyclic nucleotide was initiated. This conclusion was based on the fact that 90% of the total radioactivity in perchloric acid extracts of plasma and urine was isolated in the cyclic nucleotide fraction by ion-exchange chromatography. Identification of the radioactivity as cyclic nucleotide was accomplished by incubating one portion of TCA-treated extract with phosphodiesterase for 1 h, and then adding an equal volume of 0.6 N perchloric acid. This mixture was applied to the appropriate column of Dowex-50 and processed as above. Such procedures resulted in virtually complete disappearance of radioactivity from the elution fraction containing the cyclic nucleotide, as compared with that from another portion, processed identically, with the omission of the incubation with phosphodiesterase. Similar results were obtained when the phosphodiesterase-treated mixtures were concentrated and analyzed after cellulose thin layer chromatography with butanol, glacial acetic acid, and water (12: 3: 5 vol/vol).

Preparation of tissue samples for determination of content of tritium in cyclic nucleotides. About 200 mg of the frozen tissue was homogenized in 2 ml 6% TCA. After removal of the precipitate by centrifugation, the supernate was washed four times with water-saturated ether, evaporated to dryness, and resuspended in 2 ml of distilled water. Samples were taken for liquid scintillation counting and for column chromatography on Dowex-50. For column chromatography, 500 μl of the extract was added to 500 μl of 0.6 N perchloric acid; this solution was applied to the appropriate column (0.5 × 4-cm for [^{3}H]cAMP, 1.0 × 7.5 cm for [^{3}H]cGMP) and eluted with distilled water. Fractions of 2 ml were collected, dried, and resuspended in a small volume of distilled water. Samples of these concentrated fractions were removed for liquid scintillation counting.

Preparation of whole organs for determination of total content of tritium. The whole organ was weighed, and about 100 g of tissue was homogenized in 200 ml of distilled water in a Waring blendor (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn.). A sufficient volume of 75% TCA was added to bring the final concentration of TCA to 5%, and homogenization was continued. After the precipitate had settled, a sample (2 ml) of supernate was removed and washed four times with water-saturated ether. After drying, the residue was resuspended in 2 ml of distilled water, and samples were removed for liquid scintillation counting. The total amount of water-soluble compounds containing tritium was then calculated.

Liquid scintillation counting. 25–400 μl of the solution to be counted was added to a glass scintillation vial (Packard Instrument Co., Inc., Downers Grove, Ill.) containing 10 ml of a scintillation fluid prepared by dissolving naphthalene (100 g) and 2,5-diphenyloxazole (5 g) in dioxane (1,000 ml). The samples were then stored in the dark for at least 36 h in order to minimize chemoluminescence.

The counting was performed by a Beckman LS-250 liquid scintillation system (Beckman Instruments, Inc., Electronic Instruments Div., Schiller Park, Ill.); each sample was counted once for 50 min or until 10,000 counts had been recorded. The two channels used were those provided by the manufacturer preset to count ^{3}H with minimal overlap of ^{14}C radioactivity, and ^{14}C without ^{3}H.

In order to correct for differences in quenching between samples of plasma, urine, and tissue extracts, samples were counted both before and after the addition of an aliquot of internal standard to each vial. Radioactivity as measured in counts per minute was then converted to disintegrations per minute on the basis of the efficiency with which the the internal standard was counted. The internal standards were [^{3}H]toluene (2,350 dpm/50 μl) and [^{14}C]-toluene (3,009 dpm/50 μl). In the samples of plasma and urine, the efficiency of counting tritium was 34%, and the efficiency of counting ^{14}C was 60%. For samples in which both ^{3}H and ^{14}C were present, the observed dpm of tritium were corrected for the overlap of ^{14}C radioactivity into the tritium channel (about 15–20%).

Calculations. Plasma clearance rates (PCR) of each cyclic nucleotide were determined by dividing the infusion rate of the ^{3}H-labeled cyclic nucleotide in disintegrations per minute per minute by the arterial level of cyclic nucleotide radioactivity attained at equilibrium in disintegrations per minute per milliliter (9). The production rate of cyclic nucleotides was calculated as the product of the arterial plasma concentration and the PCR. Renal plasma flow was calculated as the urinary clearance of infused PAH divided by its extraction ratio (10). Plasma and urine PAH were determined by the method of Bratton and Marshall (11) as modified by Smith, Finkelstein, Aliminosa, Crawford, and Graber (12). The glomerular filtration rate was taken as the urinary clearance of infused creatinine (10). Plasma and urine creatinine concentrations were measured with a Technicon autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (13).

Renal extraction ratios for both ^{3}H-labeled and en-

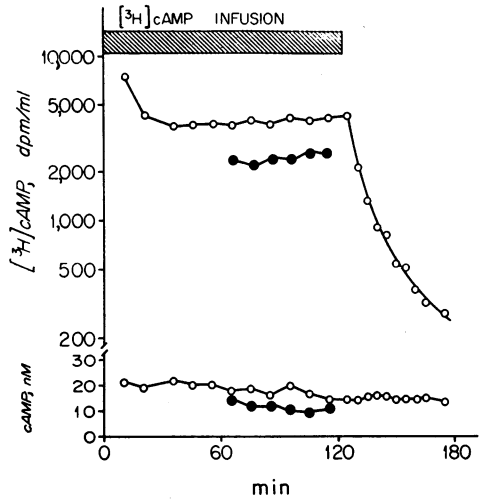

FIGURE 1 Concentrations of [^{3}H]cAMP and cAMP in arterial and renal vein plasma of a dog during and after constant intravenous infusion of [^{3}H]cAMP (Exp. 6). A priming dose of [^{3}H]cAMP (0.04 mCi) was given at the initiation of the infusion (time = 0), and [^{3}H]cAMP was subsequently infused at a rate of about 0.75 μCi/min. Clearance studies were done during the period from 60 to 120 min after the start of the infusion. The infusion was terminated at 120 min, and levels of [^{3}H]cAMP decreased rapidly. ○ represents arterial concentration; ● represents renal vein concentration.

dogenous cyclic nucleotides were determined from the formula:

$$E_{cyc} = (RA_{cyc} - RV_{cyc})/RA_{cyc},$$

where E_{cyc} = renal extraction ratio of cyclic nucleotide; RA_{cyc} = renal arterial plasma cyclic nucleotide concentration, and RV_{cyc} = renal venous plasma cyclic nucleotide concentration.

In order to assess that fraction of the entire PCR of each nucleotide which was the result of renal elimination, the renal extraction value has been converted to a clearance rate to facilitate comparison with both the PCR and the urinary clearance rate for each nucleotide. This clearance rate has been termed the total kidney clearance (TKC) and was the total volume of plasma cleared of cyclic nucleotide by the kidney per unit time. It was calculated by the following formula:

$$TKC = RPF \cdot E_{cyc},$$

where TKC = total kidney clearance; RPF = renal plasma flow; and E_{cyc} = renal extraction ratio of cyclic nucleotide. In contrast, the urinary clearance of cyclic nucleotide was determined as follows:

$$UC_{cyc} = \frac{U_{cyc} \cdot \dot{V}_u}{RA_{cyc}},$$

where UC_{cyc} = urinary clearance of cyclic nucleotide; U_{cyc} = urinary concentration of cyclic nucleotide; RA_{cyc} = renal arterial plasma cyclic nucleotide concentration; and $\dot{V}_u$ = urine flow rate.

Statistical analysis of the data was performed using the Student's grouped t test of observations of all experimental clearance periods (14).

RESULTS

Plasma clearance and production rates

Typical curves, depicting levels of plasma cyclic nucleotide radioactivity after infusion of ^{3}H-labeled cAMP and ^{3}H-labeled cGMP are presented in Figs. 1 and 2. Steady state had been achieved by 60 min in all cases. In each experiment, radioactive cyclic nucleotides accounted for less than 3% of the plasma and urine concentrations of total cyclic nucleotides. When plotted on semilogarithmic graph paper, the disappearance curves of cyclic nucleotide radioactivity in each case were multiexponential, i.e. nonlinear, indicating that the clearance of these compounds is multicompartmental (9). The PCR of both cyclic nucleotides were approximately equal (271±27 ml/min for cGMP and 261±17 ml/min for cAMP), while the production rate of cGMP was about two-thirds that of cAMP (3.78±0.49 nmol/min vs. 5.85± 0.60 nmol/min).

Renal extraction and TKC

Table I contains information about the net renal extraction of [^{3}H]cGMP and endogenous cGMP from the plasma. The concentration of arterial plasma cGMP was 14.7±1.8 nM, which was significantly greater than the concentration in renal venous plasma, 6.8±1.0 nM ($P < 0.001$). Thus, the resulting extraction ratio of endogenous cGMP was 0.556±0.019, which was slightly

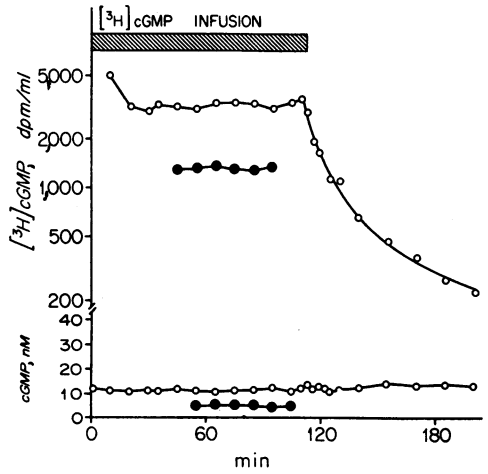

FIGURE 2 Concentrations of [^{3}H]cGMP and cGMP in arterial and renal vein plasma of a dog during and after constant intravenous infusion of [^{3}H]cGMP (Exp 4). A priming dose of [^{3}H]cGMP (0.04 mCi) was given at the initiation of the infusion (time = 0), and [^{3}H]cGMP was subsequently infused at a rate of about 0.75 μCi/min. Clearance studies were done during the period from 50–110 min after the start of the infusion. The infusion was terminated at 110 min and levels of [^{3}H]cGMP decreased rapidly. ○ represents arterial concentration, ● represents renal vein concentration.

TABLE I

*Parameters of Renal Handling of cGMP and [^{3}H]cGMP in Dogs**

Exp.	Arterial plasma concentration cGMP	Renal plasma flow	Glomerular filtration rate	Renal vein plasma concentration cGMP	TKC‡ cGMP	Arterial plasma [^{3}H]cGMP	Renal vein plasma [^{3}H]cGMP	TKC‡ [^{3}H]cGMP	Urinary clearance [^{3}H]cGMP	Urinary clearance cGMP
	nM	*ml/min*	*ml/min*	*nM*	*ml/min*	*dpm/ml*	*dpm/ml*	*ml/min*	*ml/min*	*ml/min*
1§	6.8±0.4	158±7	39.1±0.1	2.6±0.1	96.9±4.6	717±61	234±3	106.0±7.0	63.0±2.7	58.5±2.6
2§	15.8±0.8	52±6	19.5±1.0	6.4±0.8	30.5±2.3	11,403±133	3,683±210	34.9±4.0	18.6±1.0	40.9±2.2
3§	13.7±0.8	107±15	20.4±0.3	7.5±0.4	48.2±7.6	420±27	180±13	58.2±4.7	45.9±8.6	44.0±2.4
4§	11.2±0.3	141±8	31.7±0.5	4.4±0.1	85.7±2.3	3,231±32	1,345±9	82.2±4.1	42.5±3.8	35.0±0.8
5§	26.1±2.1	95±6	24.8±0.4	12.8±1.6	48.4±0.9	3,357±54	1,427±54	54.3±1.2	34.5±1.2	46.6±2.4
Mean±SE‖	14.7±1.8		27.1±2.0	6.8±1.0	61.9±6.9			67.1±6.8	40.9±4.2	45.0±2.3

* All data are for one kidney.
‡ TKC = renal plasma flow × extraction ratio.
§ The mean and SEM of the three determinations are given for each experiment.
‖ These figures are the mean and SEM for all 15 determinations in the five experiments.

less than the extraction ratio of ^{3}H-labeled cGMP, 0.615±0.016.

The renal extractions of cAMP and [^{3}H]cAMP may be compared in Table II. Endogenous arterial and renal venous plasma cAMP concentrations were 23.3±2.3 nM and 18.2±2.6 nM, respectively, yielding an extraction ratio of 0.250±0.41. This was substantially less than the corresponding ratio for [^{3}H]cAMP, which was 0.352± 0.022 ($P < 0.001$). If it is assumed that the uptake by the kidney of endogenous cAMP and ^{3}H-labeled cAMP from the arterial plasma were equivalent, these data indicate that addition of endogenous cAMP to the renal venous plasma from an unlabeled intracellular renal pool occurred, thus diminishing the arteriovenous concentration difference of the endogenous cAMP across the kidney, and accounting for the substantial decrease in the extraction ratio of endogenous cAMP relative to [^{3}H]-cAMP. The fact that the specific activity of the [^{3}H]-cAMP in renal venous plasma was 86±3% of the value in arterial plasma is consistent with the conclusion that some addition of unlabeled cAMP of renal origin to renal venous plasma had occurred. Similarly, the specific activity of [^{3}H]cGMP in renal venous plasma was 88±4% of the value in arterial plasma, indicating that addition of unlabeled cGMP of renal origin to renal venous plasma had also occurred.

Mechanism of urinary excretion

The urinary clearances of ^{3}H-labeled cGMP and endogenous cGMP by a single kidney are presented in Table I. These two clearances, 40.9±4.2 and 45.0±2.3 ml/min, respectively, were not significantly different from each other; both, however, were 50% greater than the glomerular filtration rate, which was 27.1±2.0 ml/min ($P <$

TABLE II

*Parameters of Renal Handling of cAMP and [^{3}H]cAMP in Dogs**

Exp.	Arterial plasma concentration cAMP	Renal plasma flow	Glomerular filtration rate	Renal vein plasma concentration cAMP	TKC‡ cAMP	Arterial plasma [^{3}H]cAMP	Renal vein plasma [^{3}H]-cAMP	TKC‡ [^{3}H]cAMP	Urinary clearance [^{3}H]cAMP	Urinary clearance cAMP
	nM	*ml/min*	*ml/min*	*nM*	*ml/min*	*dpm/ml*	*dpm/ml*	*ml/min*	*ml/min*	*ml/min*
6§	18.0±1.0	127±4	27.6±0.8	10.4±1.4	54.2±7.2	4,057±148	2,396±29	52.0±2.9	22.6±1.9	24.1±1.0
7§	27.8±2.1	72±7	16.7±0.7	23.3±1.2	11.0±3.8	12.396±716	8,297±63	23.2±0.7	16.6±1.2	16.8±1.8
8§	19.3±0.2	96±8	27.1±1.2	12.6±0.7	32.7±1.5	6,901±251	3,728±87	43.6±1.8	25.6±1.6	30.7±2.1
9§	13.6±0.7	147±5	33.5±1.2	10.4±0.9	30.8±16.1	3,525±78	2,402±56	44.4±2.9	35.8±2.6	42.6±4.8
10§	38.0±1.0	103±12	18.3±0.6	34.5±1.2	8.8±3.0	2,432±48	1,816±51	25.4±0.7	21.9±1.7	18.0±0.9
Mean±SE‖			24.6±1.7	18.2±2.6	27.5±5.3			37.7±3.1	23.7±1.9	27.2±2.6

* All data are for one kidney.
‡ TKC = renal plasma flow × extraction ratio.
§ The mean and SEM of the three determinations are given for each experiment.
‖ These figures are the mean and SEM for all 15 determinations in the five experiments.

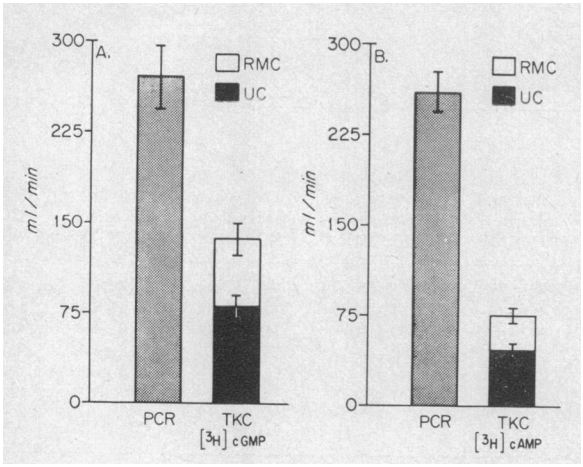

FIGURE 3 PCR, TKC, urinary clearances (UC), and renal metabolic clearances (RMC) of [^{3}H]cGMP (A) and [^{3}H]cAMP (B). The rate in milliliters per minute is shown on the ordinate. The height of the vertical bar represents the mean of the values for 15 clearance periods and the vertical line represents one SEM. The bar for TKC is divided to show the components of urinary clearance and renal metabolic clearance. The top vertical bar is the SEM value of TKC; the bottom vertical bar is the standard error of the mean value of UC. Values for one kidney have been doubled in order to approximate the total renal function of the animal.

0.01). This suggests that urinary excretion of cGMP in the dog may involve a significant element of tubular secretion in addition to glomerular filtration. In two dogs (No. 2 and 5), urinary clearance of unlabeled cGMP was substantially greater than the urinary clearance of [^{3}H]cGMP, suggesting addition of unlabeled cGMP to urine. Overall, specific activity in urine was only 91% of that in the aorta.

The urinary clearance rates for both ^{3}H-labeled and endogenous cAMP by a single kidney are presented in Table II. By grouped statistics, the urinary clearance of labeled and unlabeled cAMP (23.7±1.9 ml/min and 27.2±2.6, respectively) were not significantly different, nor was the difference between either of these and the GFR (24.6±1.7 ml/min) significant. (Paired analysis indicated a significant ($P < 0.01$) difference between the clearances of ^{3}H-labeled and unlabeled cAMP.) The similarity of these three clearances rates indicated that in dogs, glomerular filtration of plasma cAMP could be the source of most of the cAMP found in the urine.

The urinary specific activity of [^{3}H]cAMP represents 89% of the arterial plasma specific activity. Thus, a small portion (11%) of the total urinary cAMP in dogs might be derived by direct addition from an intracellular renal pool. It has been reported that in humans, as much as one-half of urinary cAMP is so derived, and that furthermore, this so-called "nephrogenous" addition may be greatly increased by parathyroid hormone (6,15).

Urinary clearance vs. TKC

[^{3}H]cGMP. The rates in ml/min for the PCR, TKC, and urinary clearance of ^{3}H-labeled cGMP are illustrated in Fig. 3A. The TKC rates were initially determined for one kidney; these were then doubled to give the approximate values for the entire kidney mass of each animal. 52% of the PCR of [^{3}H]cGMP could be accounted for by the TKC (137±13 ml/min). Only 60% (82±8 ml/min) of the TKC was due to urinary clearance. This discrepancy implies that 40% of the TKC, or fully 20% of the PCR of [^{3}H]cGMP, was the result of metabolism of ^{3}H-labeled cGMP within the renal tissue.

[^{3}H]cAMP. In Fig. 3-B are shown the PCR, TKC, and urinary clearance for ^{3}H-labeled cAMP. The TKC of [^{3}H]cAMP (75±6 ml/min) was equal to 30% of the PCR. In the case of this cyclic nucleotide also, only 64% (57±4 ml/min) of the TKC was due to urinary clearance. Therefore, 36% of the TKC, or fully 11%

TABLE III

Radioactivity in Plasma and Tissues after Intravenous Infusion of [^{3}H]cGMP

Exp.	Tritium					
	Plasma	Kidney		Liver	Lung	Skeletal muscle
		Cortex	Medulla			
	dpm/μl/mCi[^{3}H]cGMP administered	*dpm/mg tissue/mCi [^{3}H]cGMP administered*				
7	0.2	2,167	—	92	58	—
8	1.0	2,680	220	840	28	84
9	0.7	3,000	630	103	43	26
10	1.3	3,220	230	502	120	—

After cessation of the intravenous infusion of [^{3}H] cGMP, a solution of isotonic sodium chloride was infused for 90 min. Plasma levels of radioactivity by this time had fallen to negligible levels. Samples of various organs were removed, homogenized in TCA (50 g/liter), and a portion of the supernate was extracted with ether. Tritium content of the supernate was determined by liquid scintillation counting.

TABLE IV

Comparison of Contributions of Urinary Clearances and Renal Metabolic Clearance to the PCR with the Urinary Content of ^3H and Kidney Content of ^3H in Dogs

		% of PCR		% of total dose
[^{3}H]cGMP	PCR*	100	total dose of ^{3}H administered‡	100
	UC*	19.6	total urinary content of ^{3}H‡	16.7
	RMC*	17.3	total kidney content of ^{3}H‡	18.2
[^{3}H]cAMP	PCR§	100	total dose of ^{3}H administered‖	100
	UC§	18.2	total urinary content of ^{3}H‖	19.3, 30.1
	RMC§	11.6	total kidney content of ^{3}H‖	13.1, 11.9
			total liver content of ^{3}H‖	26.2, 26.0
			total content of lung of ^{3}H‖	6.8, 1.6

Urinary clearance and renal metabolic clearance for two kidneys were calculated as described in the text, and are expressed as a percent of the PCR. The tissue and urinary contents of ^{3}H are expressed as the percent of the total dose administered. Contents of tritium in tissues and urine were determined as described in the text.

* Clearance data for [^{3}H]cGMP were the averages of four periods from one experiment.

‡ Kidney and urinary contents of tritium were determined in the same experiment from which the clearance data for [^{3}H]cGMP were obtained.

‡ Clearance data for [^{3}H]cAMP were the averages of all the experiments.

‖ Kidney, urinary, liver, and lung contents of tritium were determined in two additional dogs which received infusions of [^{3}H]cAMP. Values for both dogs are indicated.

of the PCR of [^{3}H]cAMP, appears to have been due to degradation by the kidney.

Recovery of tritium in tissues and urine after the infusion of ^{3}H-labeled cyclic nucleotide

Further identification of organs responsible for the elimination of cyclic nucleotides from plasma was achieved by removal of organs after the infusion of ^{3}H-labeled cyclic nucleotides and the determination of their content of tritium. This procedure allowed not only confirmation of the locations of the metabolism of the plasma cyclic nucleotides, but permitted the estimation of the quantitative significance of the various organs involved.

All of the tissues sampled showed some accummulation of tritium, as indicated by the fact that at the time of sampling, tissue levels of tritium were usually 20–100 times in excess of levels in plasma. Table III shows the content of tritium in several tissues at the conclusion of four separate experiments in which [^{3}H]cGMP was infused. The kidney cortex accumulated considerable amounts of tritium, nearly 10 times the amount taken up by the medulla. The other organs showed less uptake on a per gram basis; however, total uptakes by the liver and skeletal muscle are probably quantitatively significant in view of the relatively large amount of hepatic and muscle tissue in the animal.

The infusion of labeled cyclic nucleotide allowed a test of the hypothesis that the kidney took up and metabolized some of the cyclic nucleotide from plasma. If the radioactivity taken up by the kidney tissue was not readily released into the plasma or urine in some form other than the tritium-labeled cyclic nucleotide infused, then the ratio of the radioactive content of the kidney to the amount infused should be identical to the ratio of the renal metabolic clearance to the PCR; and similarly, the ratio of the amount of tritium excreted in the urine to the total dose of ^{3}H-labeled cyclic nucleotide administered should be identical to the ratio of the urinary clearance to the PCR. Table IV shows that for both cyclic nucleotides the fraction of the PCR due to renal metabolic clearance was essentially the same as the fraction of the total dose of tritium contained in the renal tissue. Similarly, for both cyclic nucleotides, the fraction of the PCR due to urinary clearance was not markedly different from the fraction of total administered tritium excreted in the urine. The data also show that the liver accumulated about one-quarter of the radioactivity of the total dose of [^{3}H]cAMP administered, while the lungs accumulated a much smaller fraction of the dose. Ion-exchange chromatography of TCA extracts of renal tissue revealed that most of the tritium was eluted in fractions other than those containing the cyclic nucleotides. This suggests that the relatively small intracellular pools of cGMP and cAMP were rapidly turned over. It is likely that cGMP and cAMP were hydrolyzed by phosphodiesterases to 5′-GMP and 5′-AMP (16), which subsequently were metabolized to other nucleotides, all

of whose intracellular concentrations were greatly in excess of those of the cyclic nucleotides (17), accounting for the fact that most of the tritium remained within the kidney and liver tissue.

DISCUSSION

Broadus and his colleagues have shown that in humans the cyclic nucleotides in plasma are in dynamic steady state with tissue pools of these nucleotides (6). Metabolic clearance rates from plasma were rapid, and less than 20% of the plasma clearance could be accounted for by urinary clearance. These studies also showed that the volume of distribution of the plasma cyclic nucleotides was significantly greater than the extracellular fluid volume and was particularly large for cGMP.

Our investigation of the clearance of plasma cyclic nucleotides by the kidney in the dog were prompted by the observation that cyclic nucleotide concentration in renal venous plasma was substantially less than that in arterial plasma (7). We have shown that in the dog the plasma cyclic nucleotides are also in a dynamic steady state. The PCR and production rates of both cyclic nucleotides are, on a per kilogram basis, similar to those reported for humans. As in humans, urinary clearance accounted for only 15–30% of the PCR of either of the nucleotides; therefore, processes other than urinary excretion accounted for the majority of the elimination of cyclic nucleotides from plasma.

TKC of plasma cyclic nucleotides, calculated on the basis of the arteriovenous plasma cyclic nucleotide extraction ratios, accounted for 52% of the PCR of cGMP and 30% of the PCR of cAMP. Urinary clearance, however, accounted for only about two-thirds of the TKC of each cyclic nucleotide. This suggested that the remainder, or fully 10% of the PCR of cAMP and 20% of the PCR and cGMP, was the result of metabolism within the renal tissue. This suggestion was supported by the observation that 12% of the radioactivity of infused [^{3}H]cAMP and 18% of that of infused [^{3}H]cGMP was recovered in the renal tissue, primarily in the form of compounds other than the cyclic nucleotides themselves. Thus, degradation within the kidney accounted for a significant portion of that fraction of the PCR that could not be accounted for by urinary clearance.

The mechanisms of the urinary excretion of cAMP and cGMP were investigated by comparing the urinary clearances of endogenous cyclic nucleotides and infused ^{3}H-labeled cyclic nucleotides with the glomerular filtration rate. Broadus et al. reported that in humans between 30–55% of the urinary cAMP is derived not from plasma, but from production and release by the renal parenchyma (6). They observed that the urinary clearance of [^{3}H]cAMP was equal to the glomerular filtration rate, while the clearance of unlabeled cAMP exceeded both these values. In our studies in dogs, we observed no significant difference between the glomerular filtration rate and the urinary clearance of both cAMP and [^{3}H]cAMP. The urinary clearance of the endogenous cAMP slightly exceeded that of the ^{3}H-labeled compound, and the specific activity of the [^{3}H]cAMP in the urine was 11% lower than in aortic plasma. Therefore, net nephrogenous addition of cAMP in dogs contributes only a minor fraction of the total cAMP excreted in the urine, and under the conditions of these experiments, glomerular filtration of plasma is probably the major mechanism by which cAMP appears in the urine in the dog. Even the administration of parathyroid homone, which markedly enhances the nephrogenously derived urinary cAMP in humans (15) and increases the urinary excretion of cAMP in rat (18), produced no striking increase in urinary cAMP in several dogs that we studied in preliminary experiments.

With regard to cGMP, the mean urinary clearance of both [^{3}H]cGMP and endogenous cGMP were similar, and both clearance rates were significantly greater than the glomerular filtration rate (Table I), indicating that tubular secretion from plasma as well as glomerular filtration is involved in the urinary excretion of cGMP.

In spite of the large clearance of plasma cyclic nucleotides by the kidney, this organ also accounts for some the specific radioactivity of both [^{3}H]cAMP and [^{3}H]-cGMP in renal venous plasma was lower than that in arterial plasma. It can be calculated that, under basal conditions, the kidneys were responsible for about 5% of the total rate of entry into plasma of cAMP and for 10% of the rate of cGMP.

That the clearance data and renal arteriovenous concentration differences are inaccurate because of possible shifts of cyclic nucleotides between plasma and blood cells appears unlikely. As discussed elsewhere (7), the concentrations in blood cells are lower than those in plasma, and there appears to be minimal exchange of ^{3}H-labeled cAMP or cGMP with the intracellular cyclic nucleotides. Further evidence to support the validity of the clearance data is the quantitative recovery of predicted amounts of tritium in urine and kidney tissue in this study after infusion of the ^{3}H-labeled cyclic nucleotides. Therefore, it is doubtful that shifts of cyclic nucleotides between blood cells and plasma occur during transit through an organ.

In addition to demonstrating the uptake of ^{3}H-labeled cyclic nucleotides by the renal tissues, these studies indicated that approximately one-quarter of the radioactivity of an administered dose of [^{3}H]cAMP is recovered in the liver and suggest that the liver is an important site

of elimination of plasma cAMP. This finding is of interest in view of the fact that the liver, under the influence of glucagon, appears to be capable of adding considerable amounts of cAMP to plasma (19). A balance between uptake and secretion of cyclic nucleotide by the liver may account for the observation reported in the accompanying paper (7), that there is no large concentration difference of cAMP between hepatic artery, superior mesenteric vein, and hepatic vein.

The large accumulation of tritium in the kidneys and liver indicate that much of the uptake of ^{3}H-labeled cyclic nucleotides is irreversible. It is likely that once within these tissues, the ^{3}H-labeled cyclic nucleotides undergo rapid metabolism and are converted to nucleotides of much greater pool size, e.g. AMP, ATP, and GMP. The tritium is effectively trapped within the tissues in short-term experiments, presumably because these other nucleotides do not exit rapidly from the cells (17).

The functional significance of uptake and metabolism of the plasma cyclic nucleotides by specific organs has not been elucidated. Since the plasma cyclic nucleotides are present in such low concentration relative to intracellular concentrations, it is unlikely that they are acting as hormones by penetrating cell membranes of selected tissues. Nevertheless, the levels of the cyclic nucleotides in plasma both in humans and in the dog seem to be maintained by mechanisms that involve both uptake and release by tissue, metabolism within tissue, urinary excretion, and possibly other mechanisms. It is of interest that the metabolic production rate of plasma cGMP is close to that of cAMP even though the tissue pools of the former nucleotide in the rat, mouse, and dog are in general an order of magnitude less in size that those of cAMP (20–23). Since apparently there is rapid release of cGMP from tissue pools, measurement of cGMP in the venous effluent of specific tissues after appropriate pharmacologic stimulation might provide a sensitive index of alteration in the intracellular cGMP concentration in these tissues, and provide insight into the biologic function of cGMP.

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