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# Renal effects of adenosine 3′,5′-cyclic monophosphate and dibutyryl adenosine 3′,5′-cyclic monophosphate: *Evidence for a role for adenosine 3′,5′-cyclic monophosphate in the regulation of proximal tubular sodium reabsorption*

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Stable water diuresis was produced in anesthetized, hydrocortisone-treated hypophysectomized dogs by infusion of 2.5% dextrose. Infusion of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) in the left renal artery decreased ipsilaterally glomerular filtration rate (GFR), cortical and non-cortical renal plasma flow, and tended to increase urine flow (V) and free-water clearance ($C_{H_2O}$) despite a decrease in mean arterial pressure. Infusion of dibutyryl adenosine 3′,5′-cyclic monophosphate (dibutyryl cyclic AMP) in the left renal artery increased V and $C_{H_2O}$ significantly ($P<0.01$) bilaterally with essentially no change in GFR, in total renal plasma flow or its cortical and non-cortical components. For each kidney the magnitude of the change in V was similar to the magnitude of the change in $C_{H_2O}$ and the change in sodium excretion was trivial. Cyclic AMP probably produced its effects on renal hemodynamics and mean arterial pressure wholly or in part through the action of metabolites such as 5′-AMP and adenosine on the renal and systemic vasculature. The absence of an effect of dibutyryl cyclic AMP on renal hemodynamics and its bilateral effect may be explained by the resistance of this nucleotide derivative to metabolism.

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# Renal Effects of Adenosine 3′,5′-Cyclic Monophosphate and Dibutyryl Adenosine 3′,5′-Cyclic Monophosphate

## EVIDENCE FOR A ROLE FOR ADENOSINE 3′,5′-CYCLIC MONOPHOSPHATE IN THE REGULATION OF PROXIMAL TUBULAR SODIUM REABSORPTION

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ABSTRACT Stable water diuresis was produced in anesthetized, hydrocortisone-treated hypophysectomized dogs by infusion of 2.5% dextrose. Infusion of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) in the left renal artery decreased ipsilaterally glomerular filtration rate (GFR), cortical and non-cortical renal plasma flow, and tended to increase urine flow (V) and free-water clearance ($C_{H_2O}$) despite a decrease in mean arterial pressure. Infusion of dibutyryl adenosine 3′,5′-cyclic monophosphate (dibutyryl cyclic AMP) in the left renal artery increased V and $C_{H_2O}$ significantly ($P < 0.01$) bilaterally with essentially no change in GFR, in total renal plasma flow or its cortical and non-cortical components. For each kidney the magnitude of the change in V was similar to the magnitude of the change in $C_{H_2O}$ and the change in sodium excretion was trivial. Cyclic AMP probably produced its effects on renal hemodynamics and mean arterial pressure wholly or in part through the action of metabolites such as 5′-AMP and adenosine on the renal and systemic vasculature. The absence of an effect of dibutyryl cyclic AMP on renal hemodynamics and its bilateral effect may be explained by the resistance of this nucleotide derivative to metabolism.

Dibutyryl cyclic AMP appears to decrease by direct cellular effect(s) proximal tubular sodium reabsorption but does not prevent virtually complete reabsorption of the increased load of sodium in the distal nephron. This effect on the kidney is qualitatively and quantitatively similar to the effect of renal arterial infusion of isoproterenol.

The results suggest that synthesis of cyclic AMP in proximal renal tubule cells in response to stimulation of beta adrenergic or other receptors can mediate a decrease in the proximal tubular reabsorption of sodium.

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## INTRODUCTION

Infusion of isoproterenol in a renal artery of the dog decreases proximal tubular sodium reabsorption ipsilaterally without a change in mean arterial pressure, in glomerular filtration rate (GFR), in total renal plasma flow (RPF),[1] or in its cortical and noncortical components (1). This apparent direct effect of isoproterenol on the proximal tubule could be explained if stimulation of beta adrenergic receptors activated the synthesis of intracellular adenosine 3′,5′-cyclic monophosphate (cyclic AMP) and this nucleotide could in turn depress sodium reabsorption. Recent studies have demonstrated the presence of adenyl cyclase in homogenates of isolated renal cortical tubules (2). The activity of this enzyme, which catalyzes the conversion of adenosine triphosphate to cyclic AMP, is increased by epinephrine and this effect of epinephrine can be inhibited by propranolol, a beta adrenergic blocking agent (2). The present studies,

[1] Abbreviations used in this paper: $C_{H_2O}$, clearance of free water; $C_{IN}$, clearance of inulin; $C_{PAH}$, clearance of para-aminohippurate; cyclic AMP, adenosine 3′,5′-cyclic monophosphate; $E_{PAH}$, renal extraction of *P*-aminohippurate; FF, filtration fraction; GFR, glomerular filtration rate; MAP, mean arterial pressure; NCPF, noncortical plasma flow; RPF, total renal plasma flow; $U_{Na}V$, sodium excretion; V, urine flow.

TABLE I

*The Effects of Infusion of Adenosine 3',5'-Cyclic Monophosphate in the*

| Experiment | Regimen | Right kidney | | | | | |
|---|---|---|---|---|---|---|---|
| | | $C_{IN}$ | $U_{Na}V$ | $U_{Osm}$ | V | $C_{H_2O}$ | $C_{PAH}$ |
| | | *ml/min* | *μEq/min* | *mOsm/kg* | *ml/min per 100 ml GFR* | *ml/min per 100 ml GFR* | *ml/min* |
| 79 | C* | 28 | 3 | 33 | 11.9 | 10.4 | 76 |
| | CAMP‡ | 29 | 5 | 36 | 11.3 | 9.7 | 76 |
| | PC§ | 30 | 10 | 40 | 11.0 | 9.3 | 73 |
| 80 | C | 21 | 42 | 59 | 11.2 | 9.0 | 68 |
| | CAMP | 20 | 20 | 68 | 7.4 | 5.7 | 48 |
| | PC | 18 | 4 | 70 | 5.8 | 4.4 | 38 |
| 81 | C | 21 | 10 | 63 | 12.6 | 8.5 | 47 |
| | CAMP | 20 | 8 | 61 | 13.0 | 10.1 | 46 |
| | PC | 19 | 11 | 67 | 14.0 | 10.5 | 37 |
| 85 | C | 30 | 7 | 42 | 7.6 | 6.3 | 71 |
| | CAMP | 52 | 8 | 41 | 4.1 | 3.5 | 70 |
| | PC | 23 | 13 | 34 | 13.9 | 12.0 | 84 |
| Mean | C | 25 ±2 | 16 | 49 | 10.8 | 8.6 | 66 ±7 |
| | CAMP | 30 ±8 | 10 | 52 | 9.0 | 7.2 | 60 ±8 |
| | PC | 22 ±3 | 10 | 53 | 11.1 | 9.0 | 58 ±12 |

$C_{IN}$, mean clearance of inulin; V, mean urine flow; $C_{H_2O}$, mean free-water clearance; $U_{Na}V$, mean sodium excretion; $C_{PAH}$, mean clearance of para-aminohippurate; NCPF, non-cortical plasma flow; MAP, mean arterial pressure.

* C, control.

‡ CAMP, infusion of adenosine 3',5'-cyclic monophosphate in left renal artery.

§ PC, post-control.

designed to determine the effect of cyclic AMP on renal function, indicate that the nucleotide can indeed depress tubular sodium reabsorption in a manner which is qualitatively and quantitatively similar to that of isoproterenol.

## METHODS

Mongrel dogs weighing 16–26 kg (mean 20.6 kg) were anesthetized with pentobarbitol, hypophysectomized with a dental drill through a buccal approach, and given hydrocortisone hemisuccinate 1 mg/hr intravenously. Catheters were placed in both ureters, in the left renal artery, in the left renal vein by way of the spermatic or ovarian vein, and in the aorta to measure mean arterial pressure (MAP) (3). Normal saline containing inulin and para-aminohippurate was infused in a femoral vein at 0.5 ml/min by a constant infusion pump. Water diuresis was produced by intravenous infusion of 2.5% dextrose solution, first 1000 ml rapidly, then at 5 ml/min. When urine flow was stable, clearance measurements were started with clearance periods 20 min in duration. After 3 control periods, either adenosine 3',5'-cyclic monophosphate (Calbiochem, Los Angeles, Calif.) (1.5 mg/ml), or $N^6$-2'-O-dibutyryl adenosine 3',5'-cyclic monophosphate (Calbiochem), (1.5 mg/ml), or sodium butyrate (1.5 mg/ml), or nothing was added to the normal saline infused in the left renal artery at 0.5 ml/min for four experimental periods each; four postcontrol periods were also obtained. The acid pH of the adenosine 3',5'-monophosphate solution was not adjusted because the hydrogen ion ($H^+$) content was considered too trivial to exert an appreciable effect when infused (3 μEq $H^+$/min). The $N^6$-2'-O-dibutyryl adenosine 3',5'-cyclic monophosphate solution was neutral.

The clearances of inulin ($C_{IN}$) and para-aminohippurate ($C_{PAH}$) were determined as previously described (3). Serum and urinary sodium were determined by internal standard flame photometry; serum and urinary osmolality were measured by a Precision osmometer (Precision Systems, Framingham, Mass.).

Free-water clearance ($C_{H_2O}$) was calculated by the conventional formula, and expressed as milliliters per minute. For purposes of comparison, the data on urine flow (V) and $C_{H_2O}$ for the right and left kidneys were also expressed as milliliters per 100 ml of glomerular filtrate to correct for small variations in $C_{IN}$. Extraction of PAH ($E_{PAH}$) was calculated from the formula

$$E_{PAH} = (RA_{[PAH]} - RV_{[PAH]})/RA_{[PAH]},$$

where $RA_{[PAH]}$ and $RV_{[PAH]}$ are the concentrations of PAH in renal arterial and venous samples, respectively. Renal plasma flow (RPF) was then calculated by the formula $RPF = C_{PAH}/E_{PAH}$. Noncortical plasma flow (NCPF) was calculated as $RPF - C_{PAH}$ and taken as an estimate of medullary plasma flow; it was assumed that $C_{PAH}$ approximates cortical plasma flow (4). The values in the tables for control, experimental, and postcontrol regimens are the means of the last two periods of each regimen. The significance

*Left Renal Artery on the Function of the Right and Left Kidneys*

| Left kidney | | | | | | | |
|---|---|---|---|---|---|---|---|
| $C_{IN}$ | $U_{Na}V$ | $U_{Osm}$ | V | $C_{H_2O}$ | $C_{PAH}$ | NCPF | MAP |
| *ml/min* | *μEq/min* | *mOsm/kg* | *ml/min per 100 ml GFR* | *ml/min per 100 ml GFR* | *ml/min* | *ml/min* | *mm/Hg* |
| 26 | 6 | 34 | 10.3 | 8.9 | 67 | 45 | 120 |
| 23 | 8 | 35 | 11.8 | 10.4 | 59 | 32 | 112 |
| 22 | 8 | 41 | 11.0 | 8.4 | 52 | 23 | 118 |
| 30 | 71 | 66 | 9.7 | 7.6 | 72 | 72 | 104 |
| 16 | 35 | 79 | 10.0 | 7.2 | 49 | 39 | 102 |
| 16 | 6 | 64 | 7.5 | 5.9 | 38 | 22 | 125 |
| 26 | 13 | 62 | 11.8 | 9.1 | 50 | 22 | 123 |
| 16 | 10 | 60 | 16.2 | 12.9 | 42 | 16 | 120 |
| 20 | 7 | 63 | 14.4 | 11.1 | 38 | 13 | 130 |
| 37 | 8 | 42 | 6.5 | 5.5 | 76 | — | 149 |
| 38 | 6 | 44 | 5.0 | 4.2 | 66 | — | 132 |
| 25 | 13 | 34 | 14.5 | 12.5 | 92 | — | 135 |
| 30 ±3 | 24 | 51 | 9.6 | 7.8 | 66 ±6 | 46 | 124 |
| 23 ±6 | 15 | 54 | 10.8 | 8.7 | 54 ±6 | 29 | 116 |
| 21 ±2 | 8 | 50 | 11.8 | 9.5 | 55 ±14 | 19 | 127 |

of the data was determined by paired analysis of the control with the experimental regimen for each kidney in each dog.

## RESULTS

The results of infusion of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) 750 μg/min, in the left renal artery are presented in Table I. Control mean values for $C_{IN}$ and $C_{PAH}$ were 25 ±2 (SE) ml/min and 66 ±7 ml/min, respectively, in the right kidney and 30 ±3 ml/min and 66 ±6 ml/min in the left kidney; with infusion of the nucleotide, mean values for $C_{IN}$ and $C_{PAH}$ were 30 ±8 ml/min and 60 ±8 ml/min, respectively (right) and 23 ±6 ml/min and 54 ±6 ml/min, respectively (left). Mean V and $C_{H_2O}$ decreased in the right kidney from control values 10.8 and 8.6 ml/min per 100 ml GFR, respectively to 9.0 and 7.2, respectively but on the left they increased from 9.6 and 7.8 ml/min per 100 GFR, respectively, to 10.8 and 8.7 ml/min per 100 ml GFR, respectively. Mean urinary osmolality was essentially unchanged by the nucleotide (control: 49 mOsm/kg [right], 51 mOsm/kg [left]; experimental: 52 mOsm/kg [right], 54 mOsm/kg [left]). In the postcontrol periods $C_{IN}$, $C_{PAH}$, V, and $C_{H_2O}$ were essentially similar in the two kidneys. Mean arterial pressure was 124 mm Hg in the control periods, decreased to 116 mm Hg with the nucleotide, and increased to 127 mm Hg in the postcontrol periods.

The results of infusion of $N^6$-2′-O-dibutyryl adenosine 3′.5′-cyclic monophosphate (dibutyryl cyclic AMP), 750 μg/min in the left renal artery, are presented for 1 study in Fig. 1. Control mean $C_{IN}$ was 19 ml/min (right kidney) and 21 ml/min (left kidney), and did not change with infusion of the nucleotide (20 ml/min in right kidney and 20 ml/min in left kidney) or in the postcontrol periods (21 ml/min in right kidney and 22 ml/min in left kidney). Control mean V and $C_{H_2O}$ values were 2.2 and 1.6 ml/min, respectively (right kidney), and 2.1 and 1.5 ml/min, respectively (left kidney). These values increased bilaterally with the nucleotide to 2.8 and 2.1 ml/min, respectively (right kidney), and to 3.1 and 2.3 ml/min, respectively (left kidney), and remained increased in the postcontrol periods. Control mean sodium excretion ($U_{Na}V$) was 3 μEq/min (right kidney) and 6 μEq/min (left kidney); $U_{Na}V$ increased slightly with the nucleotide to 6 μEq/min (right kidney) and 12 μEq/min (left kidney).

TABLE II

*The Effects of Infusion of Dibutyryl Adenosine 3',5'-Cyclic Monophosphate in the*

| Experiment | Regimen | Right kidney | | | | | |
|---|---|---|---|---|---|---|---|
| | | $C_{IN}$ | $U_{Na}V$ | $U_{Osm}$ | V | $C_{H_2O}$ | $C_{PAH}$ |
| | | *ml/min* | *μEq/min* | *mOsm/kg* | *ml/min per 100 ml GFR* | *ml/min per 100 ml GFR* | *ml/min* |
| 57 | C* | 32 | 12 | 50 | 5.4 | 4.4 | 79 |
| | DBCAMP‡ | 30 | 25 | 37 | 9.7 | 8.2 | 74 |
| | PC§ | 26 | 25 | 35 | 10.9 | 9.5 | 61 |
| 59 | C | 30 | 8 | 56 | 7.1 | 5.7 | 76 |
| | DBCAMP | 34 | 18 | 46 | 8.4 | 7.1 | 82 |
| | PC | 26 | 23 | 36 | 12.5 | 10.9 | 62 |
| 60 | C | 25 | 4 | 38 | 7.9 | 6.8 | 65 |
| | DBCAMP | 26 | 8 | 40 | 9.4 | 8.0 | 59 |
| | PC | 23 | 18 | 38 | 13.7 | 11.7 | 55 |
| 61 | C | 31 | 4 | 54 | 5.6 | 4.4 | 82 |
| | DBCAMP | 33 | 22 | 56 | 6.4 | 5.1 | 80 |
| | PC | 30 | 36 | 52 | 8.8 | 7.1 | 71 |
| 62 | C | 19 | 3 | 79 | 11.7 | 8.3 | 43 |
| | DBCAMP | 20 | 6 | 66 | 14.1 | 10.6 | 38 |
| | PC | 21 | 8 | 48 | 12.9 | 10.5 | 40 |
| 75 | C | 22 | 6 | 63 | 5.2 | 3.9 | 57 |
| | DBCAMP | 26 | 10 | 53 | 7.1 | 5.6 | 69 |
| | PC | 28 | 13 | 46 | 7.9 | 6.4 | 62 |
| 78 | C | 26 | 3 | 41 | 10.8 | 9.3 | 86 |
| | DBCAMP | 24 | 2 | 37 | 13.2 | 11.5 | 80 |
| | PC | 25 | 4 | 35 | 12.3 | 10.7 | 82 |
| Mean | C | 26 ±2 | 6 | 54 | 7.7 | 6.1 | 70 ±6 |
| | DBCAMP | 28 ±2 | 13 | 48 | 9.8 | 8.0 | 69 ±6 |
| | PC | 26 ±1.3 | 18 | 41 | 11.3 | 9.5 | 62 ±6 |
| *P* | C vs DBCAMP | >0.2 | | | <0.01 | <0.01 | >0.7 |

$C_{IN}$, mean clearance of inulin; V, mean urine flow; $C_{H_2O}$, mean free-water clearance; $U_{Na}V$, mean sodium excretion; $C_{PAH}$, mean clearance of para-aminohippurate; MAP, mean arterial pressure.

* C, control.

‡ DBCAMP, infusion of dibutyryl adenosine 3',5'-cyclic monophosphate in left renal artery.

§ PC, post-control.

The results of all the studies are summarized in Table II. Control mean $C_{IN}$ and $C_{PAH}$ values were 26 ±2 and 70 ±6 ml/min, respectively, in the right kidney and 24 ±1.6 and 68 ±7 ml/min, respectively, in the left kidney. With infusion of dibutyryl cyclic AMP, mean $C_{IN}$ and $C_{PAH}$ values did not change significantly: (28 ±2 and 69 ±6 ml/min, respectively (right kidney); 24 ±1.3 and 68 ±5 ml/min, respectively (left kidney), Table II). Control mean V and $C_{H_2O}$ values were 7.7 and 6.1 ml/min per 100 ml GFR, respectively (right kidney), and 7.4 and 5.9 ml/min per 100 ml GFR, respectively (left kidney); increased significantly ($P < 0.01$) bilaterally with the nucleotide to 9.8 and 8.0 ml/min per 100 GFR, respectively (right kidney), and 10.7 and 8.8 ml/min per 100 ml GFR, respectviely (left kidney) and tended to increase slightly in the postcontrol periods. Mean urinary osmolality was 54 mOsm/kg (right kidney) and 58 mOsm/kg (left kidney), and tended to decrease bilaterally throughout the study. Control mean $U_{Na}V$ was 6 μEq/min (right kidney) and 5 μEq/min (left kidney), and increased slightly with the nucleotide to 13 μEq/min (right kidney) and 15 μEq/min (left kidney). MAP showed a small but significant increase from 121 to 129 mm Hg.

*Left Renal Artery on the Function of the Right and Left Kidneys*

| Left kidney | | | | | | |
|---|---|---|---|---|---|---|
| $C_{IN}$ | $U_{Na}V$ | $U_{Osm}$ | V | $C_{H_2O}$ | $C_{PAH}$ | MAP |
| *ml/min* | *μEq/min* | *mOsm/kg* | *ml/min per 100 ml GFR* | *ml/min per 100 ml GFR* | *ml/min* | *mm Hg* |
| 29 | 8 | 51 | 5.7 | 4.6 | 82 | 115 |
| 26 | 22 | 37 | 10.6 | 9.7 | 78 | 125 |
| 21 | 24 | 37 | 12.5 | 10.7 | 60 | 128 |
| 26 | 3 | 61 | 5.7 | 4.5 | 69 | 98 |
| 28 | 14 | 46 | 9.9 | 8.1 | 78 | 104 |
| 24 | 16 | 35 | 12.2 | 10.6 | 68 | 111 |
| 18 | 3 | 40 | 9.3 | 7.8 | 64 | 127 |
| 19 | 10 | 42 | 10.6 | 9.0 | 54 | 128 |
| 22 | 14 | 36 | 12.2 | 10.5 | 54 | 120 |
| 24 | 6 | 57 | 5.8 | 4.6 | 62 | 132 |
| 26 | 24 | 60 | 7.2 | 5.5 | 62 | 140 |
| 21 | 28 | 55 | 9.6 | 7.6 | 58 | 137 |
| 21 | 6 | 85 | 10.0 | 7.5 | 46 | 107 |
| 20 | 12 | 66 | 15.4 | 11.6 | 50 | 127 |
| 22 | 10 | 50 | 12.6 | 10.3 | 46 | 140 |
| 24 | 8 | 70 | 4.6 | 3.3 | 60 | 142 |
| 28 | 16 | 52 | 7.3 | 5.8 | 69 | 145 |
| 24 | 14 | 46 | 8.4 | 6.8 | 60 | 144 |
| 27 | 4 | 41 | 10.4 | 8.9 | 92 | 123 |
| 23 | 5 | 37 | 14.0 | 12.2 | 88 | 135 |
| 22 | 8 | 34 | 15.1 | 13.3 | 86 | 140 |
| 24 ±1.6 | 5 | 58 | 7.4 | 5.9 | 68 ±7 | 121 |
| 24 ±1.3 | 15 | 49 | 10.7 | 8.8 | 68 ±5 | 129 |
| 22 ±0.4 | 16 | 42 | 11.8 | 10.0 | 62 ±6 | 131 |
| >0.9 | | | <0.01 | <0.01 | >0.6 | <0.02 |

Infusion of sodium butyrate, 750 μg/min in the left renal artery, did not affect $C_{IN}$, $C_{PAH}$, V, $C_{H_2O}$, or $U_{Na}V$. Since the results of these three studies with sodium butyrate were similar to the results of four studies in which only normal saline was infused in the left renal artery, the two groups of results were combined for analysis (Table III). Control mean values for $C_{IN}$, $C_{PAH}$, and V were 27 ±2.3 ml/ min. 68 ±6.7 ml/min, and 8.1 ml/min per 100 ml GFR, respectively (the right kidney) and 29 ±1.7 ml/min, 70 ±5.7 ml/min, and 7.8 ml/min per 100 ml GFR, respectively (left kidney) and did not change significantly: 28 ±1.9 ml/min, 66 ±3.0 ml/min, and 9.3 ml/min per 100 ml GFR, respectively (right kidney); 34 ±3.7 ml/min, 68 ±3.4 ml/min and 7.7 ml/min per 100 ml GFR, respectively (left kidney). MAP showed a small but significant ($P < 0.01$) increase from 126 (control) to 131 mm Hg (experimental), as it did in the studies with dibutyryl cyclic AMP.

## DISCUSSION

Infusion of cyclic AMP into the left renal artery of hypophysectomized dogs during stable water diuresis was associated with a decrease in mean arterial pressure

TABLE III

*The Effects of Infusion of Normal Saline Alone and Sodium Butyrate in Normal*

| Experiment | Regimen | Right kidney | | | | | |
|---|---|---|---|---|---|---|---|
| | | $C_{IN}$ | $U_{Na}V$ | $U_{Osm}$ | V | $C_{H_2O}$ | $C_{PAH}$ |
| | | *ml/min* | *μEq/min* | *mOsm/kg* | *ml/min per 100 ml GFR* | *ml/min per 100 ml GFR* | *ml/min* |
| 63 | C* | 37 | 6 | 82 | 7.9 | 5.6 | 62 |
| | NS‡ | 34 | 6 | 62 | 9.3 | 7.1 | 69 |
| | PC§ | 24 | 17 | 47 | 15.5 | 13.0 | |
| 64 | C | 23 | 6 | 50 | 11.4 | 9.4 | 73 |
| | NS | 22 | 9 | 40 | 13.8 | 11.8 | 68 |
| | PC | 23 | 11 | 38 | 12.0 | 10.3 | 60 |
| 65 | C | 23 | 6 | 50 | 6.1 | 5.1 | 62 |
| | NS | 35 | 14 | 38 | 6.6 | 5.6 | 73 |
| | PC | 40 | 22 | 36 | 7.0 | 6.0 | 59 |
| 66 | C | 21 | 9 | 101 | 7.1 | 4.5 | 58 |
| | NS | 24 | 10 | 72 | 9.3 | 6.8 | 62 |
| | PC | 28 | 9 | 53 | 9.8 | 7.9 | 52 |
| 82 | C | 32 | 5 | 70 | 5.9 | 4.4 | 98 |
| | B‖ | 26 | 4 | 82 | 4.9 | 3.5 | 63 |
| | PC | 26 | 4 | 106 | 3.5 | 2.2 | 54 |
| 83 | C | 26 | 8 | 45 | 9.9 | 8.2 | 51 |
| | B | 28 | 14 | 38 | 12.2 | 10.4 | 52 |
| | PC | 30 | 24 | 37 | 13.6 | 11.6 | 46 |
| 84 | C | 28 | 7 | 50 | 8.3 | 6.7 | 74 |
| | B | 30 | 10 | 48 | 8.7 | 7.1 | 72 |
| | PC | 34 | 16 | 50 | 7.9 | 6.4 | 68 |
| Mean | C | 27 ±2.3 | 7 | 64 | 8.1 | 6.3 | 68 ±6.7 |
| | Exp. | 28 ±1.9 | 10 | 54 | 9.3 | 7.5 | 66 ±3.0 |
| | PC | 29 ±2.4 | 15 | 52 | 9.9 | 8.2 | 56 ±3.7 |
| *P* | C vs Exp | >0.6 | | | >0.05 | | |

Exp, mean of the values for NS and B; $C_{IN}$, mean clearance of inulin; V, mean urine flow; $C_{H_2O}$, mean free-water clearance; $U_{Na}V$, mean sodium excretion; $C_{PAH}$, mean clearance of para-aminohippurate; MAP, mean arterial pressure.

* C, control.

‡ NS, infusion of normal saline in left renal artery.

§ PC, post-control.

‖ B, infusion of sodium butyrate in normal saline in left renal artery.

and a decrease in $C_{IN}$, $C_{PAH}$, and NCPF in the left kidney (Table I). Urine flow from the left kidney increased in three of the four studies (the exception was S-85 in which MAP decreased 17 mm Hg) whereas urine flow from the right kidney decreased in three of the four studies.

Cyclic AMP and its metabolites, 5′-AMP and adenosine, have been reported to produce vasodilatation of systemic arteries such as the mesentric and femoral arteries but constriction of the renal artery (5). The metabolism of cyclic AMP in blood is rapid (6), and this suggests that metabolites with similar effects on the arterial tree may mediate wholly, or in part, the changes in mean arterial pressure and in renal hemodynamics associated with the nucleotide.

Cyclic AMP through intracellular effects may also have decreased the proximal tubular reabsorption of sodium and increased urine flow from the left kidney (7) despite decreases in arterial pressure and renal plasma flow. The validity of such an interpretation is

*Saline in the Left Renal Artery on the Function of the Right and Left Kidney*

| Left kidney | | | | | | |
|---|---|---|---|---|---|---|
| $C_{IN}$ | $U_{Na}V$ | $U_{Osm}$ | V | $C_{H_2O}$ | $C_{PAH}$ | MAP |
| *ml/min* | *μEq/min* | *mOsm/kg* | *ml/min per 100 ml GFR* | *ml/min per 100 ml GFR* | *ml/min* | *mm Hg* |
| 34 | 4 | 81 | 8.6 | 6.2 | 67 | 106 |
| 36 | 5 | 63 | 8.9 | 6.7 | 70 | 113 |
| 33 | 16 | 46 | 11.1 | 9.4 | | 116 |
| 28 | 9 | 43 | 10.5 | 8.8 | 84 | 133 |
| 34 | 10 | 40 | 9.6 | 8.3 | 78 | 140 |
| 22 | 12 | 36 | 13.5 | 11.8 | 62 | 140 |
| 30 | 9 | 50 | 5.4 | 4.3 | 55 | 141 |
| 38 | 17 | 38 | 6.4 | 5.6 | 69 | 150 |
| 33 | 25 | 36 | 8.8 | 7.6 | 50 | 150 |
| 22 | 12 | 84 | 7.3 | 5.1 | 62 | 114 |
| 26 | 10 | 65 | 8.8 | 6.6 | 61 | 116 |
| 29 | 12 | 50 | 9.7 | 7.9 | 54 | 125 |
| 33 | 5 | 69 | 5.9 | 4.4 | 94 | 120 |
| 28 | 4 | 83 | 4.8 | 3.4 | 68 | 131 |
| 27 | 4 | 97 | 4.2 | 2.7 | 60 | 135 |
| 24 | 9 | 46 | 9.6 | 8.1 | 54 | 142 |
| 26 | 9 | 40 | 10.4 | 8.8 | 54 | 142 |
| 22 | 12 | 40 | 10.9 | 9.2 | 46 | 142 |
| 30 | 5 | 48 | 7.4 | 6.1 | 74 | 125 |
| 52 | 8 | 44 | 5.2 | 4.4 | 78 | 126 |
| 52 | 15 | 44 | 5.8 | 4.9 | 71 | 125 |
| 29 ±1.7 | 8 | 60 | 7.8 | 6.1 | 70 ±5.7 | 126 |
| 34 ±3.7 | 9 | 53 | 7.7 | 6.3 | 68 ±3.4 | 131 |
| 31 ±4.3 | 14 | 50 | 9.1 | 7.6 | 57 ±4.2 | 133 |
| >0.1 | | | >0.8 | | | <0.01 |

open to question, however, because of the effects of cyclic AMP on the vascular system.

The $N^6$-2′-O-dibutyryl-derivative of cyclic AMP appears to exert an effect similar to that of the parent compound in a number of tissues, but it is more resistant to conversion to 5′-AMP by phosphodiesterase (8). The precise mechanism of action of dibutyryl cyclic AMP is unknown. It may substitute for cyclic AMP or, as recently indicated, it may inhibit cyclic nucleotide phosphodiesterase (9). In the latter case, dibutyryl cyclic AMP would act in a manner similar to that of the methylxanthines and depend on endogenously generated cyclic AMP for its effect.

When dibutyryl cyclic AMP was added to the left renal artery infusate, urine flow and $C_{H_2O}$ increased bilaterally, significantly ($P < 0.01$), without a change in the clearances of inulin or PAH and with only trivial increases in sodium excretion; these changes persisted after the nucleotide was stopped (Table II). When the left renal artery infusate contained smaller concentra-

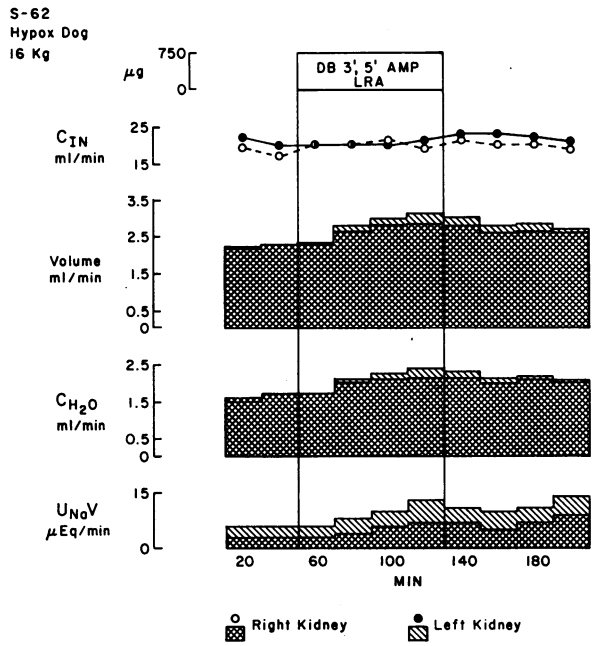


FIGURE 1 The effect of infusion of dibutyryl adenosine 3',5'-cyclic monophosphate (DB 3',5' AMP) in the left renal artery (LRA) of a water-loaded hypophysectomized (hypox) dog on $C_{IN}$ (clearance of inulin), volume (urine flow), $C_{H_2O}$ (clearance of solute-free water), and $U_{Na}V$ (sodium excretion) in the right and left kidney.

tions of dibutyryl cyclic AMP the increase in urine flow was less but still bilateral. A similar situation was encountered during studies on the effects of renal arterial infusion of dibutyryl cyclic AMP on phosphate excretion: an amount of the nucleotide which appreciably increased phosphate excretion by the infused kidney also increased phosphate excretion by the contralateral kidney (10). One possible explanation for the bilateral renal effects of dibutyryl cyclic AMP is that neither the derivative nor cyclic AMP itself crosses plasma membranes readily and a blood concentration many times greater than the concentration of nucleotide found in the cell is required to establish a physiologically effective intracellular concentration (8). Thus, much of the dibutyryl cyclic AMP infused in a renal artery probably leaves that kidney and is available for accumulation in other tissues such as the contralateral kidney. The resistance of dibutyryl cyclic AMP to breakdown would facilitate its intracellular accumulation and is very likely the reason for persistence of its effects after it was stopped (8). This characteristic of the molecule could also explain why no changes in renal hemodynamics were observed with its infusion.

Infusion of sodium butyrate into the left renal artery did not have any effect different from that of normal saline alone, infused in the left renal artery (Table III). The combined results of these two groups of experiments, which do not contain nucleotide in the renal artery infusate but are similar in other respects, are presented as control for the studies with dibutyryl cyclic AMP. The mean urine flow, $C_{H_2O}$, and $U_{Na}V$ for the left kidney before the infusion of dibutyryl cyclic AMP are similar to the mean urine flow, $C_{H_2O}$, and $U_{Na}V$ for the left kidney in those seven studies in which no nucleotide was given (Fig. 2). Administration of dibutyryl cyclic AMP produced an increase in V and an increase in $C_{H_2O}$ of similar magnitude with only a trivial increase in $U_{Na}V$, whereas similar clearance periods from the studies

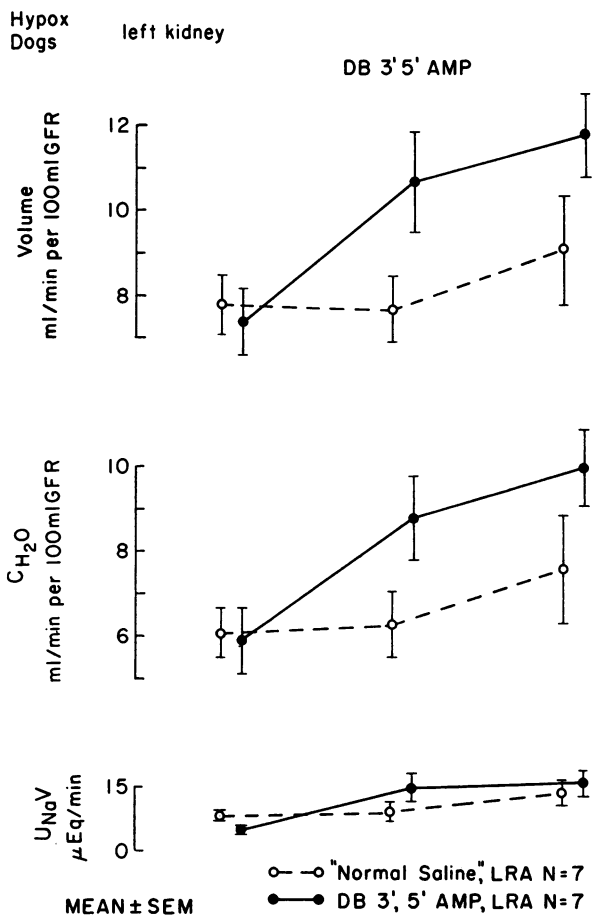


FIGURE 2 The effect of infusion of dibutyryl adenosine 3',5'-cyclic monophosphate (DB 3',5' AMP) and of "normal saline" (the combined results of three studies with sodium butyrate and four studies with normal saline alone) in the left renal artery (LRA) of hypophysectomized (hypox) dogs on volume (mean urine flow), $C_{H_2O}$ (mean free-water clearance) and $U_{Na}V$ (mean sodium excretion) in the left kidney. The values presented are the means for the following regimens: control; infusion of DB 3',5' AMP and "normal saline" (similar clearance periods from seven studies in which no nucleotide was given); postcontrol.

TABLE IV

*Effect of Infusion of Dibutyryl Adenosine 3',5'-Cyclic Monophosphate in the Left Renal Artery on the Hemodynamic Function of the Left Kidney*

| | $C_{PAH}$ | $E_{PAH}$ | NCPF | RPF | FF |
|---|---|---|---|---|---|
| | *ml/min* | | *ml/min* | *ml/min* | |
| Mean control ±SEM | 68 ±7 | 0.70 ±0.013 | 28 ±2 | 96 ±8 | 0.35 ±0.026 |
| Mean change with, dibutyryl 3',5'-cyclic AMP ±SEM | 0 ±2 | +0.01 ±0.011 | 0 ±1 | +1 ±1 | 0 ±0.003 |

$C_{PAH}$, clearance of *p*-aminohippurate; $E_{PAH}$, renal extraction of *p*-aminohippurate; NCPF, non-cortical plasma flow; RPF, total renal plasma flow; FF, filtration fraction.

in which no nucleotide was given, showed no change in V, $C_{H_2O}$, and $U_{Na}V$ (Fig. 2).

An increase in urine flow and in $C_{H_2O}$ without a change in GFR and with only a minimal change in $U_{Na}V$, provides indirect evidence that dibutyryl cyclic AMP decreased the proximal tubular reabsorption of sodium and water, and increased the volume of proximal fluid delivered distally to the diluting site (presumably the ascending limb of the loop of Henle) where the increased load of sodium was largely reabsorbed (7). A depression of proximal tubular sodium and phosphate reabsorption by dibutyryl cyclic AMP also has been observed in micropuncture studies (11). An absence of change in cortical and noncortical blood flow and in filtration fraction (Table IV), and only a small increase in mean arterial pressure similar to that observed in the studies without nucleotide in the infusate, suggest that direct cellular effects of dibutyryl cyclic AMP rather than changes in Starling forces in the peritubular capillaries were the basis for the decrease in proximal tubular sodium reabsorption.

In previous studies, infusion of a beta adrenergic stimulator (isoproterenol) into a renal artery decreased the proximal tubular reabsorption of sodium and water, increased $C_{H_2O}$ with essentially no change in sodium excretion, and did not appear to alter Starling forces in the peritubular capillaries—a pattern of effect qualitatively similar to that produced by dibutyryl cyclic AMP in the present studies (1). The quantitative effects of the chosen doses of isoproterenol and the nucleotide were also similar: proximal tubular reabsorption, as estimated from the changes in V, decreased 44% with the former and 45% with the latter. The ability of beta adrenergic stimulation to activate renal cortical adenyl cyclase (2) and to increase the synthesis of cyclic AMP in many tissues (12), and the similarity of effects of isoproterenol and dibutyryl cyclic AMP in the kidney provide strong circumstantial evidence in support of the concept that the generation of intracellular cyclic AMP is an intermediary event in the depression of proximal tubular reabsorption of sodium by beta adrenergic stimulation.

Parathyroid hormone and glucagon have both been shown to stimulate adenyl cyclase in homogenates of renal cortical tubules via receptor(s) other than beta adrenergic receptors (2). The decrease in tubular reabsorption of sodium and phosphate, which occurs with parathyroid hormone (11) and glucagon (13), can also be produced by dibutyryl cyclic AMP (10, 11). These results suggest that it is not beta adrenergic stimulation per se but rather the production of cyclic AMP intracellularly that is responsible for the decrease in sodium reasbsorption. The series of reactions initiated by cyclic AMP formation that lead to changes in tubular reabsorption are at present unknown.

A considerable body of data indicates that changes in peritubular capillary Starling forces could mediate the decrease in proximal tubular sodium reabsorption which occurs when extracellular fluid volume is expanded (14–16). In some studies, however, apparent dissociation between peritubular capillary forces and proximal tubular sodium reabsorption has been observed (17, 18). Such a discrepancy could be explained if expansion of the volume of extracellular fluid leads to release of a "natriuretic substance" into the circulation (19); it could also be explained if expansion of extracellular fluid volume leads to the formation of cyclic AMP in proximal renal tubule cells by stimulation of beta adrenergic or other receptors.

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