# THE RENAL ACTIONS OF ADENOSINE

By

Lois Johanna Arend

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

1987

## ABSTRACT

## THE RENAL ACTIONS OF ADENOSINE

By

Lois Johanna Arend

Renal function is regulated by neural, humoral, and physical factors. Intrinsic factors are also responsible for the control of renal function. Many hypotheses have been formulated for the intrinsic regulation of renal function. One of these hypotheses involves adenosine, acting as a retaliatory metabolite. That is, as renal cellular metabolism is stimulated, and ATP hydrolysis is increased, adenosine is formed and acts to return metabolism to a normal state. Many previous studies have demonstrated an effect of exogenous adenosine on renal function consistent with the hypothesis that adenosine is an intrinsic regulator of renal function.

The present studies were designed to test the effects of endogenously-produced adenosine on renal function, and to investigate the cellular mechanisms involved in some of the renal actions of adenosine.

Increasing the intrarenal concentration of adenosine by several pharmacological and pathophysiological manuevers

led to pronounced changes in renal function. These effects could be attenuated or inhibited by the competitive adenosine receptor antagonist, theophylline. These studies support the hypothesis that intrarenally-produced adenosine can alter renal function.

To investigate the cellular mechanisms of the actions of adenosine, cortical collecting tubule cells (RCCT) were used as an epithelial model. The presence of adenosine receptors in these cells and the second messenger systems to which they are coupled were studied with the use of non-transported, non-metabolized adenosine analogs. RCCT cells were demonstrated to possess $A_1$ and $A_2$ adenosine receptors, coupled to the production of cAMP, and a receptor of unknown subtype coupled to the mobilization of intracellular calcium. This latter receptor may be a previously unidentified adenosine receptor. Alternatively, because it has characteristics similar to the $A_1$ receptor, it may be a second signal transducing mechanism coupled to the $A_1$ receptor.

These studies support the hypothesis that adenosine is involved in the regulation of renal function. This conclusion is based on the ability of adenosine, produced by the kidney, to alter renal function, and on the presence of adenosine signal transducing mechanisms in one population of renal cells.

To my grandmothers
Helen, Lois, and Marcella

## ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge the love and understanding given to me by my parents. Without their support this work would mean much less.

I would like to thank the members of my Guidance Committee: Drs. Steven R. Heidemann, S. Richard Heisey, William L. Smith, William S. Spielman, and Lynne C. Weaver, for their advice and encouragement.

In particular, I thank Bill, my advisor, colleague, and friend, for providing a challenging, feracious environment in which to nurture my research skills.

## COPYRIGHT ACKNOWLEDGEMENTS



This work was supported in part by a USPHS Predoctoral Traineeship, NIH (HL07404), and by a University Graduate Fellowship awarded by the Michigan State University College of Natural Science.

## TABLE OF CONTENTS


| | Page |
|---|---|
| List of Tables | vii |
| List of Figures | viii |
| I. Introduction | 1 |
| II. Literature Review | 4 |
| A. Adenosine Biochemistry | 4 |
| A.1. Adenosine metabolism | 4 |
| A.2. Adenosine receptors | 6 |
| B. Effect of Adenosine on Renal Function | 10 |
| B.1. Hemodynamics | 10 |
| B.2. Renin release | 12 |
| B.3. Neurotransmitter release | 15 |
| B.4. Sodium and water excretion | 16 |
| B.5. Endogenous adenosine | 18 |
| C. Cellular Mediation of the Actions of Adenosine | 21 |
| C.1. Non-epithelial cells | 21 |
| C.2. Epithelial cells | 24 |
| III. The Renal Effects of Increasing Endogenous Adenosine | 28 |
| A. Dipyridamole decreases glomerular filtration in the sodium-depleted dog | 28 |
| A.1. Introduction | 28 |
| A.2. Methods | 30 |
| A.3. Results | 41 |
| A.4. Discussion | 55 |
| B. Increasing intrarenal adenosine by maleic acid decreases GFR and renin release | 65 |
| B.1. Introduction | 65 |
| B.2. Methods | 67 |
| B.3. Results | 71 |
| B.4. Discussion | 78 |
| C. A role for intrarenal adenosine in the renal hemodynamic response to contrast media | 88 |
| C.1. Introduction | 88 |
| C.2. Methods | 90 |

## TABLE OF CONTENTS, CONT'D.


| | Page |
|---|---|
| C.3. Results | 93 |
| C.4. Discussion | 99 |
| IV. The Renal Cellular Actions of Adenosine | 106 |
| A. A1 and A2 adenosine receptors in rabbit cortical collecting tubule cells | 106 |
| A.1. Introduction | 106 |
| A.2. Methods | 107 |
| A.3. Results | 111 |
| A.4. Discussion | 117 |
| B. Adenosine signal transduction in the rabbit cortical collecting tubule: receptor-mediated calcium mobilization | 124 |
| B.1. Introduction | 124 |
| B.2. Methods | 126 |
| B.3. Results | 131 |
| B.4. Discussion | 143 |
| V. Summary and Conclusions | 148 |
| Appendix A. Abbreviations and Definitions of Compounds Used | 154 |
| Appendix B. Derivation of Equation for Calculation of Intracellular Calcium | 156 |
| Literature Cited | 158 |

## LIST OF TABLES


| Table | | Page |
|---|---|---|
| 1. | Classification of adenine receptors..... | 8 |
| 2. | Effect of dipyridamole on renal function in sodium-depleted and sodium-loaded dogs and the effect of theophylline on the renal responses to dipyridamole..... | 47 |
| 3. | Time course of effect of maleic acid in sodium-depleted and sodium-loaded dogs..... | 72 |
| 4. | Effect of theophylline on the maleic acid-induced changes in renal function of sodium-depleted and sodium-loaded dogs..... | 73 |
| 5. | Effects of contrast media on renal function, and the effects of theophylline and dipyridamole on the renal responses to contrast media..... | 98 |
| 6. | Effect of adenosine analogs on hormone-stimulated cAMP production in RCCT cells..... | 116 |
| 7. | Effect of pertussis toxin on stimulation of cAMP production by $N^6$-cyclohexyladenosine (CHA) in RCCT cells..... | 119 |
| 8. | Effect of C-101 on stimulation and inhibition of cAMP production by adenosine analogs in RCCT cells..... | 140 |

## LIST OF FIGURES


| Figure | | Page |
|---|---|---|
| 1. | Dilution curves for adenosine and ara-H in plasma from a representative single-injection, multiple-tracer experiment..... | 43 |
| 2. | Dilution curves for adenosine and ara-H in the urinary effluent..... | 44 |
| 3. | Comparison of glomerular filtration rate (GFR) before, during, and after the intrarenal infusion of dipyridamole in sodium-depleted dogs..... | 46 |
| 4. | Renal perfusion pressure (RPP), renal blood flow (RBF), and glomerular filtration rate (GFR) in five sodium-depleted dogs before, during, and after the intrarenal infusion of dipyridamole.... | 51 |
| 5. | Mean arterial pressure (MAP), renal blood flow (RBF), and glomerular filtration rate (GFR), in five sodium-depleted dogs before and during the intrarenal infusion of dipyridamole, and during the intrarenal infusion of theophylline and dipyridamole, simultaneously..... | 53 |
| 6. | Effect of theophylline on the maleic acid-induced changes in glomerular filtration rate (GFR) and renal blood flow (RBF) in five sodium-depleted dogs..... | 74 |
| 7. | Effect of theophylline on the maleic acid-induced changes in glomerular filtration rate (GFR) and renal blood flow (RBF) in five sodium-loaded dogs..... | 75 |
| 8. | Effect of theophylline on the maleic acid-induced decrease in renin release presented as percent of control..... | 79 |
| 9. | Effect of maleic acid on arterial adenosine, renal venous adenosine, and the urinary excretion of adenosine in six sodium-depleted and ten sodium-loaded dogs..... | 80 |

## LIST OF FIGURES, CONT'D.


| Figure | | Page |
|---|---|---|
| 10. | Representative renal blood flow tracing of the responses to contrast media injection (small arrows) in the control state, and in the presence of dipyridamole..... | 95 |
| 11. | Effect of contrast media on glomerular filtration rate (closed bars) and renal blood flow (hatched bars) in the control state, (CON), and during the infusion of theophylline (THEO) or dipyridamole (DP)..... | 96 |
| 12. | Effect of adenosine analogs on total RCCT cell cAMP production, expressed as percent of control (basal)..... | 113 |
| 13. | Effect of pretreatment with pertussis toxin on the modulation by CHA (50 nM) of 1 uM AVP-stimulated cAMP production in RCCT cells..... | 118 |
| 14A. | Effect of adenosine analogs on intracellular free calcium in primary RCCT cells, expressed as percent change from control..... | 133 |
| 14B. | Effect of adenosine analogs on intracellular free calcium in first-passage RCCT cells, expressed as percent change from control..... | 134 |
| 15. | Representative fluorescence tracing of response of intracellular calcium to adenosine analogs... | 135 |
| 16. | Effect of DPX on the NECA-induced increase in intracellular calcium..... | 137 |
| 17. | Effect of C-101 on the CHA-induced increase in intracellular calcium..... | 138 |
| 18. | The response of intracellular calcium to NECA in the presence and absence of extracellular calcium..... | 140 |
| 19. | Effect of pertussis toxin on the calcium responses to the adenosine analogs, CHA and NECA..... | 142 |
| 20. | Model cell of adenosine receptor systems..... | 150 |

# I. INTRODUCTION

Adenosine is a ubiquitous compound that has a variety of actions in many tissues and is thought to be involved in metabolic control of organ function. That is, adenosine production results from the breakdown of ATP during periods of increased cellular work and is thought to act in a negative feedback manner to reduce energy consumption. Specifically, in the kidney, it has been hypothesized that adenosine acts as an intrinsic regulator of renal function (Osswald 1980; Spielman and Thompson 1982). Because renal energy utilization is almost entirely a result of active sodium transport by the tubular epithelium and because the rate of tubular transport of sodium is determined by the rate of sodium filtered, a phenomenon known as glomerulotubular balance, it was postulated that increased ATP hydrolysis, secondary to stimulated transport, elevated adenosine levels that decrease GFR, filtered load, and thereby ATP hydrolysis toward normal.

In determining a role for adenosine in the metabolic control of renal function it is necessary to determine if the exogenous administration of adenosine produces the proposed changes in renal function. Several previous

studies to characterize the effects of exogenous adenosine support the hypothesis that adenosine may act as an intrinsic regulator of renal function (reviewed in Spielman and Thompson 1984, Osswald 1984). These studies are discussed in the second section of the literature review (II.B.1-4). It must also be proven that adenosine can be produced in the kidney in sufficient amounts to elicit the necessary changes in renal function, that there is a system for the regulation of adenosine levels by degradation or cellular removal, and that renal cells possess adenosine receptors coupled to signal transduction mechanisms.

This dissertation extends what is known about the functional effects of intrarenal adenosine by describing three studies in which endogenous adenosine concentrations were increased and the changes in renal function observed. This dissertation further characterizes the renal actions of adenosine by detailing two studies directed at understanding the mechanism by which adenosine acts to control renal cellular function. These studies identify the presence of adenosine receptors in rabbit cortical collecting tubule cells and the effects of adenosine on two second messenger systems in those cells.

The literature review is composed of three sections, the first being a general review of the adenosine molecule,

its biochemistry, and the present state of understanding of the adenosine receptor systems. The other two sections correspond generally to the two diverse areas of study. Each of the five projects comprising this study is represented by a chapter, consisting of a separate introduction, methods, results, and discussion with specific points relevant to each project. A general discussion of the importance of adenosine in the regulation of renal function completes the thesis.

# II. LITERATURE REVIEW

## A. Adenosine Biochemistry

### A.1. Adenosine metabolism

Adenosine, a purine nucleoside that is found in all cells, is an intermediate in the pathway of adenine nucleotide synthesis and degradation. Adenosine consists of a ribose moiety and an adenine purine ring, and is a precursor to, and metabolite of, ATP and the other adenine nucleotides. Adenosine is also a component of S-adenosyl homocysteine (SAH).

Adenosine is produced by the action of 5' nucleotidase to hydrolyze adenine nucleotides (Baer et al 1966) and by the hydrolysis of SAH by SAH hydrolase (Schrader, Schutz, and Bardenheuer 1981). Both of these enzymes are present in the kidney (Eloranta 1977; Miller 1978; Schatz 1977). Despite the favorable equilibrium of the hydrolase reaction toward degradation of SAH and production of adenosine, SAH is not likely to be a major source of adenosine since the SAH hydrolase is inhibited by its products, adenosine and inosine. The major source of adenosine is therefore presumed to be the adenine nucleotides.

The extracellular concentration of adenosine depends upon the balance between its rate of production and its rate of removal. The removal of adenosine from the extracellular space is primarily accomplished by cellular uptake followed by deamination to inosine by adenosine deaminase or phosphorylation to AMP by adenosine kinase. Adenosine deaminase is also present in the interstitium (Conway and Cooke 1939; Trams and Lauter 1974; Baer and Drummond 1968). Movement of adenosine into and out of the cell is governed by bi-directional facilitated diffusion. The adenosine carrier has been extensively characterized in red blood cells (Kolassa and Pfleger 1975; Kubler and Bretschneider 1963), hepatoma cells (Plagemann 1971; Plagemann and Wohlhueter 1980; Plagemann and Richey 1974), aortic endothelial and smooth muscle cells (Pearson et al 1978), and platelets (Sixma et al 1976). It has been suggested that the nucleoside carrier is closely linked to the membrane-bound 5'-nucleotidase, thereby producing and transporting adenosine in one step (Fox and Kelley 1978).

Relatively little is known about the adenosine uptake system in the kidney. Saturable, temperature-sensitive adenosine uptake has been demonstrated in renal brush border and antiluminal membrane vesicles (Trimble and Coulson 1984). Thompson et al (1985) provided evidence

that the kidney possesses an adenosine uptake mechanism that is sensitive to dipyridamole, a nucleoside uptake blocker. As discussed in III.A., elevation of endogenous adenosine levels in the kidney by blocking uptake results in profound changes in renal function, suggesting that the control of extracellular adenosine concentrations is an important part of the renal actions of adenosine.

A.2. Adenosine receptors

Purine compounds have been recognized for over fifty years to have effects on organ function. In 1929, Drury and Szent-Gyorgyi first reported that adenosine produced vasodilation of the coronary arteries, lowered blood pressure, induced sleep, and reduced intestinal motility. Much of the work with purines in the next 20-30 years centered on adenine nucleotides since it had been shown that tissue adenosine concentrations were very low, suggesting that adenosine was not a physiologically important compound. However, in 1963, Berne reported an increase in the amounts of inosine and hypoxanthine, degradation products of adenosine, in coronary sinus blood during hypoxia. In addition, Haddy and coworkers demonstrated that physiological concentrations of adenosine produce vasodilation in a number of organs (Haddy and Scott 1968) and vasoconstriction in the kidney (Haddy et al

1965). Subsequent studies have demonstrated actions of adenine compounds on the liver, skeletal muscle, vascular smooth muscle and many other tissues. It was suggested that ATP functioned as a neurotransmitter to produce many of the effects attributed to adenosine in these organs (Burnstock 1972, 1976, 1978). Later, it was established that many of these actions were due to the direct stimulation of extracellular receptors by adenosine.

Purinergic (P) receptors activated preferentially by adenosine over the adenine nucleotides were termed $P_1$ receptors, while those activated to a greater degree by adenine nucleotides than by adenosine were labelled $P_2$ receptors (Table 1, Burnstock 1978). Purinergic receptors have been further characterized by their responses to xanthine derivatives and their effects on cAMP production. The actions of adenine compounds at $P_1$, but not $P_2$ receptors are antagonized by xanthine derivatives. Activation of $P_1$ receptors evokes changes in cellular cAMP production, while $P_2$ receptors do not. Subsequently, it has been demonstrated that $P_1$ receptors can be further subdivided into two extracellular receptors, $A_1$ (or $R_i$) and $A_2$ (or $R_a$) receptors (VanCalker 1979; Londos 1980), and an intracellular receptor, the P-site, with a distinguishing feature being the direction of the change in cAMP

Table 1. Classification of adenine receptors

| | $P_1$ | | | $P_2$ |
|---|---|---|---|---|
| Specificity | ado>AMP>ADP>ATP | | | ATP>ADP>AMP>ado |
| | P-site | $A_1$ | $A_2$ | |
| Location | intracellular | extracellular | extracellular | extracellular |
| Requires intact ribose moiety | no | yes | yes | |
| Requires intact purine moiety | yes | no | no | |
| Sensitive to methylxanthines | no | yes | yes | no |
| Effect on adenylate cyclase activity | inhibit | inhibit | stimulate | no effect |
| $K_m$ | 1-10 mM | 1-10 nM | 1 uM | |
| Analog potency | DDA>DA>ado | CHA>ado>NECA | NECA>ado>CHA | |

production. Activation of the $A_1$ receptor and the intracellular P-site is associated with an inhibition of cAMP production, presumably through a decrease in the activity of the enzyme adenylate cyclase. Stimulation of adenylate cyclase activity and subsequent increase in cAMP production characterizes activation of the $A_2$ receptor. Both extracellular receptors ($A_1$ and $A_2$) are sensitive to xanthine derivatives, while the intracellular P-site is not. The extracellular inhibitory receptor ($A_1$) is a high affinity receptor with a Km of approximately 1-10 nM, while the stimulatory ($A_2$) receptor has lower affinity for adenosine with an approximate Km of 1-10 uM (reviewed by Daly 1982). The intracellular site is activated only at concentrations of adenosine in the millimolar range, and is possibly mediated by intracellular conversion of adenosine to 5'AMP.

The study of adenosine's actions at the $P_1$ receptors is hindered because adenosine is rapidly removed from the extracellular medium by cells and metabolized. Furthermore, the study of adenosine receptors is facilitated by compounds with greater specificity for one receptor type. Consequently, adenosine derivatives have been synthesized which are not substrates for the nucleoside carrier and have more specificity of binding to

either the $A_1$, $A_2$, or P-site adenosine receptor. The most commonly used of these compounds are: 5'-N-ethylcarboxamideadenosine (NECA), $N^6$-cyclohexyladenosine (CHA), $N^6$-phenylisopropyladenosine (PIA), and 2-chloroadenosine. The rank order of potency for these analogs at the $A_2$ receptor is: NECA>2-chloro>CHA=PIA and at the $A_1$ receptor is: CHA=PIA> 2-chloro>NECA. 2'5' dideoxyadenosine (DDA) and 2' deoxyadenosine (DA) act at the P-site to inhibit cAMP production (Londos and Wolff 1977).

## B. The Effect of Adenosine on Renal Function

### B.1. Hemodynamics

Adenosine is a smooth muscle relaxant in most organs (Harvey 1960; Haddy and Scott 1968; Walter and Bassenge 1968; Kraupp 1969; Ally and Nakatsu 1976). Adenosine causes vasodilation of intestinal smooth muscle (Ally and Nakatsu 1976) and most vascular beds, including the portal vein, coronary arteries, and renal interlobular artery (Walter and Bassenge 1968). However, bolus injections of adenosine into the renal artery result in vasoconstriction (Hashimoto and Kumakura 1965; Osswald 1975; Osswald et al 1975; Osswald et al 1978; Sakai et al 1981; Scott et al 1965; Spielman and Osswald 1978; Thurau 1964). Furthermore, intrarenal infusion of adenosine produces a biphasic renal blood flow response (Osswald et al 1978; Spielman et al

1980; Tagawa and Vander 1970). That is, immediately following initiation of an adenosine infusion, renal blood flow is reduced, but with continued infusion, blood flow returns to the pre-infusion level or above. This biphasic renal blood flow response to an adenosine infusion is not completely understood, but has been suggested to be the result of differential effects of adenosine on afferent and efferent arteriolar resistances (Thompson et al 1985), or a differential effect on nephrons in the outer and inner cortex (Spielman et al 1980). As demonstrated by microsphere studies, after the initial vasoconstriction has waned and blood flow is at or above control, inner cortical blood flow is increased, while that to the outer cortical nephrons is unchanged (Spielman et al 1980).

Adenosine infusion results in a decrease in glomerular filtration rate (GFR) that is sustained throughout the period of the infusion, despite the return of blood flow to or above control. The decrease in GFR, as well as the biphasic blood flow response, may be due to an action of adenosine to differentially alter afferent and efferent arteriolar resistances. Thompson et al (1985) demonstrated that there is no change in filtration fraction as renal blood flow declines at the beginning of the infusion, but

filtration fraction decreases as renal blood flow returns to control. These results suggest that adenosine produces an initial constriction of the afferent arteriole, leading to both the decrease in renal blood flow and glomerular filtration rate, followed by a dilation of the efferent arteriole. Efferent arteriolar vasodilation results in a subsequent return of renal blood flow but a maintained decrease in glomerular filtration rate due to decreased filtration pressure in the glomerulus. Whether adenosine alone produces both the constriction of the afferent arteriole and the dilation of the efferent arteriole, or whether another hormone contributes to this response, is yet to be determined.

B.2. Renin release

In addition to changes in renal hemodynamics, adenosine infusion into the kidney results in a pronounced decrease in the release of renin (Osswald et al 1978, 1982; Tagawa and Vander 1970; Spielman 1984). A decrease in renin release has also been demonstrated in renal slices (Murray and Churchill 1984, 1985; Churchill and Churchill 1985) and in isolated perfused afferent arterioles (Itoh et al 1985). Recently, Kurtz et al (1987) observed an

adenosine-induced decrease in renin production by isolated, cultured juxtaglomerular cells.

The effect of adenosine on renin release, as well as renal blood flow and glomerular filtration rate, demonstrates a sodium-dependency. That is, the adenosine-induced changes in renal hemodynamics and renin release were found to be greatly enhanced by prior sodium-depletion of the animal and could be abolished by feeding the animal a diet high in sodium prior to experimentation (Thurau 1964; Osswald et al 1975). This sodium-dependent response is also observed in the renal responses to increased endogenous adenosine concentrations (III.A-C). Because renal renin concentrations, and therefore, angiotensin concentrations, are altered by changing sodium intake, an interaction of adenosine with the renin-angiotensin system was proposed. Indeed, the adenosine-induced vasoconstriction and decrease in GFR can be attenuated by the administration of angiotensin antagonists (Spielman and Osswald 1979) and converting enzyme inhibitors (Hall and Granger 1986). Additionally, as recently observed by Edwards (personal communication), in the presence of adenosine, angiotensin results in afferent arteriolar vasoconstriction, an effect of angiotensin that is not

normally observed. These results suggest that the vasoconstrictive action of adenosine may be mediated by angiotensin. However, Spielman (1984) reported that angiotensin concentrations in renal lymphatic fluid of the dog were not increased, but actually decreased, during an adenosine infusion, suggesting that angiotensin does not mediate the vasoconstriction. Therefore, the exact relationship between adenosine and the renin-angiotensin system has yet to be determined.

The mechanism of the adenosine-induced decrease in renin release has been studied at several levels. In whole-animal experiments, Arend et al (1984) reported that adenosine inhibition of renin release is independent of the hemodynamic actions of adenosine and is not affected by the administration of the organic calcium influx blocker, verapamil. These results suggest a direct action of adenosine on the renin-producing juxtaglomerular cells. It is generally believed that renin secretion from the juxtaglomerular cell is inversely linked to changes in intracellular calcium (reviewed in Fray 1980). That is, an increase in intracellular calcium in the juxtaglomerular cell results in a decrease in renin release and vice versa. This hypothesis extends from the results of several studies

in which the intracellular concentration of calcium was changed with an accompanying inverse change in renin release. Agents such as catecholamines, which are known to act through calcium in other cells can inhibit renin secretion (Fray and Park 1979). Changes in the calcium concentration of an intrarenal infusate (Vandongen and Peart 1974; Fray 1977; Baumbach and Leyssac 1977) leads to an opposite change in renin release. An increase in the intracellular calcium concentration by the addition of calcium ionophores (Baumbach and Leyssac 1977; Fynn et al 1977) or by membrane depolarization with potassium (Ettienne and Fray 1979; Fray and Park 1979) lead to a decrease in renin secretion. Very recently, Kurtz et al (1987) reported that in isolated juxtaglomerular cells, adenosine increases the production of cyclic 3',5' guanosine monophosphate (cGMP), but not cAMP or calcium, in these cells. These results suggest that cGMP may be the intracellular mediator of the adenosine-induced decrease in renin release.

B.3. Neurotransmitter release

A further action of adenosine in the kidney is its ability to block the release of neurotransmitter from sympathetic neurons (Hedqvist and Fredholm 1978). This

effect of adenosine is not confined to renal sympathetic neurons, but is a widely observed phenomenon at other sympathetic nerve terminals (reviewed by Fox and Kelley 1978). It was observed by Phillis and Edstrom (1976) that adenosine depresses electrical activity of cortical neurons, resulting in speculation that adenosine interferes with neurotransmission by an antagonism of calcium influx (Silinsky 1981).

The sympathetic nerves in the kidney innervate the afferent and efferent arterioles, the juxtaglomerular cells, and the renal tubules. Thus, adenosine inhibition of renal neurotransmitter release has been proposed as a possible mechanism of action for the changes in hemodynamics, renin release, and excretion that occur with adenosine infusions. However, because adenosine decreases GFR, renin release, and excretion in chronic denervated kidneys (Cook and Churchill 1984), where renal nerves are not functional, inhibition of neurotransmitter release by adenosine may be involved in modulating, rather than mediating, some of the actions of adenosine.

### B.4. Sodium and water excretion

Adenosine infusion results in a fall in urine output and a decrease in sodium excretion (Churchill 1982; Osswald

et al 1978). This could occur by a direct action of adenosine on transepithelial solute and water movement and this action will be discussed in greater detail in the section on the cellular actions of adenosine (II.C.2). Alternatively, because excretion is tightly coupled to filtration, the decrease in water and solute excretion may be an indirect result of the action of adenosine to decrease renal blood flow and glomerular filtration rate. The question of how adenosine exerts its action on excretion has not been adequately addressed, primarily due to the difficulty of separating this effect from the other actions of adenosine, thus providing a rationale for investigating the direct action of adenosine on isolated, segment-specific cells of the kidney. Further support for a direct effect of adenosine on the tubular epithelium comes from studies performed in renal cell lines (Lang et al 1985) and other epithelial tissue (Stoff et al 1979; Forrest et al 1980; Poeschla et al 1982; Dobbins et al 1984; Grasl and Turnheim 1984; Dillingham and Anderson 1985) as discussed in Sections II.C.2 and II.C.3.

B.5. Endogenous adenosine

A crucial point in defining a role for adenosine in the regulation of renal function is to demonstrate that

adenosine is present in the kidney and that maneuvers resulting in an increase in intrarenal adenosine produce changes in renal function consistent with the hypothesis. It was suggested by Scott et al in 1965 that adenosine might be involved in the vascular changes following brief ischemia of the kidney because the hemodynamic responses to occlusion of the renal artery and an intrarenal injection of adenosine were similar.

The presence of adenosine in the kidney was established in the 1970's by several groups (Osswald et al 1977; Miller et al 1978). These studies demonstrated adenosine in the normal kidney and increased concentrations of adenosine following ischemia in the kidney. Ischemia was induced by occluding the renal artery for varying times (15 sec - 10 min), and adenosine and ATP concentrations were measured in whole kidney tissue. Following ischemia, ATP concentrations decline and adenosine concentrations increase proportional to the length of the induced ischemia. These increased concentrations of adenosine were accompanied by a brief reduction in renal blood flow, which was attenuated by the adenosine receptor antagonist, theophylline. These results suggested that during ischemia, ATP is broken down to adenosine. Adenosine

accumulates in the kidney and subsequently acts to reduce blood flow when the occlusion is released, possibly to allow ATP concentrations to be restored before energy-requiring transport begins again. Similar results were reported by Osswald et al, in 1980, following the intrarenal infusion of hypertonic saline. Hypertonic saline infusion caused a vasoconstriction and an inhibition of renin release, both antagonized by theophylline. Adenosine concentrations were increased and accompanied by a decrease in the tissue content of ATP. This suggests that as the transport load of the kidney increases and ATP is hydrolyzed to adenosine, adenosine acts to return filtered sodium toward normal by decreasing blood flow and GFR.

These studies have, as a common goal, to investigate the way in which the kidney can regulate its own blood flow and glomerular filtration. One way the intrinsic regulation of renal blood flow and GFR could occur is by the process of tubuloglomerular feedback (TGF). In TGF, a signal, probably a chemical mediator, transmits information through the macula densa area of the distal tubule, to the glomerulus and the afferent and efferent arterioles. In this way, each nephron controls its own blood flow and

filtration rate. Micropuncture studies show that increases in tubular fluid flow rate lead to a decrease in single nephron filtration rate, suggesting changes in blood flow and glomerular dynamics of that nephron (Schnermann et al 1970). Therefore, a more direct assessment of the role of adenosine in intrinsic regulation of the kidney is to employ the method of micropuncture to study the TGF response in regard to adenosine. Osswald et al (1980) and Schnermann et al (1977) have shown that theophylline interferes with the normal TGF response. That is, in the presence of theophylline, an increase in tubular perfusion rate did not result in a change in filtration in the perfused nephron. In addition, in the latter study the TGF response was enhanced by blocking adenosine uptake with dipyridamole. These results suggest that adenosine may be involved in regulation of renal function as a mediator of the tubuloglomerular feedback response.

## C. Cellular Mediation of the Actions of Adenosine.

### C.1. Non-epithelial cells

The nature of the adenosine receptor and the post-receptor mechanisms responsible for the actions of adenosine have been studied extensively in non-renal

tissues and cells. Many reports have demonstrated an action of adenosine on cellular adenylate cyclase activity and/or cAMP production. Others have correlated these biochemical effects with specific biological aspects of the tissue or cells.

An effect of adenosine on the adenylate cyclase enzyme was demonstrated independently by two groups (Sattin and Rall 1970; Shimizu and Daly 1970). They reported that adenosine and some adenine nucleotides increased cAMP concentrations in brain slices. Since then, an effect of adenosine on adenylate cyclase activity and cAMP production has been documented in a wide variety of neural tissue, including neuroblastoma (Blume et al 1973), astrocytoma (Clark et al 1974), and glioma (Clark and Seney 1976) cells. In addition to nervous tissue, adenosine-sensitive cAMP production has been demonstrated in platelets (Haslam and Lynham 1972), adipocytes (Fain 1973; Fain et al 1972; Schwabe et al 1973; Hjemdahl and Fredholm 1976), hepatocytes (Lund et al 1975), lung tissue (Palmer 1971), fibroblasts (Bruns 1980), lymphocytes (Wolberg et al 1975), myocardium (Huang and Drummond 1976), and coronary arteries (Kukovetz et al 1978). The majority of these studies demonstrate stimulation of adenylate cyclase activity

and/or production of cAMP, suggesting the presence of $A_2$ adenosine receptors. Inhibitory ($A_1$ or P-site) adenosine receptors have been demonstrated in cultured astroblasts (VanCalker et al 1979), myocardium (Dobson 1978), platelets (Haslam and Lynham 1972; Londos and Woff 1977), hepatocytes (Lund et al 1975), and adipocytes (Fain 1973; Fain et al 1972; Schwabe et al 1973; Hjemdahl and Fredholm 1976). Adipocytes appear to have only inhibitory adenosine receptors. However, neuroblastoma cells, hepatocytes, myocardium, and platelets exhibit both stimulation and inhibition of cAMP production in response to adenosine, suggesting the presence of both $A_1$ and $A_2$ receptors.

Some functional correlates to the biochemical effects of adenosine have been defined. The majority of this work has focused on the action of adenosine to inhibit lipolysis in adipose tissue. Dole (1961) reported that adenosine and adenine nucleotides inhibited hormone-stimulated lipolysis in rat adipose tissue. Fain and coworkers (1972, 1973) demonstrated the inhibition of norepinephrine-stimulated adenylate cyclase activity, cAMP accumulation, and lipolysis in fat cell ghosts by adenosine, 2'deoxyadenosine, and 2'5'dideoxy adenosine.

Adenosine has also been shown to alter the pancreatic secretion of both insulin and glucagon. Adenosine has been reported to inhibit (Ismail 1977) and stimulate (Feldman and Jackson 1974; Capito and Hedeskov 1976) the secretion of insulin and to stimulate the secretion of glucagon (Weir et al 1975). Mast cell histamine release is also stimulated by adenosine (Sullivan et al 1976). Stimulation of steroidogenesis in adrenal and Leydig tumor cells has been demonstrated with adenosine (Wolff and Cook 1977; Kowal and Fiedler 1969). In these studies, tumor cell dihydroprogesterone production, adenylate cyclase activity, and cAMP accumulation were stimulated by adenosine and inhibited by theophylline. The adenosine concentration range for this effect (EC50 = 10 uM) suggests mediation by the $A_2$ receptor. An important metabolic action of adenosine has been suggested to be linked to the immunosuppression associated with adenosine deaminase deficiency. Wolberg et al (1975) demonstrated that micromolar concentrations of adenosine stimulated lymphocyte cAMP production that was associated with a decrease in the degree of lysis of tumor cells.

The inhibition of neurotransmitter release from sympathetic nerve terminals (Hedqvist and Fredholm 1978)

and the stimulation of glucose oxidation in hepatocytes (Souness and Chagoya de Sanchez 1981) by adenosine are not associated with changes in cAMP production. These observations have prompted the speculation that adenosine may act to produce these and other changes through a system other than the adenylate cyclase-cAMP pathway. A possible alternative is the involvement of calcium: either stimulation of calcium movement across the cell membrane, or a mobilization of calcium from intracellular stores. Whether these cAMP-independent actions of adenosine are mediated through an extracellular receptor and whether this receptor is one of the currently recognized adenosine receptors is unknown and will be discussed further in IV.B.

C.2. Epithelial cells

a. non-renal epithelia. Adenosine and various adenosine derivatives have been reported to alter the function of renal and non-renal transporting epithelia. In 1979, Spinowitz and Zadunaisky demonstrated adenosine-stimulated electrogenic chloride transport in amphibian corneas as evidenced by an increase in chloride flux accompanied by increases in short circuit current and potential difference when micromolar concentrations of adenosine were added. Concurrently, Stoff et al (1979)

reported adenosine regulation of chloride transport in the shark rectal gland. Following these studies, Forrest and coworkers (Forrest et al 1980, 1982, 1984; Poeschla et al 1982, 1985; Dobbins et al 1985; Kelley et al 1984, 1985) have shown the presence of both $A_1$ and $A_2$ adenosine receptors in the shark rectal gland, which inhibit and stimulate chloride transport, respectively. The inhibition of chloride secretion by adenosine can be blocked by treatment with pertussis toxin (Kelley et al 1985) and, therefore, is very likely to be mediated by the inhibitory guanine nucleotide binding protein, $N_i$. Accompanying the alterations in chloride secretion were changes in cAMP production by the shark rectal gland. Increases in cAMP accumulation and adenylate cyclase activity were observed with micromolar concentrations of adenosine and adenosine analogs. Inhibition of cAMP accumulation was demonstrated at low (nanomolar) concentrations of adenosine. Recently, Kelley et al (1986) have suggested that the inhibition of chloride secretion may also involve a cAMP-independent mechanism because the 2-chloroadenosine-induced decrease in cAMP production was reversed by addition of forskolin, a non-receptor mediated activator of adenylate cyclase, but the accompanying inhibition of chloride secretion was not

affected. These results suggest the involvement of an additional second messenger system in the inhibition of chloride secretion in the shark rectal gland.

In addition to actions of adenosine on non-mammalian epithelia, studies that involve rabbit ileum and colon have demonstrated similar effects of adenosine on ion movement. In 1984, Dobbins et al reported adenosine regulation of chloride secretion in the rabbit ileum accompanied by an increase in cAMP production, and Grasl and Turnheim (1984) reported stimulation of electrolyte secretion by adenosine in the rabbit colon.

b. <u>renal epithelia</u>. The study of the regulation of ion movement by adenosine in renal epithelium has been limited primarily to cell lines, such as toad (A6) and porcine (LLC-PK) kidney cells. Lang et al (1985) reported that adenosine stimulated the short-circuit current related to sodium ion transport in the A6 cell line. In LLC-PK cells (Roy et al 1984), the adenosine analog PIA stimulates the activity of the adenylate cyclase enzyme at high concentrations. At low concentrations, PIA inhibited basal adenylate cyclase as well as arginine vasopressin-stimulated adenylate cyclase activity. Dillingham and Anderson (1985) recently reported that NECA stimulates

water movement in the isolated perfused rabbit cortical collecting tubule and that the P-site agonist, 2'5'dideoxyadenosine inhibits vasopressin-stimulated water movement, providing support for adenosine as a regulator of salt and water transport in the kidney.

## III. THE RENAL EFFECTS OF INCREASED ENDOGENOUS ADENOSINE

### A. Dipyridamole decreases glomerular filtration in the sodium-depleted dog

(reproduced with permission, Am. Heart Assoc.; Circ. Res. 56:242, 1985)

#### A.1. Introduction

Work in several laboratories has raised the possibility that intrarenally produced adenosine plays a role in the intrinsic control of glomerular filtration rate (GFR) and renin release (Spielman and Thompson 1982). It has been postulated that extracellular adenosine, produced from cellular 5'-adenosine monophosphate (5'-AMP), presumably, but not necessarily, as a product of the adenosine triphosphate (ATP) hydrolysis associated with active transepithelial transport, acts to alter renal function. Stated simply, increased active solute reabsorption, secondary to an increased GFR, would increase adenosine production which would, in turn, act to constrict the afferent arteriole and dilate the efferent arteriole (Osswald et al 1978b) and thereby reduce glomerular capillary hydrostatic pressure and GFR. Evidence in support of this hypothesis has come from studies that have evaluated the renal action of exogenously administered

adenosine (Osswald et al 1975, Spielman et al 1980) or, in a few cases, with the use of methylxanthines as adenosine antagonists (Osswald et al 1980, Gerkens et al 1983). For the most part, however, only circumstantial evidence has been obtained that supports a role for endogenously produced adenosine in the control of renal function. A more direct assessment of the actions of intrarenal adenosine would be to increase endogenous concentrations of adenosine, through pharmacological or physiological perturbations, while monitoring renal function.

In a variety of nonrenal tissues, extracellular adenosine has been reported to enter cells by facilitated diffusion through a nucleoside uptake mechanism, where it is deaminated to inosine or phosphorylated to 5'-AMP (Arch and Newsholme 1978). This nucleoside uptake mechanism is inhibitable by a variety of pharmacological agents (Paterson 1979), such as nitrobenzylthioinosine (NBTI) and dipyridamole. The hypothesis tested in this study was that inhibition of cellular uptake of adenosine increases the extracellular concentrations of adenosine, which in turn alters renal function. The present investigation was thus undertaken (1) to identify the presence of a renal uptake mechanism for adenosine, (2) to determine whether its inhibition with dipyridamole leads to increased

extracellular adenosine, and (3) to determine whether increasing endogenous concentrations of adenosine by inhibition of cellular uptake results in changes in renal function that mimic the effects of exogenously administered adenosine.

A.2. Methods

Preparation: Experiments were performed on dogs of either sex, weighing 12-20 kg. The animals were allowed free access to water and were deprived of food 24 hours before the experiment. On the day of the experiment, the animals were anesthetized with sodium pentobarbital (30 mg/kg, iv) and maintained with periodic small doses as needed. The trachea was exposed and cannulated, and each animal was mechanically ventilated with a Harvard respirator. Minute volumes were initially selected from the nomogram of Kleinman and Radford (1964). Body temperature was monitored with a rectal temperature probe and maintained at 37 C by a circulating water-heating pad and a radiant heat lamp, when necessary. Catheters (PE 240) were placed in the femoral arteries for measurement of blood pressure (Statham strain gauge, P23Db, Hewlett-Packard recorder, 7754B) and for blood sampling, and in the femoral veins for systemic infusions and anesthetic administration.

A retroperitoneal flank incision was made to expose the left renal artery for placement of a noncannulating electromagnetic flow probe (Zepeda Instruments). The flow probe was calibrated by renal artery cannulation at the end of each experiment. A small curved infusion needle (22-gauge) was placed into the renal artery distal to the flow probe. The patency of the needle was maintained by a constant infusion at a rate of 1.38 ml/min. Another small curved needle (20-gauge) was placed in the renal vein for sampling of the renal effluent. The dogs were then placed in a metal frame which held them in a position approximating their normal standing posture. After the completion of all surgical procedures, at least 1 hour was allowed for attainment of steady state, as determined by the stability of blood pressure, renal blood flow, and blood gas measurements.

Analytical: Inulin concentrations in plasma and urine were determined by the anthrone method described by Davidson and Sackner (1963). Plasma and urine sodium and potassium concentrations were determined by flame photometry.

One and one-half milliliter aliquots of plasma and urine taken during each clearance period were processed and analyzed for adenosine as follows. The blood samples (3-4

ml) were immediately diluted in 300 ul of a solution containing 32 uM dipyridamole, to block adenosine uptake by red blood cells, and 3 uM erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), an inhibitor of adenosine deaminase. The blood samples were then centrifuged and a 1.5 ml plasma aliquot was removed and placed in 60 ul of 70% perchloric acid. Similarly, 1.5 ml aliquots of urine were placed in 60 ul perchloric acid. All samples were then centrifuged at 10,000 g for 30 minutes to remove the protein precipitate. A 1.2 ml aliquot of the perchloric acid extract was removed and neutralized with a concentrated solution of potassium carbonate (7 M). The potassium perchlorate precipitate was removed by centrifugation and a 200 ul aliquot of the neutralized acid extract was used for high-pressure liquid chromatography (HPLC) analysis of adenosine. Four control tubes were prepared from a single arterial or venous blood sample and processed with each sample set. Two tubes received known amounts of adenosine, and the third was used to measure endogenous levels. In the final tube, the collecting solution was replaced with 2-5 units of adenosine deaminase activity in saline. The adenosine values of these tubes were used to ensure the identity of the adenosine peak and to calculate the recovery of adenosine for each experiment.

The neutralized acid extract was injected (WATERS-WISP) onto a C18 reverse-phase column (Beckman Ultrasphere ODS) and eluted using an isocratic (9-12% of a 70% solution, balance was 4 mM $K_2HPO_4$) elution (1-1.5 ml/min). A fraction ($\pm$2.5 min) of the eluate containing adenosine, as determined by the retention time of standards (and verified by the recovery tubes), was collected using a timer (ChronTrol), valve (The Lee Co. #LFAA1201418A), and fraction collector (Eldex Universal) system. The adenosine fraction was concentrated to dryness under reduced pressure using a vortex evaporator (Buchler Instruments). The samples were resuspended in 1 ml of water and the adenosine converted to inosine by the addition of 0.5 unit of adenosine deaminase and incubated for 15 min at room temperature. The adenosine deaminase was inactivated by the addition of 1 ml of HPLC grade methanol, and the samples were once again concentrated to dryness under reduced pressure, and a 200 ul aliquot was injected on a C18 reverse-phase column and eluted as described above. We quantified the inosine peak by measuring the area under the curve (WATERS data module) and relating this to standards run under the same condition. Recoveries for all experiments were 100$\pm$7%, and individual values were not corrected for recovery.

Indicator Dilution Curves: To determine whether renal adenosine uptake is blocked by dipyridamole, we employed a single-injection, multiple-tracer, indicator-dilution technique. This technique involves the simultaneous injection of radiolabeled adenosine, together with a radiolabeled reference, in this case a nontransported, nonmetabolized analog of adenosine, ara-H, and collection of serial samples of the urinary and renal venous effluents. Comparison of the tracer adenosine to the reference in urine and renal venous blood yields important information about the renal handling of adenosine (Thompson et al 1985). Based on knowledge of adenosine transport in other tissues, we hypothesized that injected adenosine can leave the vascular compartment by simple diffusion into the interstitial space, as well as by facilitated diffusion into cells via the nucleoside carrier, whereas the reference, ara-H, can leave the vascular compartment only by simple diffusion. Thus, the difference in the recovery of tracer adenosine and the recovery of the reference was taken as the uptake and metabolism of adenosine by cells (Thompson et al 1985). The [U-$^{14}$C]-adenosine (278 mCi/mmol) was obtained from Amersham. The 9-β-D-arabino-furanosyl-[adenine-2-$^{3}$H] (ara-A) (32.6 Ci/mmol) was obtained from ICN. The ara-A was converted to 9-β-D-

arabino-furanosyl hypoxanthine-[2-$^{3}$H] (ara-H) by treatment with adenosine deaminase (Sigma Chemical) and subsequent purification on a HPLC reverse phase column. Injectate containing [$^{3}$H]ara-H (2 uCi/ml) and [$^{14}$C]adenosine (0.34 uCi/ml) was made up in 1.5 ml of normal saline. The bolus was quantified by diluting 100 ul of the injectate with 900 ul saline and counted for radioactivity. Bolus injections (2-4 sec) of 1 ml were made directly into the renal artery cannula while a constant infusion rate of 1.38 ml/min was maintained to flush the cannula. Immediately before initiation of the bolus injection, sampling was begun from both ureters and the renal vein. Before each injection, renal vein plasma and urine samples were obtained for determination of blank activity. Urine samples were collected for an average of 30 seconds (range 15-60 sec) to obtain approximately 1 ml of urine per sample depending on urine flow. Contiguous 10-second withdrawals via the renal vein catheter (average 20 ml/min) were made starting with the injection and lasting 2 minutes after completion of the injection. All samples were corrected for the blank values immediately before the injection, and results were normalized as the plasma or urine concentration (% of injectate/ml).

Urine samples (0.7-1.2 ml) for radioactivity determinations were collected directly into preweighed scintillation vials. After sample collection, the volume was determined gravimetrically. In a few cases where the sample volume was less than 0.7 ml, 0.5 ml of saline was added after volume determination.

Statistical: Statistical analysis was performed by analysis of variance using the Student-Newman-Keuls' test for multiple comparisons and by Student's paired t-test when appropriate. A P value of $<0.05$ was considered statistically significant. Data are presented as mean $\pm$ 1 SEM.

Experimental: Group I - Effect of dipyridamole on the renal uptake of adenosine and the concentration of adenosine in plasma and urine (n=5).

To determine whether renal adenosine uptake is blocked by dipyridamole, we employed a single-injection, multiple-tracer, indicator-dilution technique. The uptake of adenosine was compared with that of a nonmetabolized analog of adenosine, ara-H, used as an extracellular reference. A group of dogs maintained on a normal laboratory diet were prepared as described above, with the following additions. A right flank incision was made to expose the right ureter, which was cannulated with PE 240 tubing. The incision was

then closed to prevent excess fluid loss. After the surgical preparation, the animals were given a saline volume expansion of 5% of body weight to initiate a brisk urine flow. An intravenous infusion of saline (4-5 ml/min) was given to maintain urine flow rate at approximately 2 ml/min. After at least 60 minutes had been allowed for stabilization of the animal preparation, renal adenosine uptake studies were performed, and samples for adenosine concentration were taken, as described above, during a control period, and after 45 minutes of dipyridamole infusion into the renal artery at 24 ug/kg/min.

Group II - Effects of an adenosine uptake inhibitor, dipyridamole, on renal function in dogs maintained on a low-sodium (Group IIa, n=9) or a high-sodium intake (Group IIb, n=5).

To determine whether increasing endogenous adenosine by inhibition of cellular uptake produced renal effects similar to those resulting from the intrarenal infusion of exogenous adenosine, we studied renal function before, during, and after the intrarenal infusion of dipyridamole. Because the effects of exogenous adenosine on renal function are enhanced by sodium depletion and attenuated or blocked by sodium loading (Osswald et al 1978a), dogs were divided into two groups and either sodium-depleted or

sodium-loaded to determine whether the renal effects of increasing endogenous adenosine by inhibition of uptake are also influenced by dietary sodium intake.

One group of dogs (Group IIa) was given 100 mg of the diuretic, furosemide (iv) on the first day of the dietary regimen to accelerate the volume-depletion desired, and maintained on a low-sodium diet (<2 mEq/day) for 10-14 days before the experiment. Dogs were allowed water ad libitum. On the day of the experiment, animals were prepared as described above. During the experiment, dextrose (5.5% in water) was infused (1.38 ml/min) into the renal artery for the control period. Urine was collected for two or three 15-minute clearance periods, and mid-point systemic arterial and renal venous blood samples were taken. This procedure was repeated during the infusion of dipyridamole (24 ug/kg/min in 5.5% dextrose) and again during dextrose infusion for the recovery period. A period of 30 minutes was allowed after the onset and termination of the dipyridamole infusion before beginning the dipyridamole and recovery clearance periods, respectively.

Another group of dogs (Group IIb) was given 15 mg deoxycorticosterone acetate each day to cause sodium retention and fed the same low-sodium food as the previous group to which had been added 200 mEq sodium (as NaCl) to

produce a high-sodium diet. This dietary regimen was continued for 10-14 days. The experimental procedure for Group IIb dogs was identical to the procedure outlined above for Group IIa except that saline (0.9%) was used in place of dextrose for the control and recovery periods and was used as vehicle for the dipyridamole.

In both groups, bolus injections of adenosine (5 and 10 nmol) were given into the renal artery during the control and dipyridamole periods to test for enhancement of the renal vasoconstriction to exogenous adenosine by dipyridamole.

Group III - Effect of dipyridamole on renal function in the sodium-depleted dog, during constant renal perfusion pressure (n=5).

To determine whether the renal actions of dipyridamole in the low-sodium dog (Group IIa) were a consequence of the decrease in arterial blood pressure, another group of dogs was sodium-depleted and prepared as described above, except that a clamp was placed around the abdominal aorta cephalad to the left renal artery. Two clearance periods were conducted during the infusion of 5.5% dextrose at the animals' ambient blood pressure. The aortic clamp was then tightened to lower the renal perfusion pressure (RPP) to 100 mm Hg, which is in the autoregulatory range and was

near the average mean arterial pressure (MAP) of the dogs in Group IIa during steady-state dipyridamole infusion. The dextrose infusion was continued, and clearance periods were conducted. Maintaining RPP at 100 mm Hg, dipyridamole was infused and clearance periods were conducted after 30 min of equilibration. The dipyridamole was replaced by dextrose, and RPP was maintained at 100 mm Hg for the recovery clearance periods.

Group IV - Effect of an adenosine antagonist, theophylline, on the dipyridamole-induced changes in renal function in the sodium-depleted dog (n=8).

To determine whether the renal effects of dipyridamole could be inhibited or reversed by an adenosine antagonist, a group of dogs (Group IV) was sodium-depleted and prepared as described above. In five dogs (Group IVa) dextrose was infused (2.76 umol/min) into the renal artery and control urine and blood samples were collected. The dipyridamole infusion was then started (24 ug/kg/min, 1.38 ml/min) while the dextrose continued (1.38 ml/min). After a 30 min equilibration period, blood and urine samples were collected. The dextrose infusion was replaced by theophylline (5 umol/min) while the dipyridamole continued, and another equilibration period was allowed before samples were collected again. In a group of three dogs (Group

IVb), the above procedure was followed, except that the theophylline and dipyridamole periods were reversed. Theophylline (5 umol/min) was infused during a dextrose infusion for the second set of samples, and then the dextrose was replaced by dipyridamole (24 ug/kg/min).

In both groups, bolus injections of adenosine (5 and 10 nmol) were given into the renal artery to test for enhancement and attenuation of the vasoconstriction by dipyridamole and theophylline, respectively.

A.3. Results

Group I - Effects of dipyridamole on the renal uptake of adenosine and the concentration of adenosine in plasma and urine.

The cumulative venous recovery of labeled adenosine and ara-H was 15.6 $\pm$ 2.7 and 50.5 $\pm$ 7.6%, respectively, during control. In the presence of dipyridamole, the recovery of adenosine increased to 48.1 $\pm$ 8.9% ($P<0.05$ compared to control) and was not different from the venous recovery of ara-H (56.4 $\pm$ 1.5%, NS) during dipyridamole. The ratio of adenosine to ara-H in the first eluting samples averaged 0.28 $\pm$ 0.03 compared to 0.91 $\pm$ 0.03 during dipyridamole ($P<0.05$), indicating that the cellular uptake of adenosine was inhibited in the presence of dipyridamole.

Representative dilution curves for the venous effluent before and during dipyridamole are shown in Figure 1.

The control urinary excretions following injection of labeled adenosine and ara-H were 10.8 ± 1.2 and 19.8 ± 1.7% of the amount injected. Excretion of the adenosine label was increased in the presence of dipyridamole to 15.2 ± 2.2% and was not different from ara-H excretion (20.1 ± 2.5%, NS). The adenosine:ara-H ratio in the early eluting samples was 0.57 ± 0.03 in control and increased to 0.82 ± 0.01 during dipyridamole ($P<0.05$). These results, similar to those obtained with renal venous plasma, indicate that this concentration of dipyridamole blocked the cellular uptake of tracer adenosine. Representative dilution curves for the urinary effluent before and during dipyridamole are shown in Figure 2.

The renal venous concentration of endogenous adenosine increased from 48 ± 9 nM in control to 108 ± 25 nM during dipyridamole. The urinary excretion of endogenous adenosine similarly increased from 595 ± 77 pmol/min to 1695 ± 207 pmol/min during dipyridamole ($P<0.05$). The concentration of endogenous adenosine in arterial blood also increased during dipyridamole, however, due to the variability of the response (in two of the five dogs

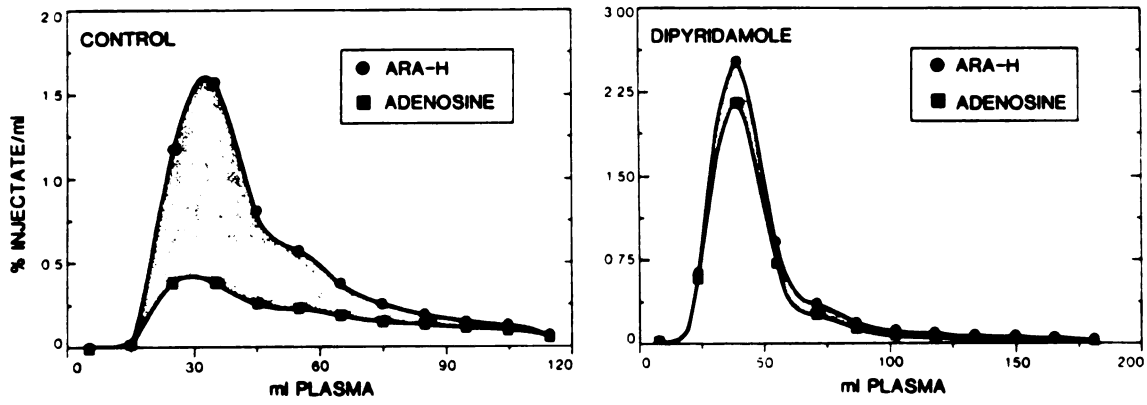


Figure 1. Dilution curves for adenosine and ara-H in plasma from a representative single-injection, multiple-tracer experiment. Data in the left panel were collected during saline infusion, and in the right panel, during dipyridamole infusion. Each data point represents the concentration of tracer, normalized as percent of injected dose, recovered in the venous effluent. The curves were generated by fitting the data with a cubic function. Total sampling time was 2 minutes.

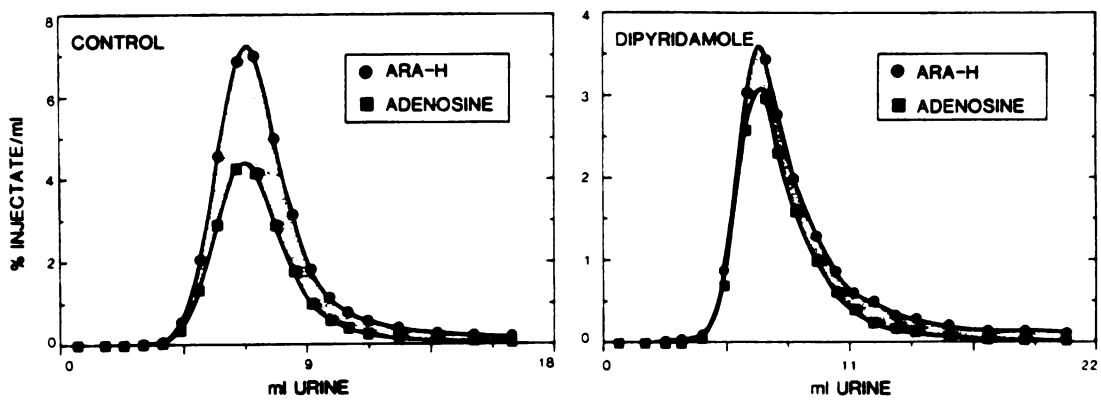


Figure 2. Dilution curves for adenosine and ara-H in the urinary effluent. See legend for Figure 1 for explanation. Total sampling time was 10 minutes.

studied, arterial adenosine decreased) this increase was not statistically significant (73 ± 26 vs 165 ± 59 nM, NS).

Group II - Effects of an adenosine uptake inhibitor, dipyridamole, on renal function in dogs maintained on a low-sodium or a high-sodium intake.

Sodium-depleted and sodium-loaded states were verified by urine flow rate, sodium excretion, and fractional sodium excretion. These indices of renal function were significantly elevated in the animals receiving a high sodium diet and were reduced in the sodium-depleted animals. Urine flow rate was 0.55 ± 0.25 ml/min in the sodium-depleted group and 1.29 ± 0.42 ml/min in the sodium-loaded group. The excretion of sodium was 4.4 ± 3.6 and 77.8 ± 25.1 mEq/min and the fractional excretion of sodium 0.296 ± 0.158 and 2.32 ± 0.61%, in the sodium-depleted and sodium-loaded dogs, respectively. The attainment of a desired sodium status was verified in all subsequent animals by these measurements.

The intrarenal infusion of dipyridamole caused a decrease in GFR in the dogs maintained on the low-sodium diet but produced no significant change in GFR when infused into the dogs that had been maintained on the high-sodium diet (Figure 3; Table 2). GFR was decreased in the low-sodium dogs (Group IIa) by an average of 58.6% (19.8 ± 1.1

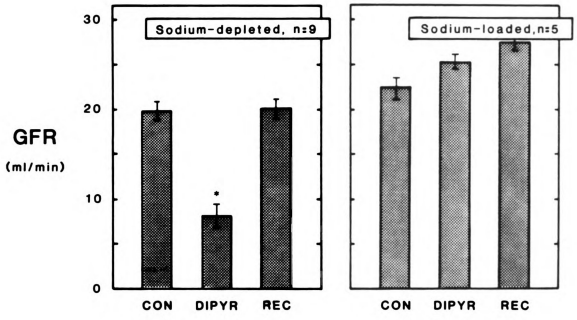


Figure 3. Comparison of glomerular filtration rate (GFR) before, during, and after the intrarenal infusion of dipyridamole (24 ug/kg/min) in sodium-depleted and sodium-loaded dogs. Each bar represents the mean $\pm$ SEM for the group. Individual animal values were taken as the mean of three clearances in each period. Asterisk indicates $P<0.05$.

Table 2. Effect of dipyridamole on renal function in sodium-depleted and sodium-loaded dogs and the effect of theophylline on the renal responses to dipyridamole.

| Group | GFR (ml/min) | RBF (ml/min) | RPP (mm Hg) | FF | RVR (mm Hg/min per ml) | $FE_{Na}$ (%) | Plasma $Na^+$ (mEq/liter) | Plasma $K^+$ (mEq/liter) |
|---|---|---|---|---|---|---|---|---|
| IIa: sodium depleted ($n$ = 9*) | | | | | | | | |
| Control | 19.8 ± 1.1 | 145 ± 10 | 121 ± 5 | 0.28 ± 0.03 | 0.91 ± 0.10 | 0.23 ± 0.15 | 134.4 ± 1.0 | 3.34 ± 0.10 |
| Dipyridamole | 8.2 ± 1.5† | 117 ± 10† | 101 ± 6† | 0.13 ± 0.02† | 0.95 ± 0.14 | 0.15 ± 0.05 | 130.7 ± 1.9 | 3.57 ± 0.12 |
| Recovery | 20.3 ± 1.1 | 135 ± 17 | 117 ± 5 | 0.27 ± 0.03 | 0.93 ± 0.13 | 0.44 ± 0.05 | 121.4 ± 3.1 | 3.52 ± 0.25 |
| IIb: sodium loaded ($n$ = 5) | | | | | | | | |
| Control | 22.3 ± 3.9 | 178 ± 24 | 102 ± 8 | 0.24 ± 0.03 | 0.62 ± 0.09 | 2.60 ± 0.68 | 148.3 ± 4.9 | 2.17 ± 0.41 |
| Dipyridamole | 25.1 ± 3.4 | 157 ± 18 | 99 ± 9 | 0.29 ± 0.03 | 0.69 ± 0.12 | 2.09 ± 0.50 | 159.3 ± 3.9 | 2.34 ± 0.45 |
| Recovery | 27.3 ± 3.4 | 133 ± 19† | 123 ± 11† | 0.39 ± 0.07 | 1.02 ± 0.15 | 2.41 ± 0.46 | 162.9 ± 4.7 | 2.59 ± 0.47 |
| III: sodium depleted, constant pressure ($n$ = 5‡) | | | | | | | | |
| Initial | 25.7 ± 3.4 | 165 ± 27 | 133 ± 3 | 0.30 ± 0.05 | 0.89 ± 0.13 | 0.77 ± 0.54 | 139.5 ± 3.4 | 3.65 ± 0.35 |
| Control | 24.4 ± 4.1 | 163 ± 34 | 100 | 0.32 ± 0.06 | 0.77 ± 0.21 | 0.16 ± 0.09† | 135.7 ± 3.1 | 3.89 ± 0.36 |
| Dipyridamole | 15.0 ± 3.6† | 167 ± 43 | 100 | 0.22 ± 0.06† | 0.86 ± 0.27 | 0.15 ± 0.09 | 130.9 ± 3.6 | 4.22 ± 0.41 |
| Recovery | 24.1 ± 3.4 | 181 ± 42 | 100 | 0.31 ± 0.07 | 0.74 ± 0.21 | 0.10 ± 0.03 | 128.2 ± 3.5 | 4.35 ± 0.55 |
| IVa: sodium depleted ($n$ = 5) | | | | | | | | |
| Control | 21.9 ± 1.0 | 154 ± 14 | 124 ± 8 | 0.29 ± 0.04 | 0.85 ± 0.14 | 0.31 ± 0.20 | 137.5 ± 2.7 | 3.31 ± 0.10 |
| Dipyridamole | 7.9 ± 2.5† | 119 ± 15† | 99 ± 8† | 0.12 ± 0.03† | 0.86 ± 0.07 | 0.18 ± 0.06 | 132.5 ± 3.4 | 3.41 ± 0.11 |
| Dipyr/Theo | 25.7 ± 1.9 | 157 ± 11 | 112 ± 7 | 0.31 ± 0.03 | 0.72 ± 0.07 | 0.85 ± 0.29 | 126.4 ± 1.9 | 3.22 ± 0.11 |
| IVb: sodium depleted ($n$ = 3) | | | | | | | | |
| Control | 31.3 ± 5.2 | 165 ± 7 | 117 ± 6 | 0.38 ± 0.08 | 0.71 ± 0.05 | 0.28 ± 0.08 | 135.5 ± 3.7 | 3.44 ± 0.25 |
| Theophylline | 30.6 ± 4.9 | 175 ± 15 | 107 ± 3 | 0.36 ± 0.08 | 0.61 ± 0.04 | 0.74 ± 0.10† | 125.5 ± 3.6 | 3.39 ± 0.18 |
| Theo/Dipyr | 25.5 ± 3.5 | 174 ± 23 | 96 ± 6† | 0.29 ± 0.05 | 0.57 ± 0.06 | 0.74 ± 0.05 | 125.9 ± 3.6 | 3.74 ± 0.24 |

vs 8.2 ± 1.5 ml/min, P<0.05), and returned to control in the recovery period (20.3 ± 1.1 ml/min). In the high-sodium dogs (Group IIb), the average GFR during the infusion of dipyridamole was not significantly different from the control GFR (22.3 ± 3.9 vs 25.1 ± 3.4 ml/min,NS). The intrarenal infusion of dipyridamole in the low-sodium dogs consistently produced a small and transient increase in renal blood flow at the onset of the infusion, which waned, and during the steady state was lower than the control blood flow (145 ± 10 vs 117 ± 10 ml/min, P<0.05). Steady state blood flow during the infusion of dipyridamole was also somewhat lower in the high-sodium dogs, albeit, not significantly (178 ± 24 vs 157 ± 18 ml/min, NS).

The intrarenal infusion of dipyridamole had no effect on the renal perfusion pressure in the dogs maintained on the high-sodium diet. In contrast, the infusion of dipyridamole resulted in a decrease of renal perfusion pressure in the low-sodium dogs (121 ± 5 to 101 ± 6 mm Hg, P<0.05). Renal vascular resistance (RVR) was not significantly different in either group of dogs during the infusion of dipyridamole. Filtration fraction (FF) decreased significantly in the sodium-depleted dogs (0.28 ± 0.03 vs 0.13 ± 0.02, P<0.05) during the infusion of

dipyridamole, but was not significantly different in the sodium-loaded dogs.

Fractional excretion of sodium and the plasma concentrations of sodium and potassium were not significantly different from control values during the infusion of dipyridamole.

In Group IIa dogs (low-sodium), injections of 5 and 10 nmol of adenosine resulted in 45 $\pm$ 8 and 56 $\pm$ 10% decrease in renal blood flow (RBF) during the infusion of dextrose; during the infusion of dipyridamole, the decreases in RBF in response to 5 and 10 nmol adenosine were significantly greater (66 $\pm$ 11 and 74 $\pm$ 11%,respectively; $P<0.05$). In the high-sodium group, RBF decreased by 14 $\pm$ 3 and 25 $\pm$ 8% during the control saline infusion and by 28 $\pm$ 5% ($P<0.05$) in response to 5 nmol adenosine and 39 $\pm$ 6% (NS) in response to 10 nmol adenosine during the dipyridamole infusion.

In some preliminary experiments, dipyridamole was infused into the femoral vein. With systemic administration, the effects of renal function were the same as that seen in the intrarenal infusion.

Group III - Effect of dipyridamole on renal function during constant renal perfusion pressure.

An initial clearance period was performed in each animal at its ambient arterial pressure. The average initial RPP for five dogs was 133 ± 3 mm Hg. Initial GFR was 25.7 ± 3.4 ml/min, and RBF was 165 ± 27 ml/min. Filtration fraction was 0.30 ± 0.05 and fractional sodium excretion was 0.77 ± 0.54% (Figure 4).

There was no effect of decreasing renal perfusion pressure (RPP) to 100 mm Hg on GFR (25.7 ± 3.4 vs 24.4 ± 4.1 ml/min, NS) or RBF (165 ± 27 vs 163 ± 34 ml/min, NS). Filtration fraction and vascular resistance remained constant as well (Table 2). With the decrease in RPP, fractional excretion of sodium decreased significantly from 0.77 ± 0.54 to 0.16 ± 0.09% ($P<0.05$).

With RPP maintained at 100 mm Hg, dipyridamole infusion resulted in a decrease in GFR from 24.4 ± 4.1 to 15.0 ± 3.6 ml/min ($P<0.05$). There was no change in RBF with dipyridamole infusion (167 ± 43 ml/min, NS); consequently, filtration fraction decreased from 0.32 ± 0.06 to 0.22 ± 0.06 ($P<0.05$). Vascular resistance and fractional sodium excretion did not change significantly from the previous period.

During the recovery period, (i.e., 30 minutes after stopping dipyridamole infusion), GFR and filtration fraction both returned to their respective control values

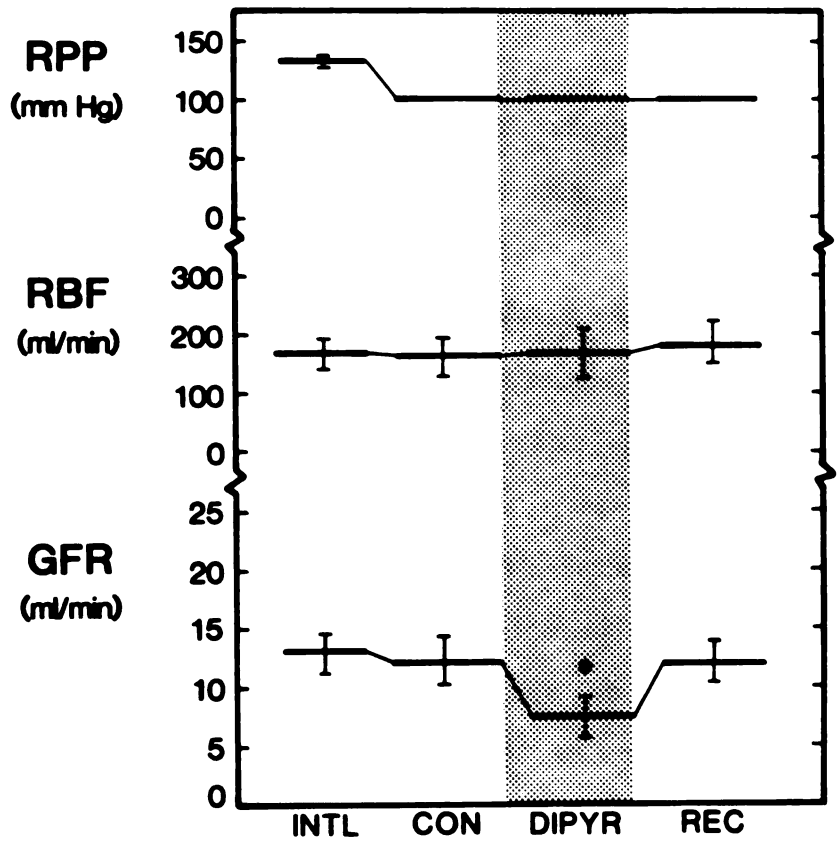


Figure 4. Renal perfusion pressure (RPP), renal blood flow (RBF), and glomerular filtration rate (GFR) in five sodium-depleted dogs before, during, and after the intrarenal infusion of dipyridamole (24 ug/kg/min) while renal perfusion pressure was maintained at 100 mm Hg. Each value represents the mean $\pm$ SEM for the group. Individual animal values were taken as the mean of three clearances in each period. Asterisk indicates $P<0.05$.

(GFR: 24.1 ± 3.4 ml/min; FF: 0.31 ± 0.07). There was no significant change in RBF, vascular resistance, or fractional sodium excretion.

Group IV - The effect of theophylline on the renal actions of dipyridamole.

The intrarenal infusion of dipyridamole in Group IV resulted in changes similar to those described for Group II dogs (Table 2; Figure 5). GFR decreased from 21.9 ± 1.0 to 7.9 ± 2.5 ml/min ($P<0.05$); likewise RBF decreased from the control value of 154 ± 14 ml/min to 119 ± 15 ml/min ($P<0.05$). Filtration fraction also decreased from 0.29 ± 0.04 to 0.12 ± 0.03 ($P<0.05$). As with Group II, MAP decreased during the dipyridamole infusion from 124 ± 8 to 99 ± 8 mm Hg ($P<0.05$). Injections of adenosine during the control dextrose infusion resulted in decreases in RBF of 35 ± 10 and 42 ± 11% for 5 and 10 nmol adenosine, respectively. In response to the same doses during the dipyridamole infusion, the decreases in RBF were significantly greater (51 ± 14% and 60 ± 12%; $P<0.05$). The concomitant infusion of theophylline resulted in the reversal of all of these effects (Figure 5). GFR increased to 25.7 ± 1.9 ml/min and RBF increased to 157 ± 11 ml/min. Neither increase was significantly different from the respective control values. The filtration fraction

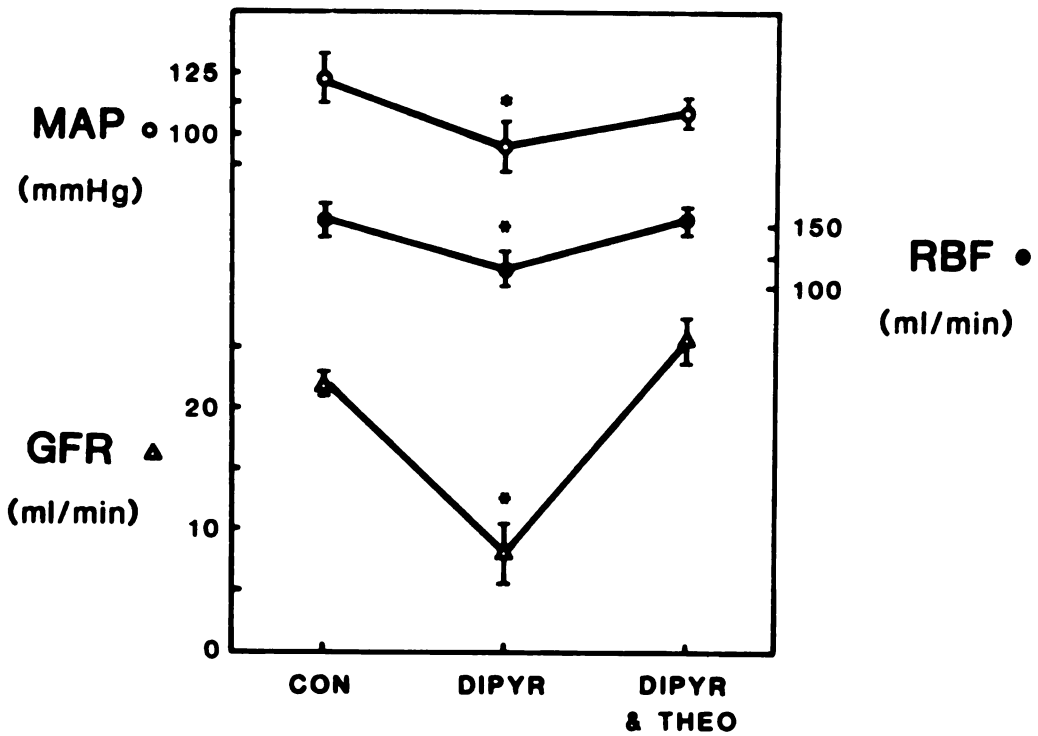


Figure 5. Mean arterial pressure (MAP), renal blood flow (RBF), and glomerular filtration rate (GFR), in five sodium-depleted dogs before and during the intrarenal infusion of dipyridamole (14 ug/kg/min), and during the intrarenal infusion of theophylline (5 umol/min) and dipyridamole, simultaneously. Each value represents the mean $\pm$ SEM for the group. Individual animal values were taken as the mean of three clearances in each period. Asterisk indicates $P<0.05$.

returned to the control value (0.31 ± 0.03) during theophylline infusion as well. Renal perfusion pressure increased from 99 ± 8 to 112 ± 7 mm Hg, a value which was not statistically different than the control value of 124 ± 8 mm Hg. The decreases in RBF in response to adenosine injections were 31 ± 15 and 43 ± 20% during the theophylline infusion, and were not significantly different than the control responses.

In an additional three dogs, theophylline was infused prior to dipyridamole to determine if the effects of dipyridamole on renal function could be inhibited by the presence of theophylline. Theophylline infusion alone did not significantly change GFR from the control value (31.3 ± 5.2 vs 30.6 ± 4.9 ml/min, NS). Likewise, RBF did not change in the steady state (165 ± 7 vs 175 ± 15 ml/min, NS), although there was an initial tendency of blood flow to increase immediately after theophylline infusion was begun. Renal perfusion pressure was also not affected by theophylline (117 ± 6 vs 107 ± 3 mm Hg, NS). Fractional sodium excretion increased from 0.28 ± 0.08 to 0.74 ± 0.10% ($P<0.05$). Theophylline infusion did not affect renal vascular resistance or filtration fraction (Table 2). The infusion of theophylline attenuated the decreases in RBF to adenosine injections as compared to control. Injections of

adenosine (5 and 10 nmol) during the dextrose infusion resulted in 44 ± 3 and 52 ± 6% decreased in RBF. During the theophylline infusion, the decreases in RBF in response to adenosine were significantly less (19 ± 5 and 33 ± 3%, $P<0.05$).

In the presence of theophylline, the infusion of dipyridamole did not significantly affect GFR (30.6 ± 4.9 vs 25.5 ± 3.5 ml/min, NS) or RBF (175 ± 15 vs 174 ± 23 ml/min, NS). Filtration fraction did not change from the value during theophylline infusion alone (0.36 ± 0.08 vs 0.29 ± 0.05, NS). Likewise, dipyridamole infusion in the presence of theophylline had no effect on renal perfusion pressure (107 ± 3 vs 96 ± 6 mm Hg, NS); however, this was significantly different from the control value of 117 ± 6 mm Hg ($P<0.05$). Vascular resistance and fractional sodium excretion were not changed with the infusion of dipyridamole (Table 2). Adenosine injections (5 and 10 nmol) during the theophylline/dipyridamole infusion resulted in 33 ± 17 and 47 ± 21% decreases of RBF which were not significantly different than the control responses.

## A.4. Discussion

The reported actions of adenosine, namely, the production of a marked decrease in GFR (Tagawa and Vander

1970), alteration of cortical distribution of blood flow (Spielman et al 1980), and a decrease in renin release (Tagawa and Vander 1970), form the basis of the hypothesis that intrarenally-produced adenosine, as the active extracellular metabolite of the ATP hydrolysis associated with transepithelial transport, is the mediator of the intrinsic regulation of renal function. However, the majority of the previous studies have involved the use of exogenous adenosine, and, whereas these studies provide important support for the role of adenosine as a regulator of renal function, alone, this evidence can be considered as merely suggestive. The use of dipyridamole, a nucleoside uptake blocker, in the present study more directly allows for the investigation of the role that endogenous adenosine may play in the regulation of renal function. The hypothesis tested in the present study was that inhibition of the cellular uptake of adenosine increases the extracellular concentration of endogenous adenosine, which, in turn, alters renal function. We therefore sought to determine the effect of dipyridamole on (1) the renal uptake of adenosine, (2) the plasma and urinary concentrations of adenosine, and (3) renal function.

For any substance to have a role as a regulatory intercellular mediator, it must have a system for inactivation, through uptake and/or metabolism. Nucleoside uptake is known to occur by facilitated diffusion in a variety of cell types (Berlin and Oliver 1975), and is inhibitable pharmacologically (Paterson et al 1975). Dipyridamole has been shown to block the uptake of adenosine by a number of cell types, including erythrocytes, platelets, and myocardial cells (Feigl 1983). In the present study, we used dipyridamole as a tool for determining whether an adenosine uptake mechanism exists in renal cells and whether blocking this uptake system increases extracellular adenosine concentrations. Dipyridamole could then be used to probe the effects of intrarenally-produced adenosine on kidney function. The difference between the recovery of adenosine and that of the reference is virtually abolished during the infusion of dipyridamole (Figures 1 and 2), and indicates that the cellular uptake of adenosine is blocked. This inhibition of the cellular uptake is apparent in both the renal venous and urinary effluents, leading to the conclusion that the kidney possesses a dipyridamole-sensitive cellular uptake mechanism for adenosine in both the vascular and tubular compartments.

Once in the cell, adenosine is metabolized to inosine or 5'-AMP by adenosine deaminase or adenosine kinase, respectively, Because adenosine metabolism is primarily intracellular (Berne and Rubio 1974) and its physiological actions are primarily extracellular (Arch and Newsholme 1978), exogenous administration of adenosine during pharmacological inhibition of the cellular uptake is generally expected to result in increased extracellular concentrations and, hence, increase its biological activity. In the heart, dipyridamole enhances the coronary vasodilation produced by adenosine (Feigl 1983). Likewise, the renal vasoconstriction produced by the intrarenal injection of adenosine is augmented in the presence of dipyridamole (Sakai et al 1981). In the present study, bolus injections of adenosine produced transient vasoconstrictions that were enhanced during the infusion of dipyridamole.

The knowledge that dipyridamole blocks the uptake and potentiates the action of exogenously administered adenosine has led investigators to use dipyridamole to enhance events suspected to be mediated by increased concentrations of endogenous adenosine (Feigl 1983). However, because the mechanism by which intracellular adenosine exits the cell remains to be defined, and because

the dipyridamole-inhibitable, facilitated diffusion is presumably a bidirectional process (Plagemann and Wohlhueter 1980), release, as well as cellular uptake, may be inhibited in the presence of dipyridamole. Thus, it is important to ascertain whether blocking cellular uptake is actually associated with increased concentrations of adenosine.

In general, it can be assumed that the concentration of adenosine in venous blood and urine can be used as an indication of the concentration of adenosine in the extracellular fluid. Measurements of adenosine in the plasma and urine before and during the administration of dipyridamole indicated that extracellular adenosine concentrations are indeed increased by dipyridamole. The concentration of adenosine in arterial blood also increased during dipyridamole, and this is presumably due to the effect of dipyridamole on adenosine uptake in other tissues, a finding not surprising in light of the decrease in arterial blood pressure. The increases in venous and urinary adenosine concentration were not, however, dependent on an increase in extrarenal adenosine levels, since the increase in venous and urinary adenosine was also seen in two of the five experiments in which the arterial concentrations were decreased or unchanged. In addition,

the arterial concentration of adenosine was increased, on the average, to slightly more than 100 nM, and the changes in renal function that occurred would require, in an infusion of exogenous adenosine, a concentration of 5 uM, or 50 times the concentration observed with the dipyridamole infusion, suggesting that the functional changes were probably elicited by increases in intrarenal adenosine levels.

Having established that (1) cellular uptake of adenosine is blocked by dipyridamole in the kidney, and (2) adenosine concentrations are increased during the administration of dipyridamole, we then sought to determine the effects of increased endogenous adenosine on renal function. The renal actions of adenosine are influenced by the dietary sodium intake of the animal (Osswald et al 1978a). That is, the intrarenal infusion of adenosine results in a decrease in GFR in sodium-depleted rats and dogs, but has relatively little, if any, effect when infused into sodium-loaded animals. Likewise, the effect of exogenous adenosine to produce a redistribution of renal cortical blood flow is influenced by the dietary sodium intake of the animal (Spielman et al 1980). The mechanism responsible for these differences in response to adenosine is unknown at present, although the enhanced renin-

angiotensin system and increased renal nerve activity present in the sodium-depleted animal are possibilities. Several recent studies have been aimed at clarifying the role of angiotensin in the renal actions of adenosine. The reduction in RBF following a brief occlusion of the renal artery - which is thought to be mediated by adenosine - as well as the decrease in RBF in response to an injection of adenosine, have been attenuated by high concentrations of the angiotensin II antagonist [$Sar^1$,$Ile^8$]AII (Spielman and Osswald 1979), suggesting an adenosine-AII interaction. However, measurements of renal lymph AII, used as an indication of interstitial AII levels, have shown that intrarenal AII did not increase, but actually decreased, during an adenosine infusion (Spielman 1984). Therefore, whereas an adenosine-AII interaction appears to exist, it may be more complicated than has been previously postulated.

To determine the renal effects of increased endogenous adenosine and the influence of sodium intake on these actions, we studied the effects of dipyridamole administration in sodium-depleted and sodium-loaded dogs, thereby comparing the renal effects of endogenous to exogenous adenosine. Animals maintained on a diet low in sodium were found to have a reversible decrease in GFR in

response to the dipyridamole infusion, whereas GFR did not change upon infusion of dipyridamole in the sodium-loaded dogs.

In addition to the decrease in GFR, renal blood flow was also reduced during the dipyridamole infusion in the sodium-depleted dog. There was, however, no calculated change in the renal vascular resistance as mean arterial blood pressure was concomitantly reduced. The filtration fraction was markedly reduced during the dipyridamole infusion, indicating that the decrease in GFR was proportionally greater than the decrease in renal plasma flow. No hemodynamic changes were observed during the infusion of dipyridamole in the sodium-loaded dogs. These effects are similar in many ways to the effects of the intrarenal infusion of adenosine, but the decrease in the arterial blood pressure, an effect not seen during the infusion of adenosine, makes the comparison somewhat complicated.

To compare the effects of dipyridamole to the effects of exogenous adenosine more directly, and to determine what contribution the decrease in arterial pressure had on the decrease in GFR, we infused dipyridamole into another series of sodium-depleted animals in which renal perfusion pressure was held constant. When renal perfusion pressure

was held constant at 100 mm Hg, dipyridamole infusion still produced a marked decrease in GFR (-38%), indicating that the effect of dipyridamole is, for the most part, independent of the decrease in arterial pressure. It should be noted here that, whereas mean arterial pressure fell during the dipyridamole infusion and was a major contributing factor to the decrease in RBF, systemic pressure did not fall below the autoregulatory range of the kidney and should not have been expected to result in a decrease in RBF. These findings suggest that dipyridamole, or endogenous adenosine, may cause an impairment of autoregulation in sodium-depleted animals. The mechanism by which this interference might occur is unknown, and may be of sufficient importance to merit further investigation.

The ability of theophylline to antagonize the actions of adenosine is well documented. In the kidney, theophylline has been shown to act as a competitive inhibitor of adenosine's actions to produce a vasoconstriction (Osswald 1975), decrease GFR, and inhibit renin secretion (Spielman 1984). To ascertain whether the decrease in GFR resulting from dipyridamole infusion in the sodium-depleted dog was mediated by increased adenosine levels, theophylline was infused before and after the dipyridamole infusion. Theophylline was able to reverse,

as well as inhibit, the dipyridamole-induced decrease in GFR, suggesting that increased endogenous adenosine was responsible for the decrease in GFR. Because in these studies an infusion of theophylline alone did not affect renal function this may be taken as evidence that adenosine is not involved in the regulation of renal function under normal circumstances. Adenosine may, indeed, be involved in the regulation of renal function only in circumstances where there is increased metabolism and energy utilization. However, because theophylline does increase urine volume and sodium excretion, and in other experiments has been shown to increase renin release, in animals without other interventions, this may suggest that adenosine is important normally as a local regulator of cellular function.

In summary, we found that, in the anesthetized dog, dipyridamole blocked the cellular uptake of adenosine, increased the extracellular concentration of adenosine, and decreased GFR in the sodium-depleted but not the sodium-loaded dog, and the effect on GFR was blocked by the adenosine antagonist, theophylline. Increasing endogenous adenosine by inhibition of cellular uptake resulted in a decrease in GFR in the sodium-depleted animal, a condition known to enhance the actions of adenosine. Although the intent of this investigation was to evaluate the renal

effects of increased endogenous adenosine through pharmacological inhibition of its cellular uptake, the marked effect of dipyridamole to decrease GFR in the sodium-depleted animal raises important issues concerning the clinical use of dipyridamole in situations where extracellular fluid volume is reduced. These findings indicate that, given an appropriate physiological or pathophysiological situation (e.g., sodium-depletion), increased concentrations of endogenous adenosine have the capability of altering GFR. Whereas the present findings are consistent with a role for intrarenally produced adenosine in the regulation of renal function, it remains to be determined whether endogenous adenosine increased by circumstances other than pharmacological ( physiological or pathophysiological) results in similar changes in renal function.

## B. Increasing intrarenal adenosine by maleic acid decreases GFR and renin release

(reproduced with permission, Springer Verlag; Kidney Int. 30:656, 1986)

### B.1. Introduction

Maleic acid administration produces a generalized tubular transport defect that results in the increased excretion of sodium, potassium, phosphate, glucose, and amino acids, in addition to increasing urinary flow rate,

and thereby closely resembles the Fanconi syndrome (Al-Bander et al 1982; Berliner et al 1950; Brewer et al 1983; Harrison and Harrison 1954; Kramer and Gonick 1973; Gmaj et al 1973). Several studies have demonstrated that, associated with the defect in reabsorption, glomerular filtration rate (GFR) is markedly reduced following maleic acid administration (Al-Bander et al 1982; Berliner et al 1950; Brewer et al 1983; Gmaj et al 1973; Gougoux et al 1976; Gunther et al 1979; Maesaka and McCaffery 1980; Osswald et al 1980). Relatively little is known about the mechanism of the decrease in GFR.

Previous work has demonstrated that maleic acid lowers renal cortical adenosine triphosphate (ATP) content (Osswald et al 1980; Kramer and Gonick 1970; Szczepanska and Angielski 1980). Because of the marked alteration of nucleotide metabolism, it has been proposed (Osswald et al 1980) that the decrease in GFR is the result of increased intrarenal concentrations of adenosine. As a means of testing this hypothesis, we sought to determine, during the intrarenal infusion of maleic acid: 1) if the decrease in GFR is greater in the sodium-depleted than in the sodium-loaded dog, thereby mimicking the effect of dietary sodium intake on the renal response to exogenous adenosine infusion (Osswald et al 1978); 2) if there is a suppression

of renin release, which is also seen with an adenosine infusion (Osswald et al 1978; Tagawa and Vander 1970; Spielman 1984); 3) if the decrease in GFR is blocked by the adenosine antagonist, theophylline; and 4) if renal venous and urinary adenosine concentrations are increased. Data are presented indicating that the decrease in GFR produced by maleic acid is mediated by adenosine.

B.2. Methods

Experiments were performed on two groups of mongrel dogs of either sex, weighing between 10 and 20 kg. One group of 10 dogs (Group I) was maintained on a low-sodium diet (Hill's Prescription Diet, h/d) of less than 2 mEq/day for seven to 10 days, and was given 100 mg furosemide, i.v., on the first day of the dietary regimen. A second group of 10 dogs (Group II) was fed the same low-sodium food as Group I, which had been supplemented with 200 mEq NaCl. This diet was maintained for 10 to 14 days, and the animals received 15 mg deoxycorticosterone pivalate (Ciba Pharmaceutical Co.) each day of the dietary regimen. All dogs were allowed free access to water. Because the transport defect produced by maleic acid occurs primarily in the proximal tubule (Al-Bander et al 1982; Brewer et al 1983; Kramer and Gonick 1973; Gmaj et al 1973; Gunther et al 1979), and lithium is handled by the proximal tubule of

the kidney (Hayslett and Kashgarian 1979), all dogs received 300 mg lithium carbonate 24 hours before the experiment. Any change in lithium excretion observed can be used as an index of proximal tubule function.

The dogs were surgically prepared as previously described (Arend et al 1984). Briefly, the dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and a tracheostomy was performed for mechanical ventilation with a Harvard respiration pump. Catheters (PE 240) were placed in both femoral arteries for monitoring systemic blood pressure and obtaining systemic blood samples, and in both femoral veins for systemic infusions and supplemental anesthetic administration . A left flank incision was made to expose the left renal artery and vein and the ureter. Small curved needles were placed in the renal artery for intrarenal infusions and in the renal vein for sampling of the renal venous effluent. The renal artery needle was kept patent by a constant infusion of saline at 1.38 ml/min. The ureter was cannulated with polyethylene tubing. An electromagnetic flow probe (Zepeda Instruments) was placed around the renal artery for measurement of renal blood flow. Systemic blood pressure and renal blood flow were recorded on a Hewlett Packard recorder. A 1% solution of inulin (Difco Laboratories) was infused at 2 ml/min into

a femoral vein throughout the experiment to achieve a constant plasma concentration of approximately 25 mg/dl. A 60 min equilibration period was allowed after completion of the surgical procedure.

Two control clearance periods were performed in each experiment, which consisted of a fifteen minute urine collection and the collection of systemic arterial and renal venous blood samples at the mid-point of each period. Saline (0.9%) was used as the intrarenal infusate for the control periods as well as for the vehicle in subsequent infusions. The saline was then replaced by maleic acid at a dose of 100 mg/kg body wt/hr (neutralized with NaOH). Ten-minute urine collections and blood samples were obtained at 60 and 80 min during the maleic acid infusion. To complete the time course of the effects of maleic acid and to serve as a time control for the theophylline study, urine and blood samples were collected at 100 min of maleic acid in five dogs from each group. In the remaining five sodium-depleted and five sodium-loaded dogs, an intrarenal infusion of theophylline (5 umol/min) was begun at 90 min of maleic acid. A five min equilibration period was allowed after which three five-minute clearance periods were performed. In all experiments, fluid losses due to the polyuric effects of maleic acid and theophylline were

replaced by the intravenous infusion of saline at a rate double to the urine flow rate of the experimental kidney. The replacement rate was twice that of urine output to account for urine formation in both kidneys, since the contralateral kidney is also affected by the maleic acid and theophylline infusions.

For these studies, adenosine and theophylline were purchased from Sigma Chemical Co. and maleic acid was purchased from J.T. Baker Chemical Co.

Inulin concentrations in plasma and urine were determined by the anthrone method, as described by Davidson and Sackner (1963). Sodium, potassium, and lithium concentrations were determined by flame photometry. Adenosine concentrations in plasma and urine during the control and maleic acid periods were determined by HPLC as previously described (Methods, III.A.). Plasma renin activity (PRA) was measured as angiotensin I by radioimmunoassay as described by Romero and Strong (1977). Renin release was calculated as the difference between arterial PRA and renal venous PRA multiplied by the renal plasma flow. No samples for measurement of adenosine concentrations were taken during the theophylline infusion.

Glomerular filtration rate was calculated as the clearance of inulin. Changes in the fractional excretion

of lithium were used as an indication of changes in proximal reabsorption.

Statistical significance was determined using two-way analysis of variance, with Tukey's w-test for multiple comparisons, and paired and unpaired t-tests where appropriate.

B.3. Results

The intrarenal infusion of maleic acid resulted in a decrease in GFR at 60, 80, and 100 min in the sodium-depleted and the sodium-loaded animals (Table 3). GFR was decreased significantly by 60 min of maleic acid and continued to decrease for the remainder of the infusion in the sodium-depleted group, however, in the sodium-loaded group GFR was not significantly decreased until 100 min. The infusion of theophylline (Table 4, Figures 6 and 7) inhibited the further decline in GFR seen at 100 min in the time course study; GFR increased to values that were not significantly different from control in both the sodium-depleted and sodium-loaded animals.

The response of RBF to maleic acid in the sodium-depleted group is shown in a representative tracing in Figure 6. There was an immediate increase in RBF at the onset of the infusion which waned after approximately 60 min and fell below the control level for the remainder of

Table 3. Time course of effect of maleic acid in sodium-depleted and sodium-loaded dogs.

| Period | GFR ml/min | RBF ml/min | MAP mm Hg | FF | RVR | $FE_{Na}$ % |
|---|---|---|---|---|---|---|
| Group 1: Sodium-depleted ($N$ = 5) | | | | | | |
| Control | 32.8 ± 3.4 | 168 ± 16 | 127 ± 5 | 0.35 ± 0.03 | 0.78 ± 0.06 | 0.24 ± 0.16 |
| Maleic acid | | | | | | |
| 60 min | 23.9 ± 4.8[b] | 192 ± 31 | 116 ± 8[b] | 0.23 ± 0.04[b] | 0.65 ± 0.07 | 12.42 ± 2.74[b] |
| 80 min | 14.9 ± 2.5[b] | 146 ± 23 | 114 ± 8[b] | 0.23 ± 0.06[b] | 0.85 ± 0.13 | 23.90 ± 3.21[b] |
| 100 min | 14.9 ± 2.6[b] | 152 ± 20 | 119 ± 6[b] | 0.20 ± 0.07[b] | 0.83 ± 0.15 | 29.05 ± 3.49[b] |
| Group 2: Sodium-loaded ($N$ = 5) | | | | | | |
| Control | 25.9 ± 4.3 | 141 ± 15 | 116 ± 1 | 0.33 ± 0.05 | 0.86 ± 0.08 | 3.08 ± 1.31 |
| Maleic acid | | | | | | |
| 60 min | 22.0 ± 5.7 | 121 ± 23 | 123 ± 2[b] | 0.33 ± 0.08 | 1.16 ± 0.27 | 26.33 ± 4.21[b] |
| 80 min | 18.1 ± 3.7 | 111 ± 22 | 121 ± 1[b] | 0.28 ± 0.06 | 1.28 ± 0.25 | 32.80 ± 3.06[b] |
| 100 min | 15.8 ± 3.4[b] | 134 ± 24 | 121 ± 1[b] | 0.29 ± 0.10 | 0.95 ± 0.14 | 29.77 ± 5.21[b] |

[a] Values are means ± SEM. Abbreviations are: GFR, glomerular filtration rate; RBF, renal blood flow; MAP, mean arterial pressure; FF, filtration fraction; RVR, renal vascular resistance; FE, fractional excretion

[b] $P < 0.05$ compared to control

Table 4. Effect of theophylline on the maleic acid-induced changes in renal function of sodium-depleted and sodium-loaded dogs.

| Period | GFR ml/min | RBF ml/min | MAP mm Hg | FF | RVR | $FE_{NA}$ % |
|---|---|---|---|---|---|---|
| **Group 1: Sodium-depleted ($N$ = 5)** | | | | | | |
| Control | 25.8 ± 4.1 | 143 ± 15 | 118 ± 5 | 0.30 ± 0.04 | 0.85 ± 0.06 | 0.76 ± 0.41 |
| Maleic acid | | | | | | |
| 60 min | 16.5 ± 3.1[b] | 139 ± 24 | 110 ± 8 | 0.22 ± 0.04 | 0.87 ± 0.13 | 29.84 ± 6.38[b] |
| 80 min | 14.2 ± 1.6[b] | 132 ± 19 | 109 ± 9 | 0.20 ± 0.03 | 0.89 ± 0.14 | 40.18 ± 7.62[b] |
| Maleic acid/theophylline | | | | | | |
| 5 min | 17.7 ± 1.9 | 140 ± 18 | 104 ± 8 | 0.25 ± 0.04 | 0.79 ± 0.11 | 56.14 ± 7.97[b] |
| 10 min | 19.2 ± 2.7 | 141 ± 18 | 105 ± 8 | 0.27 ± 0.06 | 0.79 ± 0.12 | 59.12 ± 11.24[b] |
| 15 min | 19.3 ± 2.8 | 140 ± 19 | 100 ± 8[b] | 0.26 ± 0.08 | 0.79 ± 0.16 | 64.30 ± 7.30[b] |
| **Group 2: Sodium-loaded ($N$ = 5)** | | | | | | |
| Control | 26.4 ± 3.6 | 129 ± 12 | 105 ± 9 | 0.37 ± 0.06 | 0.83 ± 0.09 | 6.13 ± 1.44 |
| Maleic acid | | | | | | |
| 60 min | 24.6 ± 3.5 | 133 ± 11 | 121 ± 9[b] | 0.33 ± 0.06 | 0.92 ± 0.06 | 34.72 ± 5.19[b] |
| 80 min | 20.2 ± 1.5[b] | 132 ± 14 | 117 ± 7[b] | 0.27 ± 0.02 | 0.93 ± 0.09 | 45.84 ± 5.41[b] |
| Maleic acid/theophylline | | | | | | |
| 5 min | 25.6 ± 2.3 | 151 ± 11 | 116 ± 6[b] | 0.29 ± 0.03 | 0.78 ± 0.06 | 48.30 ± 4.77[b] |
| 10 min | 24.1 ± 2.2 | 148 ± 11 | 112 ± 6 | 0.29 ± 0.04 | 0.77 ± 0.06 | 53.04 ± 6.43[b] |
| 15 min | 24.1 ± 2.2 | 146 ± 14 | 109 ± 8 | 0.29 ± 0.01 | 0.76 ± 0.07 | 49.78 ± 5.21[b] |

[a] Values are mean ± SEM. Abbreviations are the same as in Table 1

[b] $P < 0.05$ compared to control

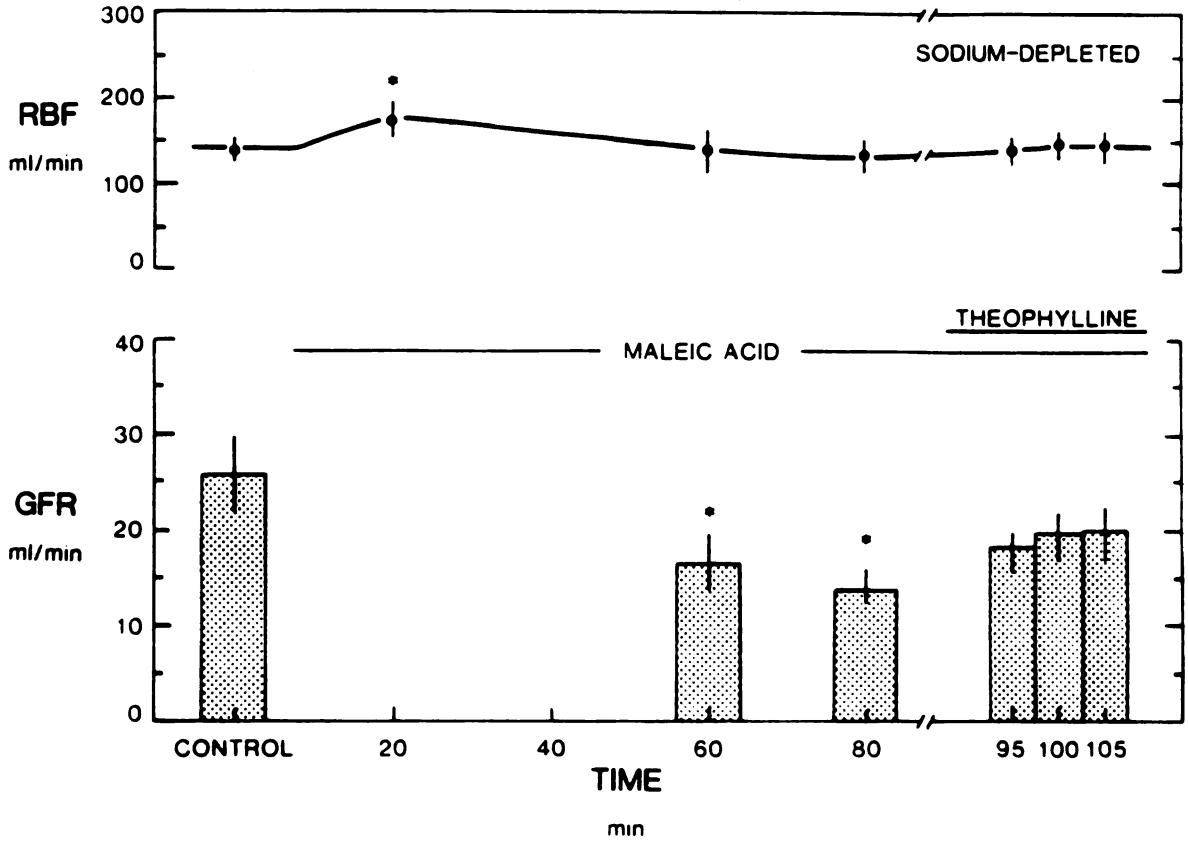


Figure 6. Effect of theophylline on the maleic acid-induced changes in glomerular filtration rate (GFR) and renal blood flow (RBF) in five sodium-depleted dogs. Each value represents the mean ± SEM for the group. Asterisk indicates $P<0.05$, compared to control.

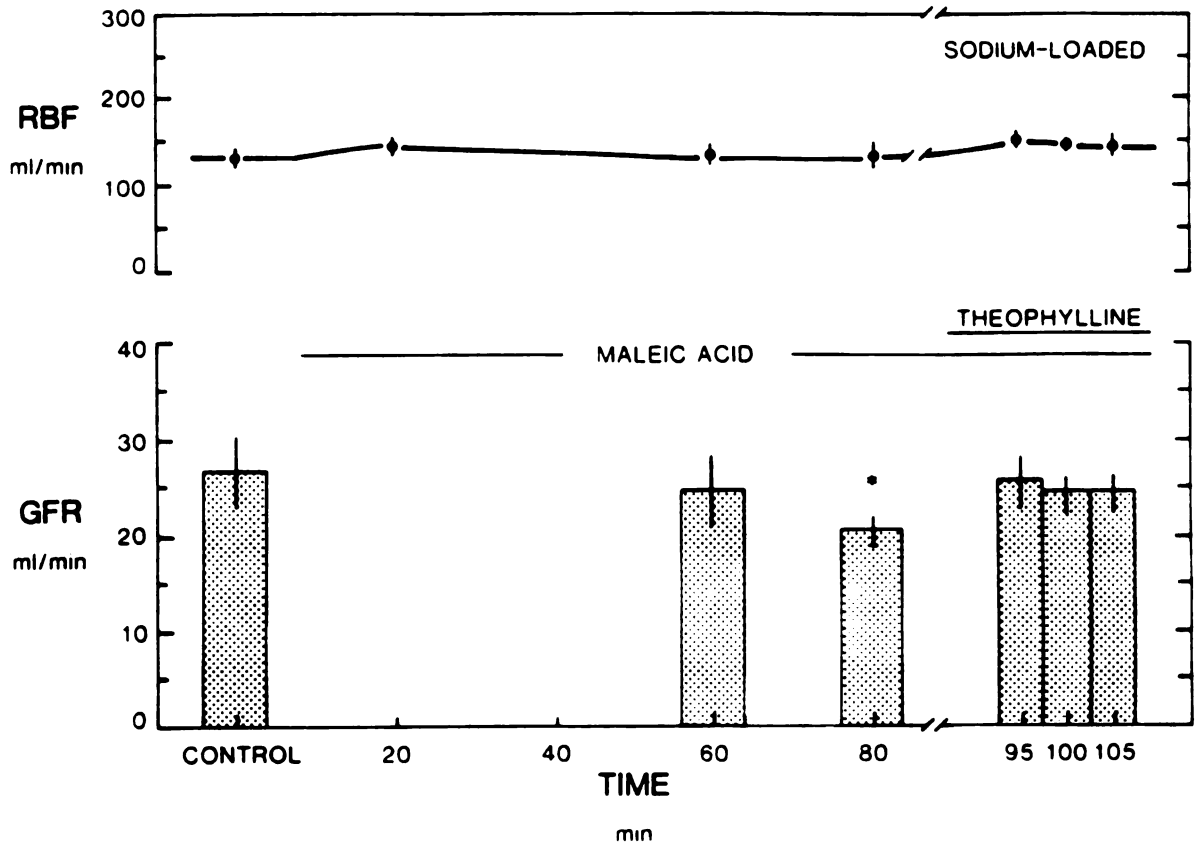


Figure 7. Effect of theophylline on the maleic acid-induced changes in glomerular filtration rate (GFR) and renal blood flow (RBF) in five sodium-loaded dogs. Each value represents the mean ± SEM for the group. Asterisk indicates $P<0.05$, compared to control.

the infusion. With the infusion of theophylline, RBF returned to the control level. In the sodium-loaded group, shown in Figure 7, RBF increased only slightly after the onset of the maleic acid infusion and was not different than the control level during the remainder of the infusion. Theophylline infusion in the sodium-loaded group resulted in a small increase in RBF. Mean arterial pressure (Table 3) decreased during maleic acid infusion in the sodium-depleted group, but increased significantly in the sodium-loaded group. Theophylline did not significantly change mean arterial pressure (Table 4) in either group.

The fractional excretion of sodium was significantly greater in the sodium-loaded animals than in the sodium-depleted animals in the control period and was significantly increased to approximately 30% in both groups, with maleic acid (Table 3). The infusion of theophylline resulted in a further increase in fractional sodium excretion. The fractional excretion of lithium also increased in both groups with maleic acid. The average plasma lithium concentration in the sodium-depleted group (N =10) was 0.56 $\pm$ 0.11 mEq/liter. There was a significant increase in the fractional lithium excretion in this group from a control value of 22.7 $\pm$ 6.4% to 64.9 $\pm$ 12.4%

($P<0.05$) after 100 min of maleate. In many of the sodium-loaded dogs, the dose of 300 mg lithium carbonate was not sufficient to produce a measurable plasma lithium concentration. Therefore, for statistical comparisons, lithium concentrations for all sodium-loaded dogs (time course and theophylline) were pooled. In six sodium-loaded dogs, the control plasma lithium concentration was 0.11 $\pm$ 0.01 mEq/liter, and the fractional excretion of lithium increased from 30.9 $\pm$ 7.7% in control to 53.3 $\pm$ 10.1% ($P<0.05$) after 60 min and to 69.1 $\pm$ 10.4% ($P<0.05$) after 80 min of maleic acid. At 100 min of maleic acid, as well as during the infusion of theophylline, the urine lithium concentration was undetectable in the sodium-loaded group. During theophylline, in the sodium-depleted group, fractional lithium excretion increased from 79.1 $\pm$ 6.9% at 80 min of maleate to 84.6 $\pm$ 9.9% (NS) after 25 min of theophylline.

The effect of maleic acid on renin release is shown in Figure 8. The data shown include all 10 of the sodium-depleted animals during the maleic acid infusion. During the control period, renin release averaged 608 $\pm$ 253 ng AI/min. Renin release decreased significantly ($P<0.05$) to 11.9 $\pm$ 7.9% of control during maleic acid and returned to

60.8 ± 9.9% of control (NS) during the theophylline infusion (N = 5).

As shown in Figure 9, the arterial concentrations of adenosine were not altered by the infusion of maleic acid in either the sodium-depleted or the sodium-loaded animals. However, the renal venous concentration of adenosine increased significantly during maleic acid infusion, from 0.05 ± 0.01 uM to 0.33 ± 0.06 uM in the sodium-depleted animals and from 0.13 ± 0.02 uM to 0.51 ± 0.10 uM in the sodium-loaded animals. In addition, the urinary excretion of adenosine increased significantly, from 0.96 ± 0.23 umol/min to 7.22 ± 2.26 umol/min in the sodium-depleted animals and from 1.21±0.17 umol/min to 18.46 ± 4.81 umol/min in the sodium-loaded animals.

B.4. Discussion

These studies demonstrate that the effects of maleic acid on renal function are dependent on the state of sodium balance of the animal and that the hemodynamic changes can be reversed by the adenosine receptor antagonist, theophylline. In addition, these changes in renal function were associated with an increase in the concentrations of endogenous adenosine.

Several studies have demonstrated that the intravenous or intrarenal administration of maleic acid results in a

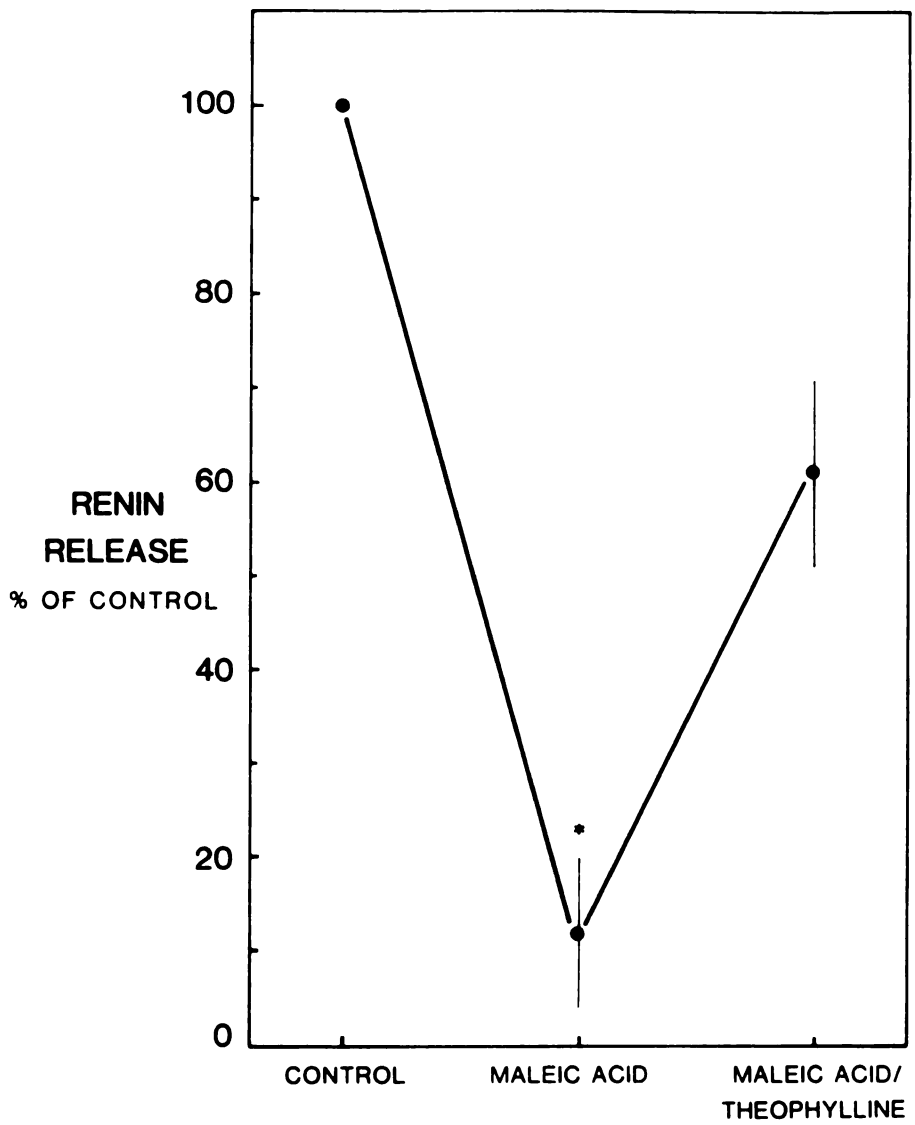


Figure 8. Effect of theophylline on the maleic acid-induced decrease in renin release presented as percent of control. Value during maleic acid represents the mean ± SEM for 10 sodium-depleted dogs, value during maleic acid and theophylline represents the mean ± SEM for five sodium-depleted dogs. Asterisk indicates $P<0.05$, compared to control.

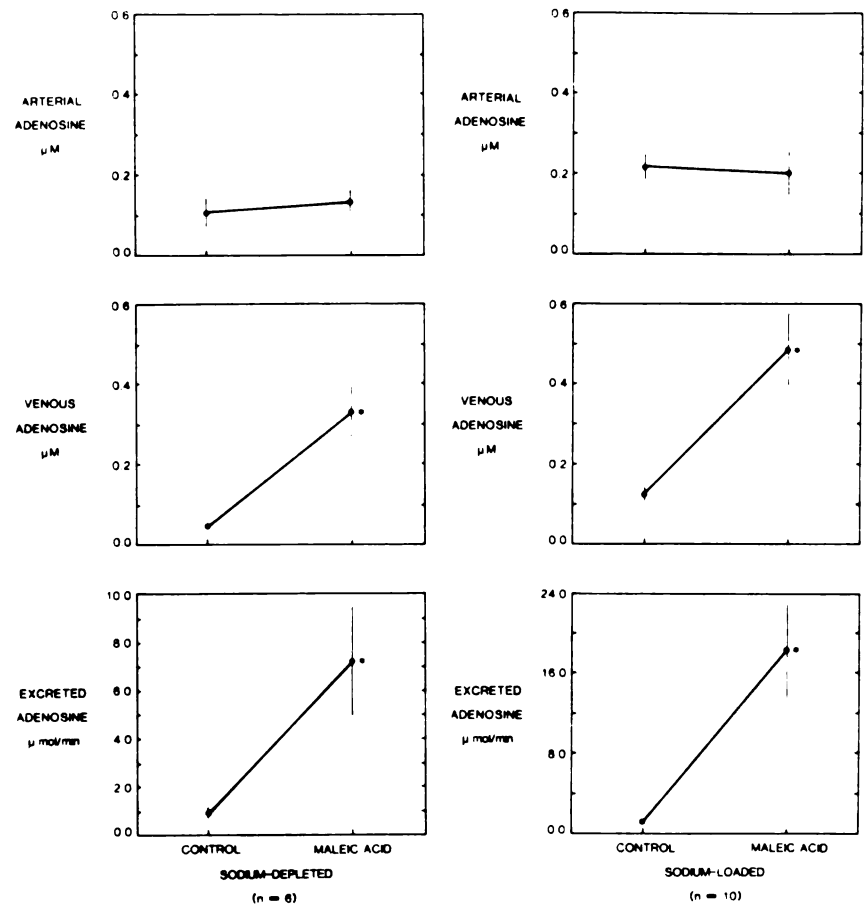


Figure 9. Effect of maleic acid on arterial adenosine, renal venous adenosine, and the urinary excretion of adenosine in six sodium-depleted and ten sodium-loaded dogs. Values are the mean $\pm$ SEM for each group. Asterisk indicates $P<0.05$, compared to control.

profound decrease in proximal and distal tubular reabsorption (Al-Bander et al 1982; Berliner et al 1950; Brewer et al 1983; Harrison and Harrison 1954; Kramer and Gonick 1973; Gmaj et al 1973). This defect in tubular transport is believed to be due to the ability of maleate to affect mitochondrial metabolic activity (Szczepanska and Angielski 1980; Angielski and Rogulski 1975; Rogulski and Pacanis 1978). The administration of maleic acid has also been shown to be associated with a decrease in cortical ATP concentrations(Osswald et al 1980; Kramer and Gonick 1970; Szczepanska and Angielski 1980). In addition, a decrease in glomerular filtration rate (GFR) has been shown to occur during the infusion of maleic acid (Al-Bander et al 1982; Berliner et al 1950; Brewer et al 1983; Gmaj et al 1973; Gougoux et al 1976; Gunther et al 1979; Maesaka and McCaffery 1980; Osswald et al 1980). While the mechanism of action of maleic acid to produce defects in tubular transport has been studied (Angielski and Rogulski 1975; Rogulski and Pacanis 1978; Angielski 1963), the action of maleic acid to cause a decrease in GFR has not been extensively examined, though a recent micropuncture study by Maesaka and McCaffery (1980) suggested that the measured decline in GFR is due to a backleak of inulin in a distal portion of the nephron. The ability of theophylline to

reverse the maleic acid-induced decrease in GFR in the present study, as well as in a preliminary study by Osswald (1980), indicates that in addition to, or alternative to a backleak of inulin, the decrease in GFR may be due to an adenosine-mediated mechanism.

The renal actions of exogenous adenosine have been shown to be dependent on the state of sodium balance of the animal. Osswald et al (1978) have shown that the effects of adenosine on GFR, renal blood flow (RBF), and renin release are enhanced by sodium restriction and attenuated by sodium loading. In addition, the renal effects of elevating endogenous adenosine have also been shown to have a sodium-dependency (Results III.A.). Therefore, as a test of the hypothesis that the decrease in GFR due to maleic acid administration is the result of an increase in endogenous adenosine, the animals in the present study were either sodium-depleted or sodium-loaded. The magnitude of the decrease in GFR was greater in the sodium-depleted than in the sodium-loaded dogs. In addition to the decrease in GFR, the change in RBF was more pronounced in the sodium-depleted animals, and there was a decrease in renin release, both of which are known to occur with the exogenous administration of adenosine (Osswald et al 1978; Tagawa and Vander 1970) as well as with increasing

endogenous adenosine concentrations (Results, III.A.). These results were true for each time point during the infusion of maleic acid and support the hypothesis that intrarenal adenosine mediates these changes. The possibility exists, however, that the metabolic effects of maleic acid are altered by the presence of a high sodium diet or volume expansion and that this alteration in the cellular effect of maleic acid is responsible for the difference in the functional responses of the two groups. The fractional excretion of sodium at 100 min was approximately 30% in both the sodium-depleted and the sodium-loaded dogs, and was actually more pronounced in the sodium-loaded animals at all time points, indicating that the difference in sodium balance apparently was not the cause of the difference in functional response between the two groups.

The animals in this study were given lithium carbonate prior to the experiments with the intention of using fractional lithium excretion as an index of proximal reabsorption, since it has been reported that lithium is handled by the kidney at this site (Hayslett and Kashgarian 1979). Previous studies attempting to localize the tubular action of maleic acid have shown that much of the transport dysfunction occurs in the proximal tubule (Al-Bander et al

1982; Brewer et al 1983; Kramer and Gonick 1973; Gmaj et al 1973; Gunther et al 1979), with some reports of dysfunction occurring distally (Al-Bander et al 1982; Bergeron et al 1976). The increased fractional excretion of lithium in the present study served as evidence that the transport defect had occurred. An interesting finding apart from the increase in fractional lithium excretion was that, given the same dose in both groups of animals, there was a consistently lower plasma lithium concentration and higher control fractional lithium excretion in the sodium-loaded groups. This may reflect an overall decrease in proximal reabsorption due to the volume-expanded state of these animals.

Concomitant with the functional changes resulting from the infusion of maleic acid, the intrarenal concentrations of adenosine were increased as measured by an increase in the level of adenosine in the renal venous and urinary effluents. In view of work by other groups (Osswald et al 1980; Kramer and Gonick 1970; Szczepanska and Angielski 1980) in which the infusion of maleic acid is shown to be associated with a decrease in cortical ATP concentrations, it was not surprising to find an increase in the concentrations of adenosine in the present study. Indeed, it was reported in a study by Osswald et al (1980) that

tissue adenosine concentration is increased following maleic acid administration in the rat. However, given the complexity of adenosine metabolism, its varied cellular sources, and the possibility of intracellular binding sites, the total tissue level of adenosine is not a reliable index of extracellular adenosine, which is the relevant pool of adenosine when proposing that adenosine may be involved in the regulation of renal function. In a recent report from this laboratory (Thompson et al 1985), it was shown that, despite similar control arterial and venous adenosine concentrations, very little of the venous adenosine was derived from the arterial supply, therefore, adenosine measured in the renal vein is primarily of renal origin. Likewise, a small portion of the adenosine measured in the urine is from renal production, the majority of adenosine in the urine being from filtration of the arterial plasma and incomplete reabsorption in the tubules. Therefore, in the present study, adenosine was measured in the renal vein and in the urine to provide an indication of changes occurring in extracellular adenosine levels. The level of adenosine in the kidney during maleic acid infusion was increased to approximately the same magnitude in both groups of dogs and, therefore, the smaller changes in renal function that occurred in the

sodium-loaded animals were not due to less adenosine being released in these dogs.

To further test the hypothesis that the increase in intrarenal adenosine mediates the decrease in GFR, RBF, and renin release seen with maleic acid, the adenosine receptor antagonist, theophylline, was used. Theophylline, given at a dose that is known to have no influence on cyclic nucleotide phosphodiesterase activity (Spielman 1984), reversed the decreases in GFR and RBF in both the sodium-depleted and the sodium-loaded dogs, and reversed the decrease in renin release in the sodium-depleted dogs. The administration of theophylline did not, however, attenuate the increase in the fractional excretion of sodium, suggesting that the defect in tubular transport caused by maleic acid is an adenosine-independent phenomenon while the hemodynamic and renin release effects may be mediated by the increase in adenosine levels. The inability of theophylline to completely reverse the decrease in GFR could be due to other factors resulting from the administration of maleic acid. As suggested above, maleic acid may cause a back leak of inulin across the damaged tubular epithelium. In addition, maleic acid has been shown previously to produce a bicarbonaturia (Berliner et al 1950; Gmaj et al 1973; Gougoux et al 1976) and may lead

to acidosis, which can adversely affect GFR. Despite these possible complications, theophylline largely returns GFR to control, suggesting a major contribution by adenosine to the decrease in GFR.

The decrease in GFR following maleic acid administration may serve to limit the polyuria associated with this nephrotoxic insult. It is tempting to speculate that the increased delivery of fluid and solute from the damaged proximal epithelia following maleic acid initiates an adenosine-mediated tubuloglomerular feedback response to reduce the filtered load in an attempt to limit the excess fluid and solute loss. Previous data have suggested a role for adenosine in mediation of the feedback response. Most relevant to the present study are the results of Schnermann and coworkers (1977) which showed that the feedback response to an increase in tubular flow rate is abolished by theophylline. Coupled with the present results, this is consistent with adenosine acting as a feedback mediator in certain circumstances.

The results of this study support the hypothesis that adenosine mediates the renal hemodynamic and renin release effects of maleic acid. However, while maleic acid is used as an experimental model of the Fanconi syndrome, this study does not necessarily imply that any changes in renal

function that occur in clinically relevant cases of the Fanconi syndrome are mediated by adenosine. What can be inferred from these results is that endogenous adenosine is capable of altering renal function in some pathophysiologic or nephrotoxic situations. It remains to be determined what role adenosine plays in the normal regulation of renal function.

## C. A role for intrarenal adenosine in the renal hemodynamic response to contrast media.

### C.1. Introduction

The effects of hypertonic contrast media administration on renal function have been well documented (Ansari and Baldwin 1976; Caldicott et al 1970; Chou et al 1971; Gerber et al 1982; Katzberg et al 1983; Larson et al 1983; Reed et al 1983; Talner and Davidson 1968). The injection of contrast media into the circulation, whether intrarenally or intravenously, results in a pronounced, though transient, decrease in renal blood flow and glomerular filtration rate (Caldicott et al 1970; Chou et al 1971; Katzberg et al 1983; Larson et al 1983; Reed et al 1983; Talner and Davidson 1968; Katzberg et al 1977). Under experimental conditions it has been shown that the hemodynamic changes exhibit a sodium-dependency, that is, the effects of contrast media on renal function are more

pronounced in animals maintained on a low-sodium diet than in those maintained on a high-sodium diet (Caldicott et al 1970; Larson et al 1983; Katzberg et al 1977; McDonald et al 1969). Several attempts have been made to elucidate the mechanism by which contrast solutions produce these changes in renal function. Alpha-adrenoceptor antagonists (Caldicott et al 1970) and angiotensin II blockers (Larson et al 1983) have been used in attempts at altering the hemodynamic effects of contrast media but did not completely prevent the vasoconstrictive response. However, in a more recent study, decreasing the availability of calcium by the use of the calcium influx blockers, verapamil and diltiazem, or by chelating extracellular calcium with EGTA, was successful in attenuating the decreases in renal blood flow (RBF) and glomerular filtration rate (GFR) due to contrast media administration in dogs (Bakris and Burnett 1985). These results suggest that the mediator of contrast-induced changes in renal hemodynamics acts through an increase in intracellular calcium, presumably in vascular smooth muscle cells.

In a recent report from this laboratory, the decrease in RBF and GFR resulting from the intrarenal administration of adenosine could be abolished by verapamil (Arend et al 1984), suggesting that the renal hemodynamic effects of

adenosine are mediated by increased calcium influx. In addition, similar to the hemodynamic actions of contrast media, the effects of adenosine on RBF and GFR are enhanced by prior sodium depletion of the animal and attenuated by sodium loading (Osswald et al 1978). Therefore, the present study was undertaken to test the hypothesis that the reduction in renal blood flow and GFR following the administration of hypertonic contrast media is mediated by an increase in endogenous adenosine levels. This hypothesis was tested by employing the nucleoside uptake blocker, dipyridamole, and the adenosine receptor antagonist, theophylline, as well as by measurements of arterial, renal venous, and urinary adenosine.

C.2. Methods

The experiments were performed on mongrel dogs of either sex weighing 10-20 kg. All animals were maintained on a low-sodium diet (Hill's Prescription Diet, h/d) for a period of 10-14 days and were given an injection of Lasix (100 mg, i.v.) on the first day of the dietary regimen. On the day prior to the experiment, each dog was fasted and allowed no water.

The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and a tracheostomy was performed for mechanical ventilation with a Harvard

respirator. The dogs were then surgically prepared as described previously (Arend et al 1984). Briefly, catheters (PE 240) were introduced into both femoral veins for systemic infusions and supplemental anesthetic administration, into one femoral artery for the collection of systemic arterial blood samples, and in one carotid artery for monitoring of systemic blood pressure. A left flank incision was made to expose the ureter and the left renal artery and vein. An electromagnetic flow probe (Zepeda Instruments) was placed around the renal artery, and renal blood flow, as well as systemic blood pressure, was recorded on a Hewlett-Packard recorder. Small curved needles were placed in the renal artery for intrarenal infusions, and in the renal vein for sampling of the renal venous effluent. The arterial needle was kept patent by a constant infusion of 0.9% saline at 1.38 ml/min. A catheter was placed in the ureter and positioned near the pelvis of the kidney for collection of urine samples. A 1% solution of inulin was infused at 2 ml/min throughout the experiments into a femoral vein.

All solutions were prepared on the day of the experiment with 0.9% NaCl as the vehicle. The contrast agent utilized in this study was Renografin-76 (meglumine/sodium diatrizoate-76%, E.R. Squibb). Inulin,

adenosine, and theophylline were obtained from Sigma. Dipyridamole was a gift from Boehringer-Ingelheim.

Inulin concentrations in plasma and urine were determined using the anthrone method of Davidson and Sackner (1963). Sodium, potassium, and lithium concentrations in plasma and urine were determined by flame photometry. Samples were assayed for adenosine concentration by HPLC using the method described previously (Methods, III.A.). Glomerular filtration was calculated as the extraction of inulin across the kidney. The data were statistically analyzed by a two-way analysis of variance, using the Student-Newman-Kuels test for multiple comparisons, or by paired t-test where appropriate. Results are reported as mean $\pm$ SEM.

Group I: The effect of intrarenal injection of contrast media on renal function (n=6). Two control clearance periods were performed to determine initial rates of glomerular filtration, renal blood flow, and other indices of renal function. These clearance periods consisted of two contiguous 15 min urine collections, with two systemic arterial and renal venous blood samples taken during each 15 min period at 5 and 10 min. Following these control periods, the contrast media (4 ml) was injected directly into the renal artery over a period of 15-30 sec.

Immediately following this injection, three 5 min clearance periods were performed, with blood samples being drawn at the midpoint of each period. A second injection of contrast media and collection of samples was repeated following approximately 30 min of recovery. Arterial and renal venous blood and urine samples for adenosine measurements were obtained during each period.

Group II: Effects of theophylline on the contrast media-induced changes in renal function (n=5). Control and contrast media clearance periods were performed as described for Group I before and during theophylline. Theophylline was infused at 5 umol/min for 30 min prior to and during the second set of control and contrast media clearance periods.

Group III: Effects of dipyridamole on the contrast media-induced changes in renal function (n=6). Two control clearance periods and one injection of contrast media, followed immediately by three 5-min periods were performed as described for Group I. The intrarenal infusion of saline was then replaced by dipyridamole (24 ug/kg/min) and after a 30 min equilibration period, two control clearance periods and one injection of contrast media were performed as before.

C.3. Results

Group I: Effect of contrast media injection on renal function. The rapid (30 sec) injection of 4 ml Renografin-76 directly into the renal artery resulted in an immediate increase in renal blood flow (RBF), resulting from the injection of a viscous solution, which lasted for the duration of the injection, followed by a 17% decrease in RBF, from 97 $\pm$ 14 ml/min to 81 $\pm$ 13 ml/min that lasted approximately 5 min. A representative RBF tracing is shown in Figure 10 (left half). An electronically-averaged RBF tracing was used for computing changes in RBF. Blood flow immediately prior to the contrast media injection was taken as the control and blood flow at the nadir of the response after the injection, was taken as the maximum decrease and used for calculation of percent decrease. In the first five minutes after contrast media injection, GFR decreased by 31% from 25 $\pm$ 4 ml/min to 17 $\pm$ 4 ml/min ($P<0.05$, Figure 11) and returned to control by the second clearance period following the injection. Data from the two control periods were pooled and those from the second and third periods following contrast injection were pooled as recovery. The responses to the second injection of contrast media were no different than the responses to the first injection and were also pooled. Intrarenal injection of contrast media

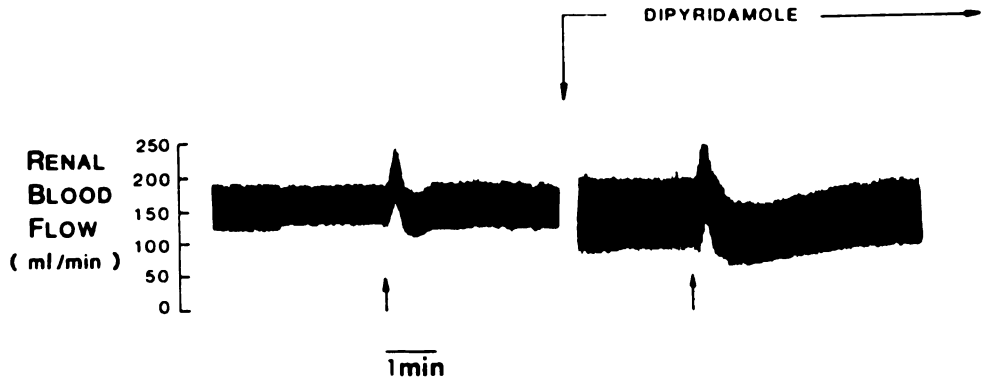


Figure 10. Representative renal blood flow tracing of the responses to contrast media injection (small arrows) in the control state, and in the presence of dipyridamole.

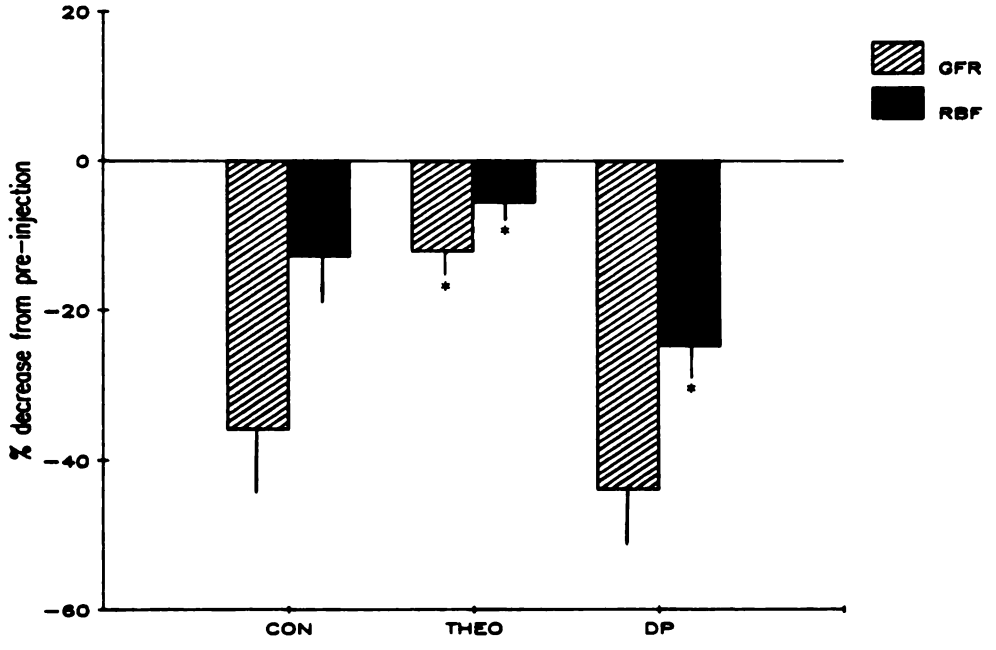


Figure 11. Effect of contrast media on glomerular filtration rate (closed bars) and renal blood flow (hatched bars) in the control state (CON), and during the infusion of theophylline (THEO) or dipyridamole (DP). Results are expressed as percent decrease from pre-contrast levels.

*, significantly different than response to contrast media in the absence of theophylline or dipyridamole.

on mean arterial pressure (MAP) or filtration fraction (FF) (Table 5). Renal vascular resistance (RVR) was increased slightly. Urine flow increased by 5-fold following contrast injection, and the excretion and fractional excretion of sodium increased significantly. Systemic arterial and renal venous adenosine concentrations were not increased following contrast media injection (136 $\pm$ 49 vs 162 $\pm$ 86 uM, NS and 237 $\pm$ 125 vs 144 $\pm$ 55 uM, NS, respectively).

The urinary concentration of adenosine was decreased by contrast media (855 $\pm$ 228 vs 357 $\pm$ 94 uM, NS), however, when factored by the large increase in urine volume and the decreased GFR, excreted adenosine (388 $\pm$ 80 vs 830 $\pm$ 23 umol/min, $P<0.05$), and fractional excretion of adenosine (15.9 $\pm$ 4.1 vs 49.6 $\pm$ 12.9%, $P<0.05$), were significantly increased.

Group II: Effect of theophylline on the contrast media-induced changes in renal function. As in Group I, contrast media injection in the absence of theophylline resulted in a significant decrease in RBF (138 $\pm$ 11 vs 114 $\pm$ 9 ml/min, $P<0.05$) and GFR (29 $\pm$ 2 vs 16 $\pm$ 1 ml/min, $P<0.05$), with little or no change in filtration fraction, mean arterial pressure, and renal vascular resistance (Table 5). The fractional excretion of sodium was

Table 5. Effects of contrast media on renal function, and the effects of theophylline and dipyridamole on the renal responses to contrast media.

| | RBF (ml/min) | GFR (ml/min) | FF | MAP (mmHg) | RVR (mmHg/ml/min) | UF (ml/min) | $E_{Na}$ (mEq/min) | $FE_{Na}$ (%) |
|---|---|---|---|---|---|---|---|---|
| **Group I: Effect of contrast media on renal function (n = 6).** | | | | | | | | |
| Control | 97±14 | 25±5 | 0.41±0.04 | 136±5 | 1.53±0.19 | 0.58±0.23 | 96±42 | 1.1±0.6 |
| Contrast media | 81±13 | 17±4* | 0.38±0.04 | 134±6 | 1.83±0.29 | 1.68±0.39* | 187±70* | 5.1±2.2* |
| **Group II: Effect of theophylline on renal responses to contrast media (n = 5).** | | | | | | | | |
| Control | 138±11 | 29±2 | 0.46±0.03 | 126±2 | 1.04±0.13 | 0.48±0.19 | --- | 1.2±0.7 |
| Contrast media | 114± 9* | 16±1* | 0.42±0.03 | 125±1 | 1.19±0.24 | 1.49±0.24* | --- | 4.6±1.8* |
| Theophylline | 131± 9 | 27±2 | 0.51±0.04 | 125±2 | 1.07±0.16 | 1.63±0.28* | --- | 4.2±1.1* |
| Contrast media | 123±10+ | 23±2+ | 0.47±0.04 | 124±2 | 1.12±0.19 | 3.39±0.21*# | --- | 8.6±1.7*# |
| **Group III: Effect of dipyridamole on renal responses to contrast media (n = 6).** | | | | | | | | |
| Control | 119±12 | 23± 3 | 0.34±0.01 | 117±8 | 1.03±0.13 | 0.54±0.12 | 45±24 | 0.8±0.4 |
| Contrast media | 104±14 | 16± 3* | 0.25±0.02* | 114±7 | 1.19±0.17 | 2.34±0.32* | 179±46* | 4.6±1.0* |
| Dipyridamole | 118±14 | 19± 2 | 0.30±0.02 | 109±6 | 1.01±0.14 | 0.54±0.31 | 18± 7 | 0.5±0.2 |
| Contrast media | 88±13*+ | 11± 2* | 0.25±0.03* | 112±6 | 1.40±0.19* | 1.49±0.29* | 75±15* | 2.2±0.5* |

RBF, renal blood flow; GFR, glomerular filtration rates; FF, filtration fraction; MAP, mean arterial pressure; RVR, renal vascular resistance; UF, urine flow rate; $E_{Na}$, excreted sodium; $FE_{Na}$, fractional sodium excretion.

*, $p < 0.05$ when compared to respective control value
+, $p < 0.05$ absolute change when compared to control change
#, $p < 0.05$ when compared to controls without theophylline

increased significantly (1.2 ± 0.7 vs 4.6 ± 1.8%, P<0.05) by contrast media. The infusion of theophylline alone resulted in a significant increase in urine flow rate and the excretion of sodium (Table 5), but did not alter RBF or GFR. In the presence of theophylline, there was no significant change in RBF (131 ± 9 vs 123 ± 10 ml/min, NS) or GFR (27 ± 2 vs 23 ± 2 ml/min, NS) with the injection of contrast media (Figure 11). The urine flow rate and excretion of sodium were significantly increased by contrast media, despite the presence of theophylline.

Group III: Effect of dipyridamole on the contrast media-induced changes in renal function. Contrast media injection resulted in a decrease in RBF of 12% (119 ± 12 vs 104 ± 14 ml/min, NS), and in GFR of 30% (23 ± 3 vs 16 ± 3 ml/min, P<0.05). Filtration fraction also decreased significantly in this group and vascular resistance, urine flow, and the excretion of sodium increased (Table 5). During the intrarenal infusion of dipyridamole, the injection of contrast media resulted in a more pronounced decrease in RBF and GFR (Figure 11). RBF decreased by 25 ± 4% (118 ± 14 vs 88 ± 13 ml/min, P<0.05; Figure 10, right half) and GFR decreased 44 ± 7% (19 ± 2 vs 11 ± 2 ml/min, P<0.05); these decreases are greater than those seen in the absence of dipyridamole. The duration of the vasocon-

striction was not lengthened by the infusion of dipyridamole; RBF returned to the control value by approximately 5 min after the injection. As in Group I, MAP and filtration fraction were essentially unchanged, while urine flow and the excretion of sodium increased after contrast media injection.

C.4. Discussion

The administration of hypertonic contrast solutions has long been recognized to produce profound changes in renal hemodynamics and in some cases to lead to the initiation of acute renal failure (Ansari and Baldwin 1976; McDonald et al 1969; Byrd and Sherman 1979). The systemic administration of contrast media, as well as injections directly into the kidney, results in a transient decrease in renal blood flow (RBF) and glomerular filtration rate (GFR) (Caldicott et al 1970; Chou et al 1971; Katzberg et al 1983; Larson et al 1983; Reed et al 1983; Talner and Davidson 1968; Katzberg et al 1977). While many attempts have been made to elucidate the mechanism of these actions, the results, for the most part, have been negative or incomplete. It is known that the large increase in urine output following contrast media administration is due to an osmotic diuretic effect in the proximal tubule (Cattell et al 1982), and it has been proposed that this increase in

tubular fluid flow is responsible for the reductions in RBF and GFR, that is, a tubuloglomerular feedback signal is being generated to reduce filtration (Reed et al 1983). In support of this idea, it has been demonstrated that the hemodynamic changes occurring following intravenous contrast administration are reduced in streptozotocin-induced diabetic rats with chronic glycosuric osmotic diuresis (Reed et al 1983), glycosuria having been associated with suppression of the feedback response (Blantz et al 1975). However, the mediator of this feedback response has not been determined. Attempts to attenuate the renal hemodynamic changes produced by contrast agents with alpha adrenoreceptor antagonists (Caldicott et al 1970) as well as angiotensin II receptor blockers and converting enzyme inhibitors (Larson et al 1983) have provided only partial protection, therefore, renal nerves and the renin-angiotensin system do not appear to play a solitary role in mediating the contrast-induced changes. An important advance in our understanding of the mechanism by which contrast media produce a decrease in RBF and GFR is the finding that the calcium-influx blockers, verapamil and diltiazem, reduce the changes in renal hemodynamics resulting from contrast media injections in anesthetized dogs (Bakris and Burnett 1985). These results

suggest that the mediator of the contrast-induced changes acts by increasing intracellular calcium, possibly by stimulating an increased influx of calcium. In addition, it was shown earlier by Larson et al (1983) that the hemodynamic changes following contrast media administration exhibit a sodium-dependency, that is, the decreases in RBF and GFR are greater in animals that were fed a low-sodium diet and are less pronounced in animals fed a high-sodium diet.

The present study was undertaken to test the hypothesis that an increase in intrarenal adenosine concentrations mediates the changes in renal hemodynamics following contrast media administration. The administration of exogenous adenosine is known to cause a brief vasoconstriction when injected into the renal artery of dogs (Arend et al 1984; Thurau 1964) and results in a biphasic change in RBF and a sustained decrease in GFR when infused intrarenally (Osswald et al 1978; Tagawa and Vander 1970). As a result of these, and other, observations, adenosine has been proposed as an intrinsic regulator of renal function (Spielman and Thompson 1982) and has been suggested as the mediator of renal function changes in some pathophysiological conditions. For example, the administration of theophylline, the adenosine receptor

antagonist, prior to occlusion of the renal artery minimized the post-occlusive reduction in RBF (Osswald 1975). In addition, the pronounced decrease in GFR that occurs following the administration of maleic acid can be attenuated by theophylline (Results III.B.; Osswald et al 1982). In both of these studies an increase in intrarenal adenosine concentrations was measured.

The renal actions of adenosine have been shown to be sodium-dependent (Osswald et al 1978). That is, sodium-depletion results in larger adenosine-induced decreases in RBF and GFR as compared with the responses in animals maintained on a normal sodium diet, while those animals maintained on a high sodium diet show smaller responses to intrarenal adenosine. Additionally, the renal hemodynamic effects of adenosine can be blocked by the administration of verapamil (Arend et al 1984). The similarities between the renal actions of contrast media and those of adenosine led to the hypothesis that adenosine mediates the hemodynamic changes due to contrast media administration.

In the present study, intrarenal injections of Renografin-76 produced changes in renal function such as those that have been reported previously (Ansari and Baldwin 1976; Caldicott et al 1970; Chou et al 1971; Gerber et al 1982; Katzberg et al 1983; Larson et al 1983; Reed et

al 1983; Talner and Davidson 1968; Katzberg et al 1977; McDonald et al 1969). In the presence of the adenosine receptor antagonist, theophylline, the changes in renal blood flow and glomerular filtration rate were substantially reduced. The concentration of theophylline used in this study is well below that which has been shown to affect cyclic nucleotide phosphodiesterase activity (Spielman 1984), and therefore, these differences in the hemodynamic response can not be attributed to theophylline-induced changes in cAMP levels. These results suggest that adenosine may partially mediate the hemodynamic effects of contrast media.

Further evidence to support this hypothesis stems from the results that the changes in renal function were potentiated when the contrast media injections were performed in the presence of an infusion of dipyridamole. In an earlier study, we demonstrated that an intrarenal infusion of dipyridamole significantly increases the intrarenal concentrations of adenosine by interfering with the cellular uptake and degradation of adenosine (Results, III.A.; Thompson et al 1985). Increasing endogenous adenosine by blocking its cellular uptake resulted in changes in renal hemodynamics and renin release similar to that seen with an infusion of exogenous adenosine. The

renal actions of exogenous adenosine are also potentiated by the presence of dipyridamole (Results, III.A.). The enhancement by dipyridamole of the contrast-induced decreases in RBF and GFR suggests that these changes are due to an increase in endogenous adenosine concentrations above that which occurred without the dipyridamole.

Following the intrarenal injection of contrast media, there was an increase in the excretion of adenosine in the urine. In a recent study on the handling of adenosine by the kidney it was shown that 50% of filtered adenosine is excreted in the urine (Thompson et al 1985). Therefore, in the present study, since there was no increase in arterial adenosine concentrations and there was a decrease in GFR, hence a decrease in filtered adenosine, it appears that the increased excretion of adenosine was due to an increase in extracellular adenosine in the kidney. The decrease in adenosine concentration of the urine likely reflects the large increase in urine volume that occurred with contrast media injection. There was, however, no increase in adenosine leaving the kidney via the renal venous blood. This does not support the hypothesis that increased renal adenosine mediates the contrast media-induced hemodynamic changes. However, the changes in RBF and GFR that occurred after injection of contrast media were of short duration,

lasting at the most 5 minutes, and it is therefore possible that there was an increase in intrarenal adenosine but it could not be detected in the renal vein due to the short time course of hemodynamic changes, or due to the rapid cellular uptake and degradation of adenosine by cells, particularly red blood cells. Alternatively, the increase in adenosine may have been small or localized and was for this reason not detectable in the renal vein.

In summary, the renal hemodynamic effects of contrast media can be attenuated by an adenosine receptor antagonist, and enhanced by an adenosine uptake inhibitor, and the urinary excretion of adenosine is increased by contrast media injection. Therefore, an increase in intrarenal adenosine may be an important factor in the evolution of the renal hemodynamic changes following the administration of hypertonic contrast media.

## IV. THE RENAL CELLULAR ACTIONS OF ADENOSINE

### A. A1 and A2 adenosine receptors in rabbit cortical collecting tubule cells: modulation of hormone-stimulated cAMP

(reproduced with permission, Am. Soc. Clin. Invest.; J. Clin.Invest. 79:710, 1987)

#### A.1. Introduction

Adenosine is an important intermediate in the pathway of purine nucleotide degradation and has been shown to modify a variety of renal functions (Osswald et al 1978; Spielman et al 1980; Tagawa and Vander 1970; Osswald et al 1978b; Spielman 1984; Hedqvist et al 1978; Churchill 1982; Coulson and Scheinman 1985). Adenosine can alter renal excretory function (Osswald et al 1978; Spielman 1984; Churchill 1982; Coulson and Scheinman 1985; Osswald 1975), but it is not clear to what extent these effects are the result of a direct tubular action or are an indirect result of altered renal hemodynamics. Recent studies by Forrest and coworkers on cultured amphibian epithelial (A6) cells (Lang et al 1985), the rectal gland of the shark (Forrest et al 1982), and the rabbit colon (Dobbins et al 1984) have demonstrated that adenosine can alter active transepithelial transport. Dillingham and Anderson (Dillingham and Anderson 1985) have shown that adenosine analogs inhibit vasopressin-stimulated water movement in

rabbit collecting tubules. These studies provide important precedents for a direct action of adenosine on mammalian renal tubular epithelium, though relatively little is known regarding the cellular mechanisms underlying the tubular actions of adenosine. The present study was undertaken to determine the effect of adenosine analogs on the production of cAMP by cultured cortical collecting tubule (RCCT) cells isolated by immunodissection from the rabbit kidney, and to test the hypothesis that adenosine modulates hormone-stimulated cAMP production. Adenosine analogs were used in this study to investigate the actions of adenosine on cAMP. The analogs used were 5'-N-ethylcarboxamide adenosine (NECA), $N^6$-cyclohexyl adenosine (CHA), and R-$N^6$-phenylisopropyl adenosine (PIA). These analogs were chosen because they are not substrates for adenosine deaminase or the nucleoside carrier, and because they each have greater potency at either the $A_1$ or the $A_2$ receptor. Data are presented indicating the presence of both inhibitory ($A_1$) and stimulatory ($A_2$) receptors for adenosine in RCCT cells and that stimulation of the $A_1$ receptor inhibits hormone-stimulated cAMP formation. The inhibition is mediated by the inhibitory guanine nucleotide-binding regulatory component of the adenylate

cyclase system and is not dependent on stimulation of prostaglandin production.

A.2. Methods

Materials. Trypsin (1:250), Dulbecco's modified Eagle's medium (DME), collagenase, fetal bovine serum (FBS), and bovine serum albumin (BSA) were purchased from Gibco Laboratories. The adenosine analogs, 5'-N-ethylcarboxamideadenosine (NECA), $N^6$-cyclohexyladenosine (CHA), and R-$N^6$-phenylisopropyladenosine (PIA), and 1,3-diethyl-8-phenylxanthine (DPX) were a gift from Warner Lambert. The phosphodiesterase inhibitor, RO 20-1724, was a gift from Hoffmann-LaRoche. Arginine vasopressin (AVP) was from Peninsula Laboratories. Pertussis toxin was from List Biochemicals. $^{125}$I adenosine 3'5'cyclicmonophosphoric acid and $^{3}$H prostaglandin $E_2$ were from ICN Biochemicals. Other chemicals were reagent grade or better obtained from standard sources.

Isolation and culture of RCCT cells. Rabbit cortical collecting tubule (RCCT) cells were isolated by immunodissection with a cell-specific mouse monoclonal antibody as described previously (Spielman et al 1986). Briefly, plastic culture dishes were coated with an $IgG_1$ (rct-30) that binds exclusively to collecting tubule cells when assayed by indirect immunofluorescence on rabbit

kidney cryotome sections (Spielman et al 1986). Both kidneys were removed from 4-5 wk old rabbits and the cortical tissue separated and minced into a brei. The tissue was digested in 0.1% collagenase for 40 min with agitation every 10 min. Following centrifugation to remove the collagenase, red blood cells were lysed with hypotonic saline and the suspension was filtered through 250 um Gelman filter holders. The cells were centrifuged and washed in 10% BSA to remove cellular debris. The resulting cell suspension was placed on the antibody-treated dishes and after 2-3 min was washed off with 10-15 ml/plate of phosphate buffered saline (PBS, composition in mM: 151 NaCl, 45 $KH_2PO_4$, and 2.5 NaOH). Freshly isolated RCCT cells were grown to confluency (4-5 days) in 100mm Costar culture dishes in DME with 10% FBS, 2mM glutamine, and 1 uM dexamethasone in a water-saturated, 7% $CO_2$ environment at 37°C. Confluent RCCT cells were detached from the dishes by treatment with trypsin (0.1%, with 0.05% EDTA in PBS, pH 7.4), transferred into 24-well culture dishes ($1x10^4$ cells/well), and cultured under the conditions described above.

Determination of total cAMP production. Treatments were done in triplicate using first passage cells grown for 5 days in 24-well dishes. Culture medium was removed and

the cells were washed once with Krebs buffer (composition in mM: 118 NaCl, 25 $NaHCO_3$, 14 glucose, 4.7 KCl, 2.5 $CaCl_2$, 1.8 $MgSO_4$, and 1.8 $KH_2PO_4$, pH 7.4). The cells were pretreated for 1 hr with 0.1 mM RO 20-1724, a phosphodiesterase inhibitor which is not an adenosine receptor antagonist (Londos et al 1978; Huang and Drummond 1976), and 1.5 units adenosine deaminase/ml Krebs at 37°C. Phosphodiesterase activity was inhibited so that increases in cAMP production could be easily detected and quantified. Adenosine deaminase was included to remove any pre-existing endogenous adenosine that might compete with the experimental agents at the receptor. After pretreatment, the buffer was aspirated and the cells were treated with the same buffer containing the various hormones and effectors at 37°C. Dimethyl sulfoxide (DMSO) was used as solvent for the adenosine analogs and antagonists and was included in the control wells. Treatment was terminated by adding an equal volume of 5% (w/v) trichloroacetic acid (TCA). The cells were frozen, thawed, and allowed to stand at 4°C for 60 min. The samples were extracted three times with 5 volumes of water-saturated ether, and the residual ether was evaporated and the samples dried under vacuum with vortexing. The samples were reconstituted with distilled water, and total cAMP was determined by

radioimmunoassay as described by Frandsen and Krishna (1976).

Determination of $PGE_2$ production. Treatments were done in triplicate using first passage cells grown for 5 days in 24-well dishes. The culture medium was removed and the cells were washed with Krebs buffer. The cells were incubated with adenosine analog in Krebs for 30 min at 37°C. At the end of the incubation period, the effector solution was removed and assayed for $PGE_2$ concentration by radioimmunoassay as described previously (Grenier et al 1981).

Protein determination. Following removal of the media for cAMP assay, the remaining liquid was aspirated and the cells were solubilized with a solution of 2% $Na_2CO_3$, 0.4% NaOH, 0.16% sodium tartrate, and 1% SDS. After 24 hr at 24°C, samples were assayed for protein using a modification of the Lowry method (Markwell et al 1978).

Statistical methods. Data were analyzed for statistical significance using two-way analysis of variance with the multiple comparisons test of Student-Newman-Kuels'. Significance is defined as $P < 0.05$.

## A.3. Results

To determine the incubation times optimal for measuring the effects of adenosine analogs, time courses of

cAMP accumulation by RCCT cells were performed. For all of the adenosine analogs, maximal increases in total cAMP production occurred between 10 and 30 min of incubation. Inhibition of cAMP accumulation by the adenosine analogs was maximal by 10 min and remained unchanged for up to 60 min. Therefore, a 30 min incubation period was used for most experiments since both inhibition and stimulation would be maximal at this time.

Each of the three adenosine analogs studied (NECA, CHA, and PIA) produced both a dose-dependent inhibition and a dose-dependent stimulation of cAMP accumulation (Figure 12) depending on the concentration of the analog. The decreases in cAMP of 30-50% from basal accumulation were consistently observed at CHA and PIA concentrations of 10-100nM. The rank order of potency for stimulation of cAMP accumulation was: NECA>PIA>CHA. The opposite order of potency was observed for the inhibition of cAMP accumulation by adenosine analogs, ie., CHA>PIA>NECA.

Further evidence for the existence of specific adenosine receptors in RCCT cell populations was obtained using the adenosine receptor antagonists, isobutylmethyl-xanthine (IBMX) and 1,3-diethyl-8-phenylxanthine (DPX). Both IBMX and DPX produced dose-dependent attenuation of the adenosine analog-induced increases and decreases in

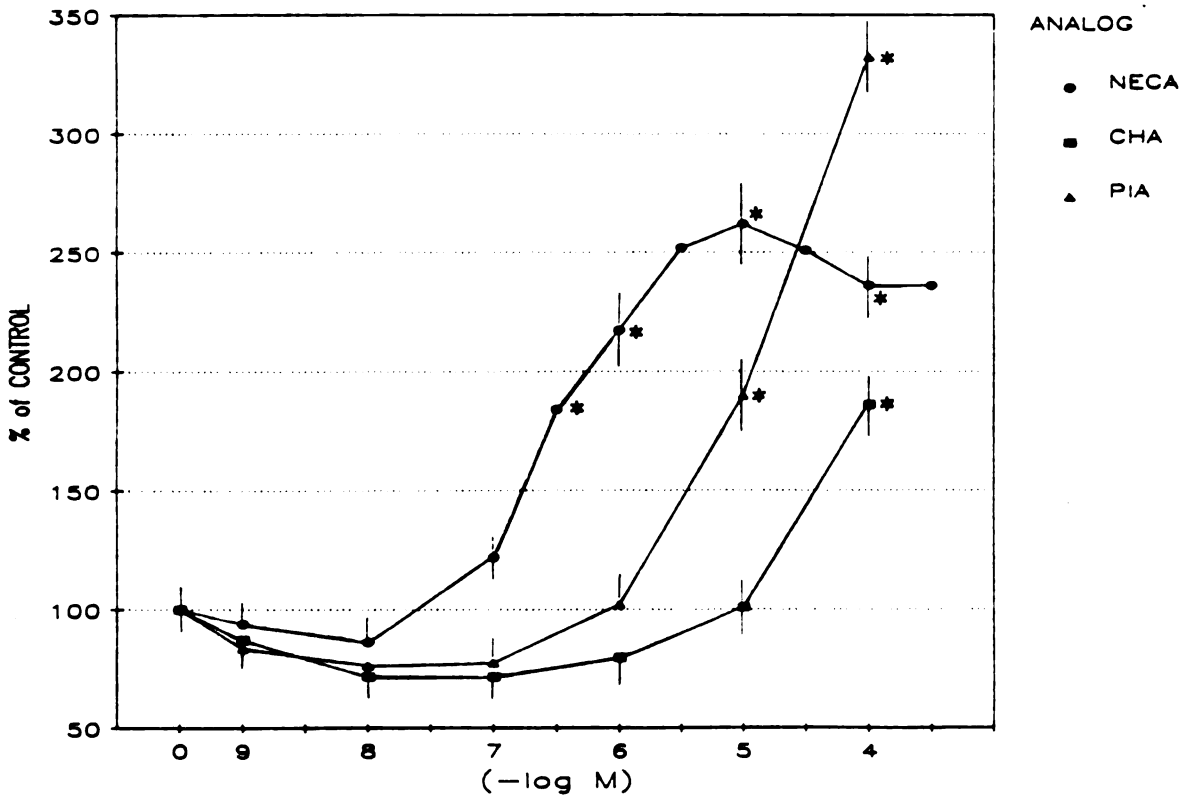


Figure 12. Effect of adenosine analogs on total RCCT cell cAMP production, expressed as percent of control (basal). Mean control values for each analog were: NECA, 341; CHA, 367; PIA, 455 pmol cAMP/mg prot/30 min. Each point is the mean of three samples from at least three different isolations.

cAMP accumulation. DPX was a more potent inhibitor of the effects of the adenosine analogs than IBMX. As little as 0.1 uM DPX produced 50% inhibition of the analog-induced stimulation of cAMP accumulation; the concentration of IBMX required to inhibit stimulation was 100 uM. Neither IBMX nor DPX affected basal cAMP concentrations, presumably since these incubations already contained a phosphodiesterase inhibitor (RO 20-1724). To investigate the potential role of adenosine in modulating hormonal regulation of tubular function, the effects of adenosine analogs on AVP- and isoproterenol-induced stimulation of cAMP accumulation by RCCT cells were studied. Treatment of RCCT cells with an inhibitory concentration of CHA (50 nM) and 1uM AVP attenuated the AVP-induced stimulation of cAMP accumulation (Table 6). Higher concentrations of CHA (100 uM), and PIA or NECA at low (50 nM) or high (100 uM) concentrations (Table 6), were much less effective in modulating AVP-induced cAMP accumulation although they produced slight inhibition. The response of RCCT cells to isoproterenol (1uM) was also attenuated by 50 nM CHA (Table 6) and, to a lesser extent, by 100 uM CHA.

Products of the cyclooxygenase pathway can inhibit the action of AVP to stimulate cAMP production in canine cortical collecting tubule cells (Garcia-Perez and Smith

Table 6. Effect of adenosine analogs on hormone-stimulated cAMP production in RCCT cells.

| Adenosine analog | | Hormone-stimulated cAMP production (pmol cAMP/mg protein) | | |
|---|---|---|---|---|
| | | None | Vasopressin | Isoproterenol |
| | | | *1 μM* | *1 μM* |
| CHA | 0 | 358±66 | 2570±722* | 3274±247* |
| | 50 nM | 126±15† | 651±77† | 1295±353† |
| | 100 μM | 675±45† | 1650±60 | 2547±493 |
| PIA | 0 | 219±13 | 1059±72* | |
| | 50 nM | 113±14† | 629±45† | |
| | 100 μM | 912±295† | 807±87 | |
| NECA | 0 | 441±31 | 2199±167* | |
| | 50 nM | 433±22 | 1979±160 | |
| | 100 μM | 1525±76† | 1607±216 | |

1984). Therefore, we tested the hypothesis that CHA stimulated the formation of prostaglandins that, in turn, mediate the attenuation of the AVP response. Treatment of RCCT cells with CHA (100 uM) failed to cause a significant increase in $PGE_2$ production (control, 115 $\pm$ 14 vs treated, 150 $\pm$ 11 fmol $PGE_2$/ug protein, NS).

To test the possibility that CHA attenuates AVP-induced increases in cAMP accumulation in RCCT cells via the inhibitory guanine nucleotide-binding protein, $N_i$, the effect of pertussis toxin was studied. Pertussis toxin catalyzes the transfer of the ADP-ribose moiety of NAD to the $N_i$ protein, thereby inactivating it and preventing the actions of inhibitory agents on cAMP production. Pretreatment of RCCT cells (12 hr) with 1 ug of pertussis toxin per ml of culture medium completely prevented the CHA-induced inhibition of cAMP accumulation, from both basal and AVP-stimulated states (Figure 13); pertussis toxin had no effect on the ability of AVP alone to increase cAMP concentrations, however, despite an increase in basal cAMP production seen in pertussis toxin-treated cells, stimulation of cAMP accumulation by higher concentrations of CHA (10-100uM) was enhanced (Table 7).

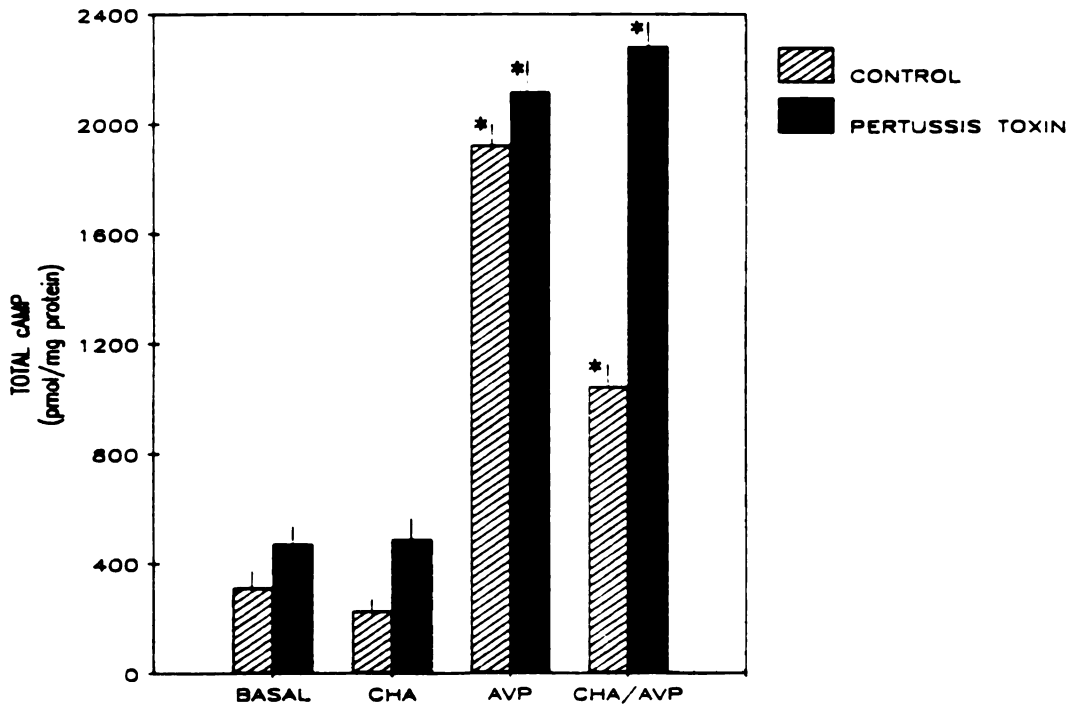


Figure 13. Effect of pretreatment with pertussis toxin on the modulation by CHA (50 nM) of 1 uM AVP-stimulated cAMP production in RCCT cells. The cells were pretreated for 12 hr with 1 ug/ml pertussis toxin. Each bar is the mean of three samples from at least three different isolations.

Table 7. Effect of pertussis toxin on stimulation of cAMP production by $N^6$-cyclohexyladenosine (CHA) in RCCT cells.

| | cAMP production | |
|---|---|---|
| | pmol/mg protein | % change from control |
| No treatment | | |
| CHA 0 | 406±38 | |
| 10 μM | 369±47 | −9 |
| 30 μM | 412±42 | +1 |
| 100 μM | 686±86* | +70 |
| Pertussis toxin | | |
| CHA 0 | 974±107 | |
| 10 μM | 912±129 | −6 |
| 30 μM | 1208±104* | +24 |
| 100 μM | 1654±162* | +70 |

## A.4. Discussion

Interest in the renal actions of adenosine stems from its possible involvement in the regulation of renal function (Spielman and Thompson 1982; Osswald et al 1980). Previous work has defined the effects of adenosine on renal hemodynamics (Osswald et al 1978b; Spielman et al 1980; Tagawa and Vander 1970), renin release (Tagawa and Vander 1970; Osswald et al 1978b; Spielman 1984), and salt and water excretion (Osswald et al 1978; Spielman 1984; Churchill 1982; Coulson and Scheinman 1985; Osswald 1975). While these actions of adenosine have been well documented, the mechanism(s) by which adenosine produces these effects in the kidney remains unclear. A primary impediment to the study of the cellular actions of adenosine in the kidney is the heterogeneous nature of renal tissue. With the advent of the technique of immunodissection, used originally by Wysocki and Sato (1978) for selective isolation of lymphocytes and modified by Garcia-Perez and Smith (1983) for use with renal tissue, large populations of homogeneous cells can be isolated and cultured for study. In the present study, rabbit cortical collecting tubule (RCCT) cells were isolated as described by Spielman et al (1986) for use in investigating the cellular mechanism of action of adenosine on renal collecting tubule epithelium.

Previous work on adenosine receptors and adenosine post-receptor mechanisms in other tissues has indicated that there are at least two types of adenosine receptors (reviewed in Daly 1982), both of which interact with the cAMP second messenger system. Several previous reports have identified the presence of both inhibitory ($A_1$) and stimulatory ($A_2$) receptors in the kidney. Stimulatory receptors have been identified in bovine renal medulla (Birnbaumer et al 1974), in cultures of rat (Newman and Levitski 1983) and toad (Lang et al 1985) kidney cortex, and in isolated rat glomeruli (Abboud and Dousa 1983), while inhibitory receptors have been reported to be present in rat renal cortex (Coulson and Harrington 1979; McKenzie and Bar 1973).

The present study provides evidence that RCCT cell populations contain both $A_1$ and $A_2$ adenosine receptors. Accordingly CHA and PIA produce an inhibition of cAMP production, while NECA produces only slight inhibition. Moreover, the rank order of potency for stimulation (NECA>PIA>CHA) and inhibition (CHA>PIA>NECA) of cAMP production seen with RCCT cells is similar to those reported for other cell types (Londos et al 1978; Londos et al 1980; Daly et al 1981; Fain and Malbon 1979; Bruns 1980). In addition, pretreatment of the cells with

pertussis toxin completely eliminated the inhibitory action of the adenosine analogs on cAMP accumulation while enhancing the stimulatory action, suggesting the presence of both receptors. Particularly relevant is the observation that 30 uM CHA, which did not increase the cAMP content of untreated RCCT cells, caused a significant increase in cAMP accumulation in cells pre-treated with pertussis toxin. In this instance, pertussis toxin appears to have prevented $A_1$ receptor-mediated inhibition of cAMP production, thereby unmasking the stimulatory effect of CHA acting via the $A_2$ receptor.

Inhibition by methylxanthines of the effects on cAMP accumulation provides additional evidence that these receptors are extracellular, as opposed to the intracellular P site receptor for adenosine, which is not affected by methylxanthines. The finding of both $A_1$ and $A_2$ receptors in cultured RCCT cells that contain both principal and intercalated cell types (Spielman et al 1986) raises the question of whether both receptor types coexist on the same cell type or if the receptor types are partitioned between subpopulations of the cultured cells. Investigation of the distribution of adenosine receptors on RCCT cells awaits separation of cultured RCCT cells into homogeneous cultures of principal and intercalated cells.

RCCT cells responded to AVP treatment as reported earlier (Spielman et al 1986), with an increase in cAMP production. CHA caused a significant reduction in the stimulatory effect of AVP. This modulation by CHA of AVP-stimulated cAMP accumulation is in accord with a preliminary study by Berl and Teitlebaum (1985) in which they report inhibition of AVP-mediated increases in papillary cell cAMP concentrations by 0.5 uM adenosine. In RCCT cells, inhibition of AVP-stimulated cAMP accumulation was observed with all three adenosine analogs at stimulatory, as well as inhibitory concentrations. Attenuation by stimulatory concentrations of the analogs was apparently mediated by activation of the inhibitory adenosine receptor ($A_1$), since activation of $A_2$ receptors would be expected to add to the increase in cAMP production and the concentrations used (100 uM) were sufficient to activate both $A_1$ and $A_2$ receptors. There does not appear to be a specific interaction between adenosine and AVP since CHA also inhibited isoproterenol-induced stimulation of cAMP production. These results indicate that adenosine can influence tubular transport processes by acting alone or by modulating the actions of other hormones.

Prostaglandin $E_2$ inhibits AVP-induced increases in cAMP by canine collecting tubule cells (Garcia-Perez and

Smith 1984), and could therefore, provide a mechanism for the CHA-induced inhibition of the AVP response in RCCT cells. However, prostaglandin ($PGE_2$) production was unaffected by 100 uM CHA, suggesting that prostaglandins do not mediate the adenosine inhibition of hormone-induced cAMP accumulation.

Hormones that act through receptors to inhibit cAMP accumulation, typically activate the inhibitory GTP-binding protein of the adenylate cyclase system (Rodbell 1980). This protein, $N_i$, is a substrate for ADP-ribosylation by pertussis toxin (Katada et al 1984), and ADP-ribosylation prevents $N_i$ from exerting its inhibitory effect on adenylate cyclase. Pertussis toxin-pretreatment of RCCT cells completely abolished the action of CHA to inhibit basal as well as AVP-stimulated cAMP production. This suggests that $N_i$ is involved in the modulation of the AVP response by CHA. However, it has been reported recently that the increase in intracellular free calcium concentrations produced by some hormones through the breakdown of membrane phospholipids is also mediated through a pertussis toxin-sensitive N protein (Verghese et al 1986). Therefore, adenosine analogs may cause an increase in intracellular free calcium concentrations that subsequently result in inhibition of the increased cAMP

produced by AVP. An interaction of calcium with AVP-stimulated cAMP has previously been reported by Teitelbaum and Berl (1986), where inhibition of AVP-induced cAMP stimulation in rat papillary collecting tubule cells was produced by treatment with the calcium ionophore, A23187. That adenosine and adenosine analogs may act through intracellular calcium is suggested by the actions of adenosine to inhibit neurotransmitter release from nerve terminals and glucose oxidation in adipose tissue, both of which are thought to be mediated by increasing intracellular calcium concen-trations (Hedqvist et al 1978; Souness and Chagoya de Sanchez 1981).

The results of the present study provide evidence for stimulatory ($A_2$) and inhibitory ($A_1$) adenosine receptors on rabbit cortical collecting tubule cells and suggest a role for adenosine in the regulation of tubular function by a direct action on cAMP production, and by modulation of hormone-induced cAMP production.

## B. Adenosine signal transduction in the rabbit cortical collecting tubule: receptor-mediated calcium mobilization

### B.1. Introduction

Adenosine is a ubiquitous compound that, among other actions, causes vasodilatation and vasoconstriction,

inhibits neurotransmission, platelet aggregation, and lipolysis, and stimulates glucose oxidation. These actions have been reviewed extensively elsewhere (Arch and Newsholme 1978; Feigl 1983; Fox and Kelley 1978). In the kidney, elevation of adenosine levels results in changes in renal blood flow, an inhibition of renin release, and decreases in the glomerular filtration rate and the excretion of sodium and water (Osswald et al 1978; Osswald et al 1978a; Spielman 1984; Spielman and Thompson 1982; Spielman et al 1980; Tagawa and Vander 1970). The cellular mechanisms involved in the renal actions of adenosine have not been well characterized. In a variety of other cell types, extensive research has revealed the existence of two extracellular receptors for adenosine through which changes in adenylate cyclase activity, and cAMP production, are mediated (Daly 1982; Fain and Malbon 1979; Londos et al 1980). These receptors are denoted $A_1$ (or $R_i$) and $A_2$ (or $R_a$), for inhibition and stimulation of cAMP, respectively (Londos et al 1980), and are thought to be coupled to guanine nucleotide binding proteins ($N_i$ and $N_s$, respectively). Both of these receptor types have been demonstrated in the renal cortex and medulla in a variety of species (Birnbaumer et al 1974; Coulson and Scheinman 1985; McKenzie and Bar 1973; Newman and Levitski 1983;

Woodcock et al 1984). A recent report from this laboratory has demonstrated the presence of $A_1$- and $A_2$-like effects of adenosine analogs in cultures of rabbit cortical collecting tubule (RCCT) cells (RESULTS, IV.A.).

Several of the actions of adenosine, namely inhibition of neurotransmitter release (Hedqvist and Fredholm 1976) and stimulation of glucose oxidation (Souness and Chagoya de Sanchez 1981), do not appear to be mediated through the adenylate cyclase-cAMP system. It has been suggested that these actions involve calcium. Therefore, the present study was designed to test the hypothesis that adenosine can modulate tubular function through changes in intracellular free calcium. Adenosine analogs were used to determine the effect of adenosine on intracellular free calcium. The sensitivity of this response to xanthines and pertussis toxin was studied, and the source of the elevation in intracellular free calcium was determined.

B.2. Methods

Immunodissection. RCCT cells were obtained as described previously (Spielman et al 1986). Briefly, polystyrene culture dishes were coated with 0.2 mg of a cell-specific mouse monoclonal antibody, $IgG_3$(rct-30), which binds exclusively to collecting tubule cells when assayed by indirect immunofluorescence on rabbit kidney

cryotome sections (Spielman et al 1986). After 1-2 hours at 24 C, unbound antibody was discarded and the plates treated with a 1% bovine serum albumin solution to saturate the exposed polystyrene sites.

Collagenase-dispersed cells from rabbit kidney cortex were overlaid on the antibody-coated plates for less than 3 min. The plates were then washed 3-5 times with buffered saline (PBS, composition in mM: NaCl, 138; $KH_2PO_4$, 1.5; KCl, 3; $Na_2HPO_4$, 8.1; pH 7.4). To the remaining bound cells was added 10 ml of a Dulbecco's Modified Eagle's media containing 10% decomplemented fetal bovine serum, 2 mM glutamine, 1 uM dexamethasone, and 50 ug/ml each of penicillin and streptomycin. Cells were incubated at 37 C in 7% $CO_2$ and grown to confluency in 3-5 days. When first-passage cells were used, cells were detached from the culture dishes by treatment with trypsin (0.1%, with 0.05% EDTA in PBS, pH 7.4). For experiments involving subsequent measurement of intracellular calcium, cells were transferred, after detachment, to 100 mm culture dishes and grown for 3-5 additional days; for experiments involving measurement of cAMP accumulation, cells were transferred to 24-well dishes and grown for 3-5 days.

Measurement of intracellular free calcium. Intracellular free calcium concentration in RCCT cells was

measured as described by Grynkiewicz et al (1985) using the fluorescent calcium chelator, fura-2. Suspensions of RCCT cells (ca. $10^7$ cells/ml) were treated with the membrane permeable acetoxymethyl ester of fura-2 (fura-2/AM, final concentration: 4 uM) for 30 min at 37 C in a shaking water bath. This procedure allows endogenous esterases to cleave the fura-2/AM molecule to produce the membrane impermeant fura-2 molecule. The cells were centrifuged at 1500 rpm, 4°C for 6-8 min, and washed twice with simplified saline solution (SSS, composition in mM: NaCl, 145; KCl, 5; $Na_2HPO_4$, 1; $CaCl_2$, 1; $MgCl_2$, 0.5; glucose, 5; HEPES, 10; pH 7.4) to remove any extracellular fura-2/AM. To measure fluorescence of the trapped fura-2, cells were diluted 1:100 with SSS at 37 C. Fluorescence was measured with an Aminco spectrofluorimeter. One milliliter samples (ca. $10^5$ cells/ml) were contained in a temperature-controlled 1 cm square quartz cuvette continuously stirred. Monochromator settings were: excitation, 342 and 385 nm with a 5 nm slit; emission, 510 nm with an 11 nm slit. Fura-2 and the fura-2/calcium complex have fluorescence maximums at different excitation wavelengths, with the free dye fluorescing at 385 nm and the calcium-bound form of the dye fluorescing at 342 nm. Therefore, increases in intracellular calcium are reflected as an increased signal

from the complexed form of the dye (342 nm) and a decreased signal from the free dye (385 nm). To estimate intracellular free calcium, the ratio (R) of fluorescence at 342 and 385 nm was used in the following equation:

$$[Ca]_i = K_d \times (R-Ro/Rs-R) \times (Fo/Fs)_{385},$$

where Ro is the ratio with zero calcium (EGTA added) and Rs is the ratio with saturating calcium (digitonin added). $[Ca]_i$ is the cytosolic free calcium concentration in nmol/l. $K_d$ is the dissociation constant for the fura-2-calcium complex, Fo is the fluorescence at 385 nm with zero calcium and Fs is the fluorescence at 385 nm with saturating calcium concentrations.

Measurement of total cellular cAMP accumulation. Treatments were done in triplicate using first passage cells grown for 4 days in 24-well dishes. Culture medium was removed and the cells were washed once with Krebs buffer (in mM: NaCl, 118; $NaHCO_3$, 25; glucose, 14; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.8; and $KH_2PO_4$, 1.8; pH 7.4). The cells were pretreated for 1 hr with 0.1 mM RO 20-1724, a phosphodiesterase inhibitor which is not an adenosine receptor antagonist, and 1.5 units adenosine deaminase/ml Krebs at 37 C. After pretreatment, the buffer was aspirated and the cells were treated with the same buffer containing the various hormones and effectors at 37 C.

Treatment was terminated by adding an equal volume of 8% (w/v) trichloroacetic acid. The cells were frozen, thawed, and allowed to stand at 4 C for 60 min. The samples were extracted three times with 5 volumes of water-saturated ether, and the residual ether was evaporated and the samples dried under vacuum with vortexing. The samples were reconstituted with distilled water and total cAMP was determined by radioimmunoassay as described by Frandsen and Krishna (1976).

Determination of cellular protein content. Following removal of the media for cAMP assay, the remaining liquid was aspirated and the cells were solubilized with a 1% sodium dodecyl sulfate solution. After 24 hr at 24 C, samples were assayed for protein using a modification of the Lowry method (Markwell et al 1978).

Statistical analysis. Data were analyzed for statistical significance using two-way analysis of variance with the multiple comparisons test of Student-Newman-Kuels'. Significance is defined as a p value of less than 0.05.

Materials. Trypsin (1:250), Dulbecco's modified Eagle's medium, collagenase, fetal bovine serum, and bovine serum albumin were purchased from Gibco Laboratories, Grand Island, NY. Fura-2 was purchased from Molecular Probes,

Junction City, OR. The adenosine analogs, 5'-N-ethylcarboxamideadenosine (NECA), $N^6$-cyclohexyladenosine (CHA), and R-$N^6$-phenylisopropyladenosine (PIA), and 1,3-diethyl-8-phenylxanthine (DPX) were a gift from Warner Lambert, Milford, CN. 8-cyclopentyl-1,3-dipropyl xanthine (C-101) was purchased from Research Biochemicals, Inc., Wayland, MA. The phosphodiesterase inhibitor, RO 20-1724, was a gift from Hoffmann-LaRoche, Nutley, NJ. Arginine vasopressin (AVP) was from Peninsula Laboratories, Inc., Belmont, CA. Pertussis and cholera toxins were from List Biological Laboratories, Inc., Campbell, CA. $^{125}$I adenosine 3'5'cyclicmonophosphoric acid was from ICN Biomedicals, Inc., Irvine, CA. Other chemicals of reagent grade or better were obtained from standard sources.

B.3. Results

The basal intracellular free calcium concentration in primary cultures of rabbit cortical collecting tubule (RCCT) cells was 165 $\pm$ 8 nM (mean $\pm$ SEM, n=67), and in first-passage RCCT cells was 342 $\pm$ 20 nM (n=64). Subsequent experiments were performed on passaged RCCT cells unless otherwise noted, to be comparable to the experiments involving cAMP production, which were performed on first-passage cells. These values for intracellular calcium concentration are only estimates of the true

cytosolic free calcium concentration in these cells since there are many shortcomings in any technique to measure intracellular ion concentrations. Because we have not corrected our results for dye leakage or sequestration, we are presenting the majority of the data as percent change from control.

The addition of the adenosine analogs NECA, CHA, and PIA, as well as native adenosine resulted in a dose-dependent, transient increase in the intracellular free calcium concentration in both primary (Figure 14A) and first-passage cells (Figure 14B). The increase occurred within 10 sec of the addition of analog and intracellular calcium returned to the control level by 2 min after analog addition (Figure 15). The ratio for the calculation of calcium in all experiments was taken at the peak of the response at 342 nm and immediately following this at 385 nm. The relative responses by the two groups of cells were equal, despite the higher absolute basal calcium concentration in the passaged cells. Reproducible changes in intracellular free calcium were observed at adenosine concentrations ranging from 10 nM to 100 uM, with an $EC_{50}$ of approximately 0.5 uM. There was no difference in the potency of the adenosine analogs to increase intracellular calcium levels.

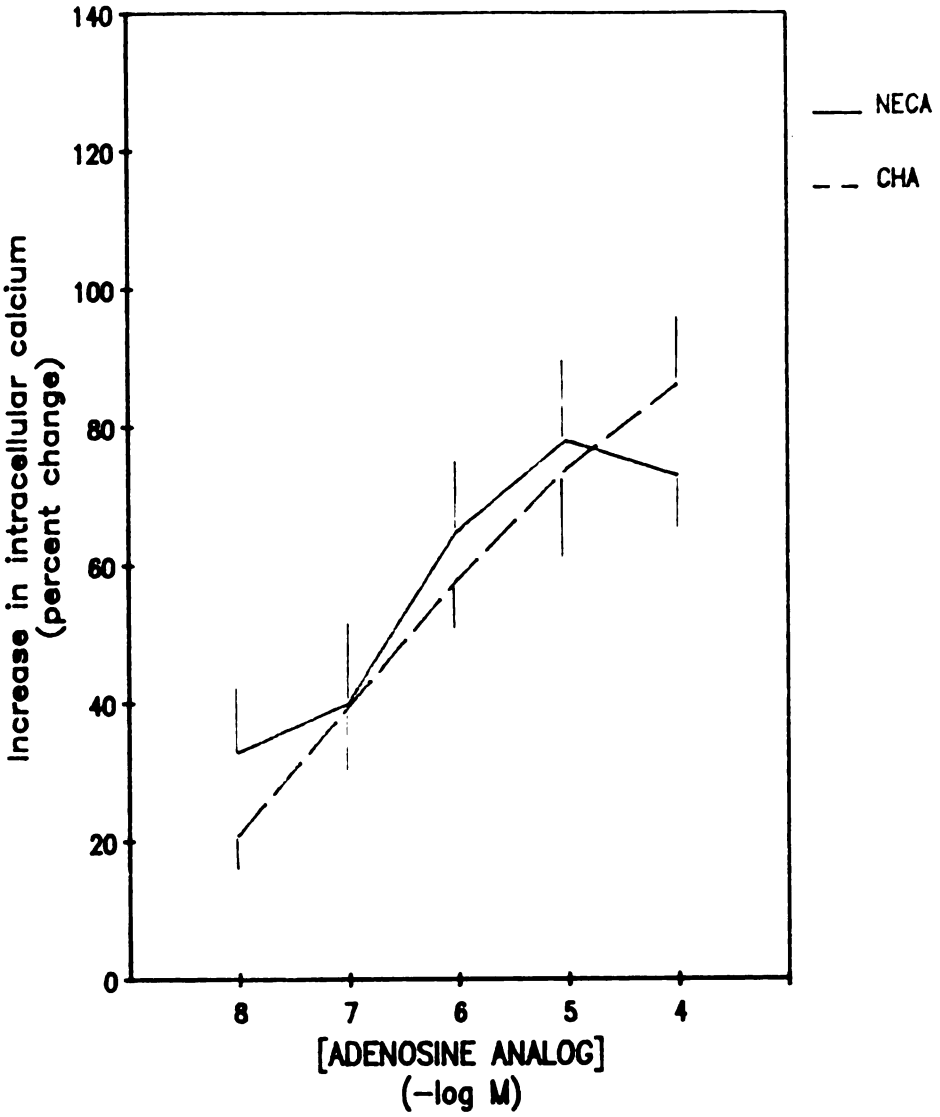


Figure 14A. Effect of adenosine analogs on intracellular free calcium in primary RCCT cells, expressed as percent change from control.

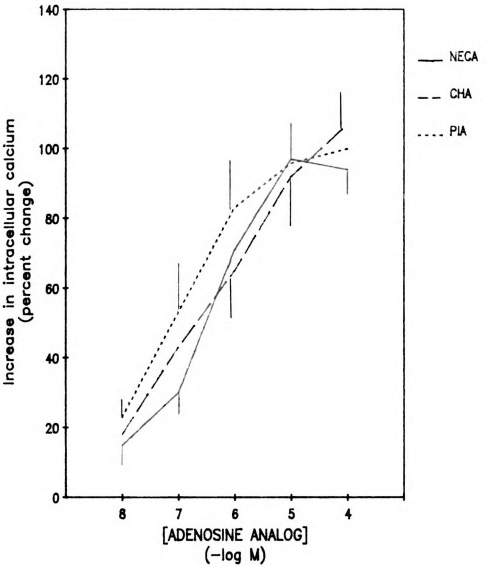


Figure 14B. Effect of adenosine analogs on intracellular free calcium in first-passage RCCT cells, expressed as percent change from control.

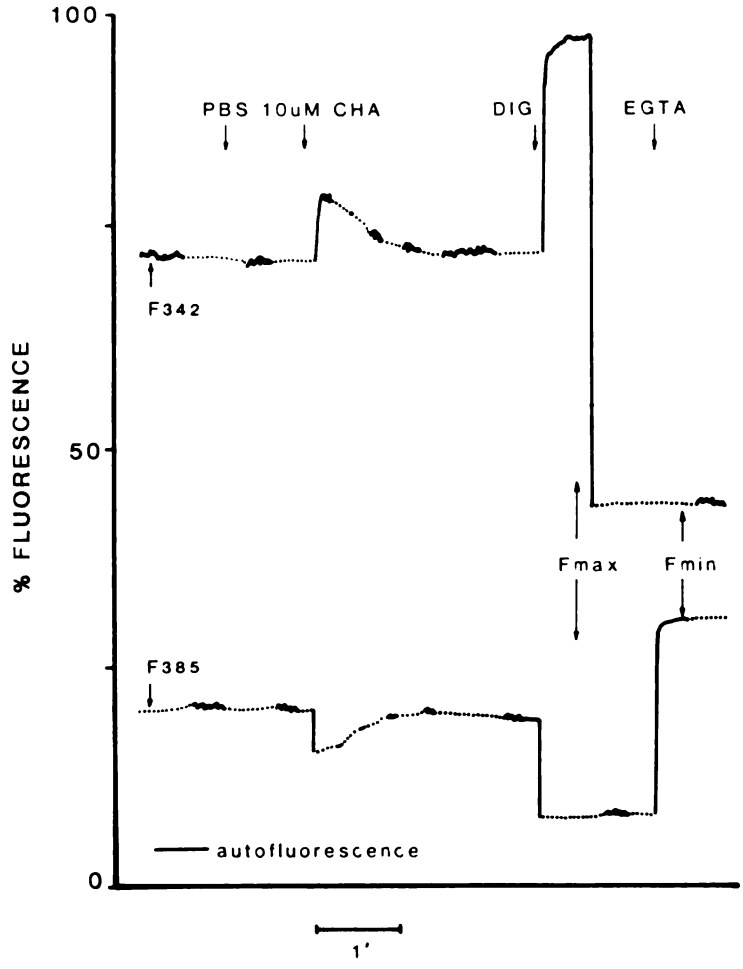


Figure 15. Representative fluorescence tracing of response of intracellular calcium to adenosine analogs. Ordinate, relative fluorescence. Abcissa, time; bar equals 1 min. Upper tracing is fluorescence at excitation wavelength of 342 nm, lower tracing is at wavelength of 385 nm. Emission wavelength was 510 nm. Digitonin (DIG) was added to a final concentration of 50 uM to determine the fluorescence maximum (Fmax) and EGTA was added to a final concentration of 3 mM to obtain the fluorescence minimum (Fmin). Autofluoresence was the fluorescence of an equal number of RCCT cells not treated with fura-2.

The effects of adenosine analogs on the intracellular free calcium concentration were antagonized by the adenosine receptor antagonists, 1,3-diethyl- 8-phenyl-xanthine (DPX, Figure 16), and the $A_1$-selective antagonist, 8-cyclopentyl-1,3-dipropylxanthine (C-101, Figure 17). The $IC_{50}$ for DPX was approximately 5 uM at adenosine analog concentrations of 1 uM, while C-101 was much more effective at inhibiting the increase in calcium, with an $IC_{50}$ of approximately 10 nM for adenosine concentrations of 1 uM. This xanthine compound (C-101) has previously been reported in rat brain membranes to antagonize $^3H$ CHA binding with an $IC_{50}$ of 0.8 nM, and $^3H$ NECA binding with an $IC_{50}$ of 500 nM, suggesting a much greater specificity of the antagonist for the high affinity $A_1$ adenosine receptor. Comparable to its reported effects on binding of adenosine analogs in brain membranes, in RCCT cells, C-101 antagonizes the inhibition of basal cAMP production by CHA (Table 8), as well as the inhibitory action of CHA on hormone-stimulated cAMP production (Table 8), but does not affect the increase in cAMP production by NECA (Table 8). The $IC_{50}$ for blocking the CHA-induced inhibition of cAMP was approximately 10 nM, similar to that for blocking the calcium response.

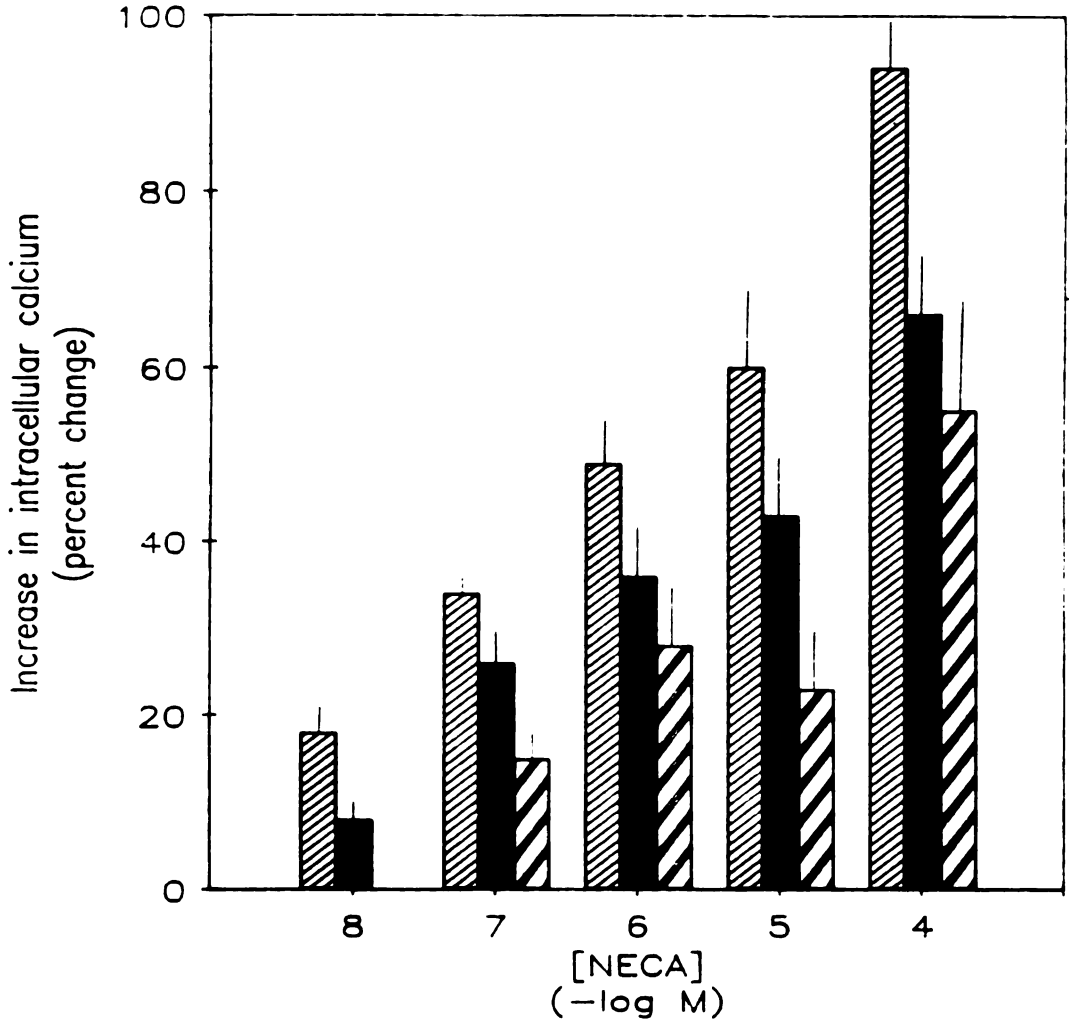


**Figure 16.** **Effect of DPX on the response to NECA. RCCT cells were treated with NECA alone (single hatch), or with concomitant addition of NECA and either 1 uM DPX (solid) or 5 uM DPX (double hatch).**

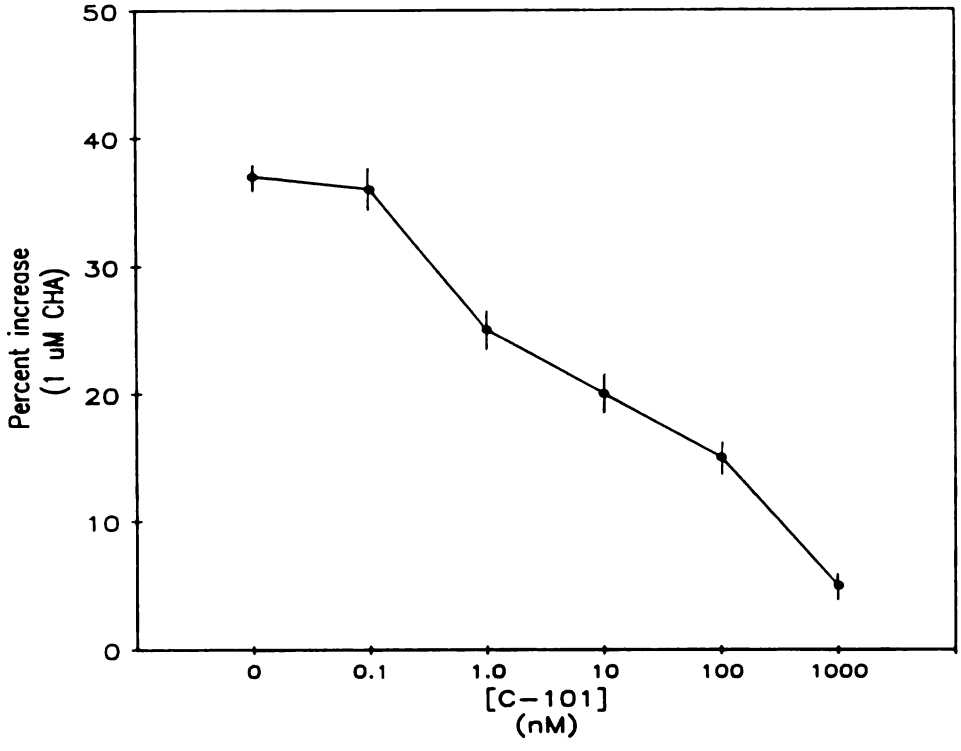


Figure 17. Effect of C-101 on the response of intracellular calcium to CHA. RCCT cells were treated with 1 uM CHA in the presence of varying concentrations of C-101. The increase in intracellular calcium in response to 1 uM CHA in the absence of C-101 was approximately 36%.

Table 8. Effect of C-101 on stimulation and inhibition of cAMP production by adenosine analogs in RCCT cells.

| | cAMP production (pmol/mg cell protein) | | | | |
|---|---|---|---|---|---|
| treatment | C-101 concentration (nM) | | | | |
| | none | 1 | 10 | 100 | 1000 |
| none | 63±10 | 62±14 | 71±11 | 79±14 | 103±12 |
| CHA 50 nM | 23± 6* | 14± 4* | 34±14 | 96± 7 | 144±11 |
| AVP 1 uM | 322±21* | 272±14* | 347±35* | 378±46* | 397± 5* |
| CHA + AVP | 198±25*# | 103±15# | 276±39* | 369±53* | 427± 6* |
| NECA 10 uM | 122±17* | ND | 140±38* | 154±21* | 226±18* |

First passage RCCT cells were incubated for 1 h with RO 20-1724 and adenosine deaminase as described in Methods. Cells were then treated for 20-30 min with either Krebs (none), adenosine analog (CHA or NECA), or vasopressin (AVP), either alone or in the presence of varying concentrations of the adenosine receptor antagonist, C-101. All incubations were performed at 37 C under a 7% $CO_2$ atmosphere. cAMP production was determined by RIA. ND, not determined.

*, P< 0.05 when compared to respective sample without treatment

#, P< 0.05 when compared to respective sample with AVP

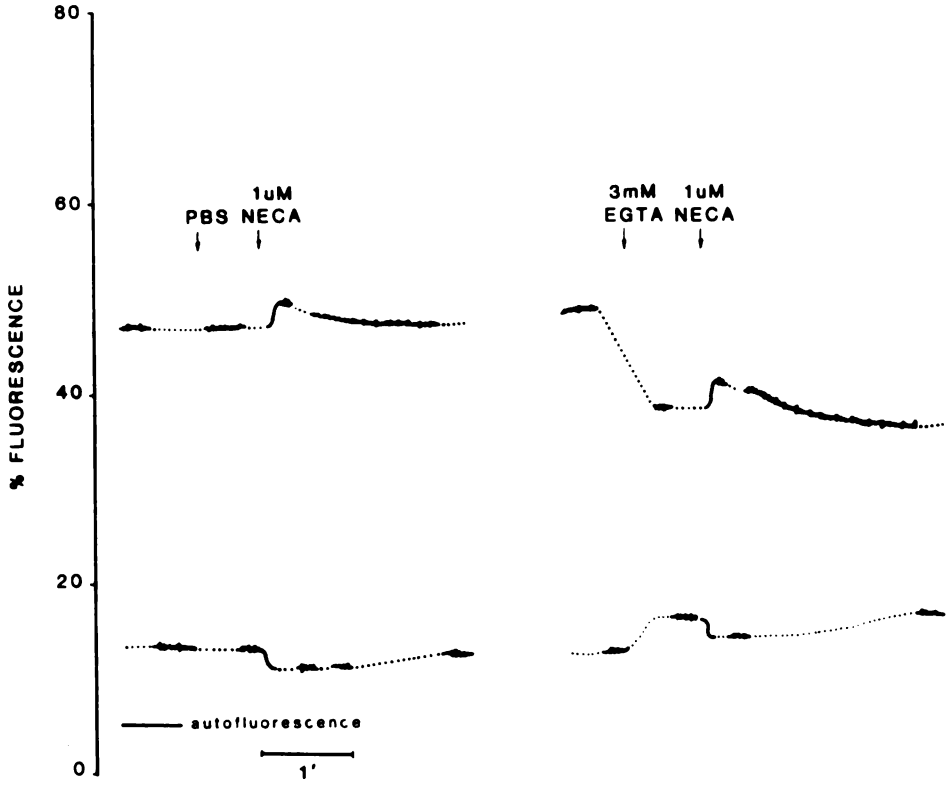


Figure 18. The response of intracellular calcium to NECA in the presence and absence of extracellular calcium. See legend to Figure 2 for explanation of tracing. Left half, response to 1 uM NECA in the presence of 1 mM extracellular calcium. Right half, response to NECA in the absence of extracellular calcium.

The source of the elevation of intracellular free calcium in RCCT cells was determined by chelation of extracellular calcium. EGTA was added to the cell suspension at a final concentration of 3 mM (media calcium concentration 1-1.5 mM), and a control fluorescence ratio was obtained immediately. Within 30 sec of the addition of EGTA the effectors were added and the experimental fluorescence ratio was obtained (Figure 18). Addition of EGTA always resulted in a lowering of the measured control calcium concentration by one-third to one-half. Despite a change in baseline calcium concentration, there was no effect of chelation of extracellular calcium on the relative increase in intracellular calcium in response to adenosine. When adenosine was added after 2-3 min of EGTA treatment, the increase in calcium was reduced by 50% or greater, suggesting an effect of EGTA on intracellular calcium stores when the cells were exposed to EGTA for an extended period of time.

It has been reported by Nakamura and Ui (1985) that treatment of mast cells with pertussis toxin inhibits $^{45}Ca$ influx and membrane phospholipid breakdown induced by the mast cell receptor agonist, compound 48/80. They suggest that a guanine nucleotide regulatory protein (N protein) may be involved in calcium-mediated cellular responses. In

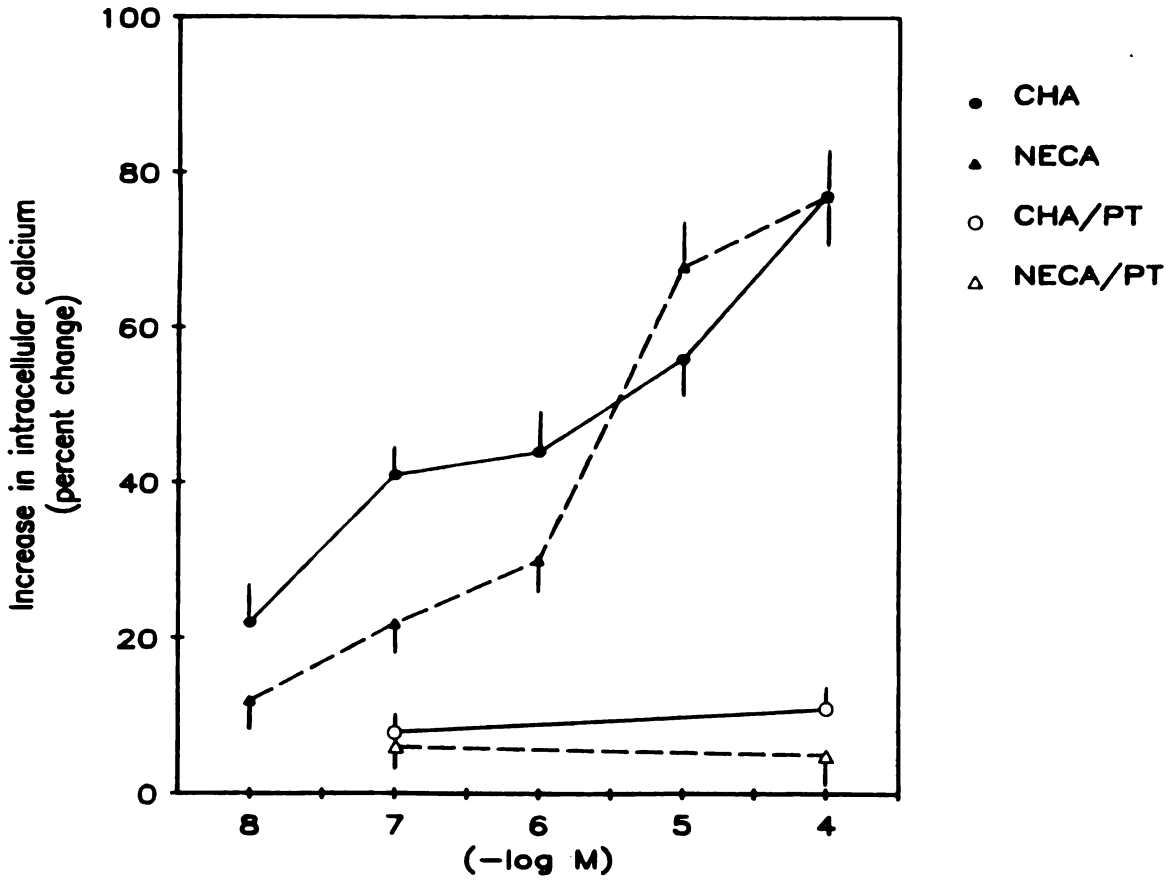


Figure 19. Effect of pertussis toxin on the responses to the adenosine analogs, CHA and NECA. Filled symbols, no pertussis toxin; open symbols, RCCT cells were pretreated for 12 h with 1 ug pertussis toxin/ml before measuring calcium.

addition, the chemotaxis response of phagocytes, which is believed to involve calcium, is inhibited by both pertussis toxin and cholera toxin (Verghese et al 1986). Therefore, the effects of these toxins on the calcium response of RCCT cells to adenosine were studied. Pretreatment for 12 hr with 1 ug/ml pertussis toxin completely blocked the increase in intracellular free calcium by the adenosine analogs NECA and CHA (Figure 19). There was no effect of 12 hr pretreatment of RCCT cells with 5 ug/ml cholera toxin on the increase in calcium in response to adenosine.

B.4. Discussion

Elevation of the intrarenal levels of adenosine results in many changes in renal function, including changes in hemodynamics, renin release, and a decrease in the excretion of solute and water (Osswald et al 1978; Osswald et al 1978a; Spielman 1984; Spielman and Thompson 1982; Spielman et al 1980; Tagawa and Vander 1970). Because of the cellular heterogeneity of the kidney and the close interaction of these aspects of renal function, it has been difficult to determine which cell types of the kidney are directly affected by adenosine and what intracellular events are responsible for transducing the actions of adenosine.

It has been demonstrated in many cell types (Daly 1982; Fain and Malbon 1979; Londos et al 1980), including renal cells (Birnbaumer et al 1974; Coulson and Scheinman 1985; McKenzie and Bar 1973; Newman and Levitski 1983; Woodcock et al 1984) that adenosine can increase and decrease the production of cAMP by altering the activity of adenylate cyclase. These studies suggest a role for cAMP as an intracellular mediator of the actions of adenosine.

A change in intracellular calcium has also been suggested to be involved in some of the actions of adenosine (Hedqvist and Fredholm 1976; Souness and Chagoya de Sanchez 1981) but an increase in calcium by adenosine has been directly demonstrated only in hepatocytes (Sistare et al 1985). A specific effect of adenosine to increase intracellular calcium in renal cells has not been demonstrated.

In the present study, we have used the fluorescent calcium indicator, fura-2, to measure intracellular free calcium concentrations in RCCT cells, and to determine what effects, if any, occur with the addition of adenosine to these cells. Adenosine, and adenosine analogs that are relatively specific for $A_1$ or $A_2$ adenosine receptors, produced dose-dependent, transient

increases in intracellular free calcium. Primary and first-passage RCCT cells responded equally to adenosine.

An increase in calcium may occur by mobilization of calcium from intracellular stores, or by stimulation of an influx of extracellular calcium. By adding EGTA to the cell suspension, extracellular calcium is removed as a possible source. Chelation of extracellular calcium did not affect the adenosine-induced increase in intracellular calcium, though the response could be significantly reduced by prolonged exposure to an extracellular medium that was calcium-free. These results suggests that the calcium is mobilized from an intracellular source. Further studies are necessary to determine the exact mechanism responsible.

In contrast to their effects on cAMP production, with NECA being more potent at eliciting an increase and CHA being more potent at the inhibitory receptor, the adenosine analogs were all equally potent at inducing an increase in intracellular calcium. This may represent an action of adenosine which is not mediated through an extracellular receptor, perhaps by changing metabolism, or by interacting with the intracellular adenosine receptor (P site). That the action of adenosine to increase intracellular calcium is mediated via an extracellular receptor is supported by the results that xanthines antagonize the effect. Xanthines

are adenosine receptor antagonists that do not alter the inhibitory effect of adenosine on cAMP mediated through the intracellular P site (Fain et al 1978).

To determine the involvement of guanine nucleotide regulatory proteins (N proteins) in the increase in calcium, RCCT cells were treated with pertussis toxin and cholera toxin. Pertussis toxin ADP-ribosylates and inactivates the inhibitory guanine nucleotide binding protein, $N_i$ (28). This protein is normally thought to be associated with receptors that, when activated, result in inhibition of cAMP production. Recently, a 40 to 41 kD protein that is both pertussis toxin and cholera toxin sensitive has been identified in leukocytes (Verghese et al 1986) and appears to be involved in calcium-mediated chemotactic responses. This protein appears to be distinct from $N_i$ and has been termed $N_c$ (Verghese et al 1986). In the present study, the action of adenosine to mobilize intracellular calcium was inhibited by pertussis toxin and not by cholera toxin, indicating coupling to an $N_i$-like protein.

The receptor-mediated mobilization of calcium showed no preference for the various adenosine analogs, in contrast to the analog selectivity for inhibition or stimulation of cAMP production, and raises the possibility

of calcium-mobilization by a previously unidentified adenosine receptor. On the other hand, our findings that a highly specific $A_1$ receptor antagonist blocks both the inhibition of cAMP production and the increase in calcium, and that both actions are pertussis toxin-sensitive seem to indicate that the same receptor, $A_1$, via coupling to $N_i$, may be responsible for both inhibition of cAMP production and calcium mobilization. The results of the present study do not provide sufficient evidence for acceptance of either of these receptor schemes for adenosine-mediated calcium mobilization.

These studies demonstrate an adenosine receptor in RCCT cells, which is responsible for mobilization of calcium from intracellular stores and is coupled to a guanine nucleotide regulatory protein. Further studies are necessary to determine what extracellular adenosine receptor mediates the changes in calcium and what role this increase in intracellular calcium has in the renal actions of adenosine.

## V. SUMMARY AND CONCLUSIONS

The studies described in this dissertation provide evidence that:

1. the elevation of intrarenal adenosine levels, by blocking its cellular uptake, or by pharmacological or pathophysiological maneuvers, leads to pronounced changes in renal function (III.A., III.B., III.C.)
2. adenosine signal transduction mediating these changes in renal function may occur through changes in the intracellular levels of cyclic AMP and/or calcium (IV.A., IV.B.)
3. an interaction between adenosine and other hormones in the kidney may be responsible for some of the renal actions of adenosine (IV.A.).

The elevation of intrarenal adenosine levels by blocking its cellular uptake and degradation, as well as by pathophysiologic manuevers can lead to changes in renal function similar to that seen with the infusion of exogenous adenosine. Elevation of endogenous adenosine reduced renal blood flow, glomerular filtration rate, and renin release. These changes mimicked those produced by

adenosine infusion by exhibiting a sodium-dependency. That is, the changes in renal function are more pronounced in animals that have been maintained on a low-sodium diet, and are reduced in those fed normal or high-sodium diets. While these studies have not proven that adenosine acts as an intrinsic regulator of renal function under normal circumstances, they have shown that endogenously-produced adenosine is capable of altering renal function, and that adenosine may be responsible for some of the changes in renal function observed under certain pathophysiological circumstances.

The hypothesis that adenosine is involved in the regulation of renal function is supported by the results reported in the second part of this dissertation which provide evidence for adenosine receptors and post-receptor systems in renal epithelial cells. Rabbit cortical collecting tubule cells were shown to posess adenosine receptors coupled to the cAMP and calcium second messenger systems. A model for the cellular actions of adenosine via these systems is shown in Figure 20. Though the three proposed receptors are depicted as being present in one cell, the data presented in this dissertation do not allow a conclusion to be drawn regarding the presence of these receptors in specific cells of the collecting tubule.

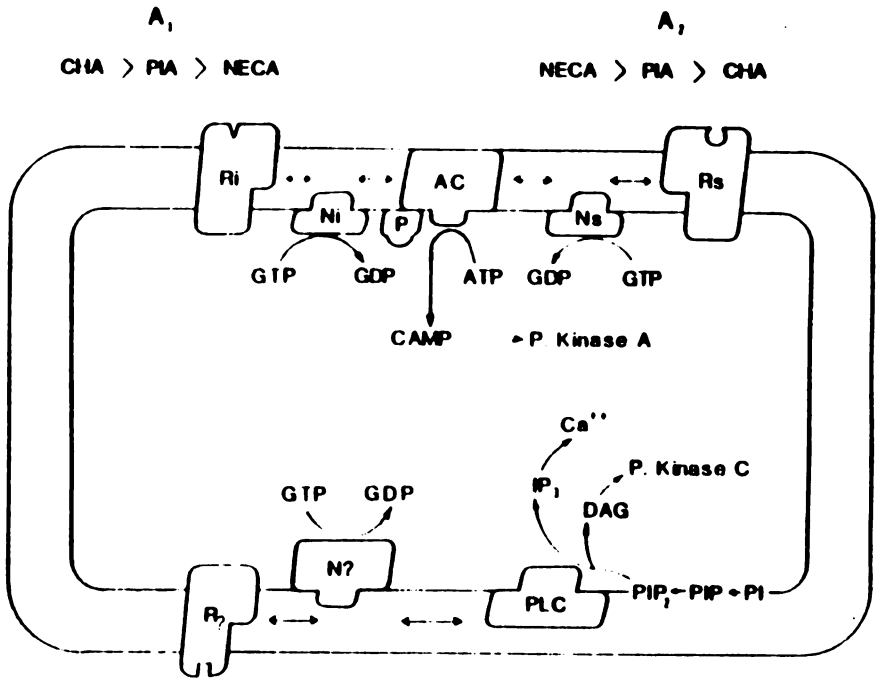


Figure 20. Model cell of adenosine receptor systems. Hypothetical design of adenosine receptor systems in RCCT cells. A,R and P: adenosine receptors; N: guanine nucleotide regulatory protein; AC: adenylate cyclase; PLC: phospholipase C; i: inhibitory; s: stimulatory; ?: unknown.

Evidence was presented that RCCT cells possess $A_1$ and $A_2$ adenosine receptors, based on the observation that adenosine analogs can inhibit and stimulate the accumulation of cAMP. Adenosine analogs were capable of increasing cAMP production at concentrations from $10^{-7}$ M to $10^{-4}$ M, and decreasing cAMP production at concentrations from $10^{-9}$ M to $10^{-7}$ M. The action of adenosine analogs to inhibit cAMP production was pertussis toxin-sensitive and this suggests the involvement of the inhibitory guanine nucleotide protein, $N_i$. A further action of adenosine analogs in these cells was mobilization of calcium from intracellular stores, at concentrations from $10^{-9}$ M to $10^{-4}$ M. This is the first report of an effect of adenosine on intracellular calcium in a renal cell, and one of the first reports of this action of adenosine in any cell type. This action of adenosine analogs was also found to be coupled to a guanine nucleotide binding protein similar to $N_i$ because treatment with pertussis toxin abolished the response.

These results suggest that changes in intracellular cAMP and/or calcium may mediate the renal actions of adenosine, though these studies can not exclude the involvement of other second messenger molecules such as cGMP in the actions of adenosine. These studies also demonstrate an interaction between the cellular actions of

adenosine and the actions of other hormones on the cAMP system. That is, vasopressin- and isoproterenol-stimulated cAMP production can be blunted by the presence of adenosine analogs. Therefore, adenosine may produce some of its actions on the kidney by modulating the effects of other intrarenal hormones.

Because the cellular actions of adenosine were studied in collecting tubule cells, a direct correlation cannot be drawn between these results and the actions of adenosine on other renal cell types such as the glomerular mesangial cells, the endothelial cells of the afferent and efferent arterioles, and the renin-secretory juxtaglomerular cells. However, the collecting tubule is an extremely hormone-sensitive segment of the nephron and can be used as a model for the study of adenosine's actions in other cell types. The results of these investigations can be used as a data-base for further studies in all of the different cells in the kidney. Furthermore, adenosine does have effects on excretion as shown in the first chapter of this dissertation as well as in several previous reports. These excretory changes may be mediated by the direct action of adenosine on the collecting tubule cells through one or more of the mechanisms described in this dissertation. The retaliatory metabolite hypothesis for adenosine, though

based on organ-level actions of adenosine, may have an analogy in the local actions of adenosine. That is, adenosine may act as a paracrine or autocrine hormone to regulate the energy utilization of neighboring cells. This action of adenosine may be important for modulatory control of transport processes initiated by other hormones acting on the collecting tubule. The analogy may be extended to include the regulation of energy-requiring processes in other cells such as glomerular mesangial and endothelial cells, or juxtaglomerular cells.

# APPENDIX A

## APPENDIX A

### Abbreviations and Definitions of Compounds Used

Adenosine deaminase (ADA): enzyme that catalyzes deamination of adenosne to inosine, used to remove endogenous adenosine from receptors for studying adenosine analogs.

Arginine vasopressin (AVP): antidiuretic hormone, known to increase cAMP production in the collecting tubule.

Cholera toxin: ADP-ribosylates and permanently activates stimulatory guanine nucleotide regulatory protein, $N_s$.

$N^6$-Cyclohexyladenosine (CHA): adenosine analog, greater potency at $A_1$ receptor.

8-Cyclopentyl-1,3-dipropylxanthine (C-101): adenosine receptor antagonist, greater potency at $A_1$ receptor.

Deoxycorticosterone pivalate: corticosteroid, promotes sodium retention, enhancing effects of high-sodium diet to produce sodium- and volume-expanded state.

1,3-Diethyl-8-phenylxnathine (DPX): adenosine receptor antagonist, similar potency for $A_1$ and $A_2$ receptors.

Dipyridamole: inhibitor of nucleoside carrier.

5'-N-Ethylcarboxamideadenosine (NECA): adenosine analog, greater potency at $A_2$ receptors.

Erytho-9-(2-hydroxy-3-nonyl)adenine (EHNA): inhibitor of adenosine deaminase, used in measurement of adenosine in blood and urine.

Furosemide, Lasix: diuretic, used to supplement and enhance low-sodium diet to obtain sodium- and volume-depleted state.

Lithium carbonate: marker of proximal tubule reabsorptive capacity.

Maleic acid: produces experimental model of Fanconi syndrome, generalized tubular transport dysfunction.

Meglumine/sodium diatrizoate-76% (Renografin 76): contrast agent used clinically for radiographic identification of blood vessels.

Pertussis toxin: ADP-ribosylates and inactivates inhibitory guanine nucleotide protein, $N_i$.

R-$N^6$-Phenylisopropyladenosine (PIA): adenosine analog, greater potency at $A_1$ receptor.

Theophylline: adenosine receptor antagonist,, similar action at $A_1$ and $A_2$ receptors, less potent than DPX.

RO 20-1724: cyclic nucleotide phosphodiesterase inhibitor that lacks adenosine receptor antagonist properties, used to allow increases in cAMP production to be detected.

# APPENDIX B

## APPENDIX B

**Derivation of the equation for calculation of intracellular calcium**

Basis: fluorescence of a compound is proportional to its concentration (calcium-bound fura-2 fluorescence is a function of the concentration of the bound dye)

Components:

concentrations of free and bound dye:
$c_f$ and $c_b$

proportionality coefficients:
$S_{f1}$, $S_{b1}$, $S_{f2}$, $S_{b2}$ where 1 = wavelength 1 (342)
2 = wavelength 2 (385)
f = free dye
b = bound dye

the proportionality coefficients are related to the extinction coefficient, excitation intensity, instrument efficiency, ln 10, and quantum efficiency.

S is measured from fluorescence intensities of solutions with known concentrations of free and calcium-bound dye, and the fluorescence intensities at wavelengths 1 and 2 are related to the proportionality coefficients and $c_f$ and $c_b$ by:

$$F_1 = S_{f1}c_f + S_{b1}c_b \qquad F_2 = S_{f2}c_f + S_{b2}c_b$$

$c_f$ and $c_b$ are related to the calcium concentration by:

$$c_b = c_f[Ca]/K_d, \text{ where } K_d = \text{the dissociation constant for fura-2/calcium}$$

the fluorescence ratio, R equals $F_1/F_2$, or:

$$R = (S_{f1}c_f + S_{b1}c_b)/(S_{f2}c_f + S_{b2}c_b)$$

replacing $c_b$ ($c_f$ cancels out):

$$R = (S_{f1} + S_{b1}[Ca]/K_d)/(S_{f2} + S_{b2}[Ca]/K_d)$$

and solving for [Ca]:

$$[Ca] = K_d \times [R-(S_{f1}/S_{f2})/(S_{f2}/S_{b2})-R](S_{f2}/S_{b2})$$

where $S_{f1}/S_{f2}$ = minimum limiting value for R with zero calcium, and $S_{b1}/S_{b2}$ = maximum limiting value for R with saturating calcium.

# LITERATURE CITED

## LITERATURE CITED


Abboud, H.E., and T.P. Dousa (1983) Action of adenosine on cyclic 3'5'- nucleotides in glomeruli. Am. J. Physiol. 244: F633-F638.

Al-Bander, H.A., R.A. Weiss, M.H. Humphreys, and R.C. Morris, Jr. (1982) Dysfunction of the proximal tubule underlies maleic acid-induced type II renal tubular acidosis. Am. J. Physiol. 243: F604-F611.

Ally, A.I., and K. Nakatsu (1976) Adenosine inhibition of isolated rat ileum and antagonism by theophylline. J. Pharmacol. Exp. Ther. 199: 208-219.

Angielski, S. (1963) The effect and metabolism of maleic acid in the kidney. Acta Biol. Med. Soc. Sci. Gedan. 7: 61-97.

Angielski, S., and J. Rogulski (1975) Metabolic studies in experimental renal dysfunction resulting from maleate administration. In S. Angielski and U.S. Dubach (eds): Biochemical aspects of kidney function. Huber: Bern, pp. 86-100.

Ansari, Z., and S.D. Baldwin (1976) Acute renal failure due to radio-contrast agents. Nephron 17: 28-40.

Arch, J.R.S., and E.A. Newsholme (1978) The control of the metabolism and the hormonal role of adenosine. Essays in Biochemistry 14: 82-123.

Arend, L.J., A. Haramati, C.I. Thompson, and W.S. Spielman (1984) Adenosine-induced decrease in renin release: dissociation from hemodynamic effects. Am. J. Physiol. 247: F447-F452.

Baer, H.P., G.I. Drummond, and E.L. Duncan (1966) Formation and deamination of adenosine by cardiac muscle enzymes. Mol. Pharmacol. 2: 67-76.

Bakris, G.L., and J.C. Burnett, Jr. (1985) A role for calcium in radiocontrast-induced reductions in renal hemodynamics. Kidney Int. 27: 465-468.

Baumbach, L., and P.P. Leyssac (1977) Studies on the mechanism of renin release from isolated superfused rat glomeruli: Effects of calcium, calcium ionophore, and lanthanum. J. Physiol. (London) 272: 745-764.

Bergeron, M., L. Dubord, C. Hausser (1976) Membrane permeability as a cause of transport defects in experimental Fanconi syndrome. J. Clin. Invest. 57: 1181-1189.

Berl, T., and I. Teitelbaum (1985) Control of prostaglandin synthesis by rat inner medullary collecting tubule cells: role of cAMP. Kidney Int. 27: 253. (Abstr.).

Berlin, R.D., and J.M. Oliver (1975) Membrane transport of purine and pyrimidine bases and nucleosides in animal cells. Int. Rev. Cytol. 42: 287-336.

Berliner, R.W., T.J. Kennedy, and J.G. Hilton (1950) Effect of maleic acid on renal funcion. Proc. Soc. Exp. Biol. Med. 75: 791-794.

Berne, R.M., and R. Rubio (1974) Adenine nucleotide metabolism in the heart. Circ. Res. 34-35: III109-III120.

Birnbaumer, L., T. Nakahara, and P.C. Yang (1974) Studies on receptor-mediated activation of adenylyl cyclases. J. Biol. Chem. 259: 7557-7566.

Blantz, R.C., D.W. Peterson, L.C. Gushwa, and B.J. Tucker (1975) Effect of modest hyperglycemia on tubuloglomerular feedback activity. Kidney Int. 22: S206-S212.

Blume, A.J., C. Dalton, and H. Sheppard (1973) Adenosine-mediated elevation of cyclic 3',5' adenosine monophosphate concentrations in cultured mouse neuroblastoma cells. J. Biol. Chem. 70: 3099-3102.

Brewer, E.D., H.O. Senekjian, A. Ince, and E.J. Weinman (1983) Maleic acid-induced reabsorptive dysfunction in the proximal and distal nephron. Am J. Physiol. 245: F339-F344.

Bruns, R.F. (1980) Adenosine receptor activation in human fibroblasts: nucleoside agonists and antagonists. Can. J. Physiol. Pharmacol. 58: 673-691.

Burnstock, G. (1972) Purinergic nerves. Pharm. Rev. 24: 509-581.

Burnstock, G. (1976) Do some nerve cells release more than one neurotransmitter? Neurosci. 1: 239-248.

Burnstock, G. (1978) A basis for distinguishing two types of purinergic receptor. In R.W. Straub and L. Bolis (eds): Cell membrane receptors for drugs and hormones: a multidisciplinary approach. New York: Raven Press, pp. 107-118.

Byrd, L., and R.L. Sherman (1979) Radiocontrast-induced acute renal failure: a clinical and pathophysiologic review. Medicine 58: 270-279.

Caldicott, W.J.H., N.K. Hollenberg, and H.L. Abrams (1970) Characteristics of response of renal vascular bed to contrast media. Evidence of vasoconstriction induced by the renin-angiotensin system. Invest. Radiol. 5: 539-547.

Capito, K., and C.J. Hedeskov (1976) Inosine-stimulated insulin release and metabolism of inosine in isolated mouse pancreatic islets. Biochem. J. 158: 335-340.

Cattell, W.R., I.K. Fry, A.G. Spencer, and P. Purkiss (1982) Excretion urography.I. Factors determining the excretion of Hypaque. British Journal of Radiology. 40: 561-571.

Chou, C.C., J.B. Hook, C.P. Hsieh, T.D. Burns, and J.M. Dabney (1971) Effects of radiopaque dyes on renal vascular resistance. J. Lab. Clin. Med. 78: 705-712.

Churchill, P.C. (1982) Renal effects of 2-chloroadenosine and their antagonism by aminophylline in anesthetized rats. J. Pharm. Exp. Ther. 222: 319-323.

Churchill, P.C., and M.C. Churchill (1985) $A_1$ and $A_2$ adenosine receptor activation inhibits and stimulates renin secretion of rat renal cortical slices. J. Pharm. Exp. Ther. 232: 589-594.

Churchill, P.C., and A.K. Bidani (1982) Hypothesis: Adenosine mediates hemodynamic changes in renal failure. Medical Hypotheses 8: 275-285.

Clark, R.B. and M.N. Seney (1976) Regulation of adenylate cyclase from cultured human cell lines by adenosine. J. Biol. Chem. 251:4239-4246.

Clark, R.B., R. Gross, J.F. Su, and J.P. Perkins (1974) Regulation of adenosine 3',5' monophosphate content in human astrocytoma cells by adenosine and the adenine nucleotides. J. Biol. Chem. 249: 5296-5303.
Conway, E.J., and R. Cooke (1939) The deaminases of adenosine and adenylic acid in blood and tissues. Biochem. J. 33: 479-492.

Coulson, R., and W.W. Harrington (1979) Renal metabolism of $N^6$,O2'-dibutyryl adenosine 3',5'-monophosphate. Am. J. Physiol. 237: F75-F84.

Coulson, R., and S.J. Scheinman (1985) Evidence for renal tubular adenosine receptors. Kidney Int. 27: 255.(Abstr.).

Daly, J.W. (1982) Adenosine receptors: targets for future drugs. J. Med. Chem. 25: 197-207.

Daly, J.W., R.F. Bruns, and S.H. Snyder (1981) Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. Life Sci. 28: 2083-2097.

Davidson, W.D., and M.A. Sackner (1963) Simplification of the anthrone method for the determination of inulin in clearance studies. J. Lab. Clin. Med. 62: 351-356.

Dillingham, M.A., and R.J. Anderson (1985) Purinergic regulation of basal and arginine vasopressin-stimulated hydraulic conductivity in rabbit cortical collecting tubule. J. Membr. Biol. 88: 277-281.

Dobbins, J.W., J.P. Laurenson, and J.N. Forrest (1984) Adenosine and adenosine analogs stimulate adenosine cyclic 3',5'-monophosphate-dependent chloride secretion in the mammalian ileum. J. Clin. Invest. 74: 929-935.

Dobson, J.G. (1978) Reduction by adenosine of the isoproterenol-induced increase in cyclic adenosine 3',5' monophosphate formation and glycogen phosphorylase activity in rat heart muscle. Circ. Res. 43: 785-792.

Dole, V.P. (1961) Effect of nucleic acid metabolites on lipolysis in adipose tissue. J. Biol. Chem. 236: 3125-3130.

Drury, A.N., and A. Szent-Gyorgyi (1929) The physiological activity of adenine compounds with special reference to their action upon mammalian heart. J. Physiol., Lond. 68: 213-237.

Eloranta, T.O. (1977) Tissue distribution of S-adenosyl methionine and S- adenosyl homocysteine in the rat. Biochem. J. 166: 521-529.

Ettienne, E.M., and J.C.S. Fray (1979) Influence of potassium, sodium, calcium, perfusion pressure, and isoprenaline on renin release induced by high concentrations of magnesium. J. Physiol. (London) 292: 373-380.

Fain, J.N. (1973) Inhibition of adenosine cyclic 3',5' monophosphate accumulation in fat cells by adenosine, $N^6$-(phenylisopropyl) adenosine, and related compounds. Mol. Pharmacol. 9: 595-604.

Fain, J.N., and C.C. Malbon (1979) Regulation of adenylate cyclase by adenosine. Mol. and Cell. Biochem. 25: 143-169.

Fain, J.N., R.H. Pointer, and W.F. Ward (1972) Effects of adenosine nucleosides on adenylate cyclase, phosphodiesterase, cyclic adenosine monophosphate accumulation, and lipolysis in fat cells. J. Biol. Chem. 247: 6866-6872.

Fain, J.N., R.E. Shepherd, C.C. Malbon, and F.J. Moreno (1978). In J.N. Dietschy (ed): Disturbances in lipid and lipoprotein metabolism. Washington, DC: Am. Physiol. Society, pp. 213-228.

Feigl, E.D. (1983) Coronary physiology. Physiol. Rev. 63: 1-205.

Feldman, J.M., and T.B. Jackson (1974) Specificity of nucleotide-induced insulin secretion. Endocrinology 94: 388-394.

Forrest, J.N., G. Kelly, D. Gifford, E. Poeschla, S. Sander, and H. Osswald (1984) Characteristics of adenosine receptors mediating chloride transport. Clin. Res. 32: 532A (Abstr.).

Forrest, J.N., E. Poeschla, and D. Rieck (1982) Ribose-specific adenosine receptors mediate cyclic AMP accumulation and chloride transport in the shark rectal gland. Kidney Int. 21: 253.(Abstr.).

Forrest, J.N., D. Rieck, and A. Murdaugh (1980) Evidence for a ribose-specific adenosine receptor ($R_a$) mediating stimulation of chloride secretion in the rectal gland of Squalus acanthias. Bull. Mt. Desert Isl. Biol. Lab. 20: 152-155.

Fox, I.H., and W.N. Kelley (1978) The role of adenosine and 2'-deoxyadenosine in mammalian cells. Ann. Rev. Biochem. 47: 655-686.

Fox, I.H., and L. Kurpis (1983). Binding characteristics of an adenosine receptor in human placenta. J. Biol. Chem. 258: 6952-6955.

Frandsen, E.K., and G. Krishna (1976) A simple ultrasensitive method for the assay of cyclic AMP and cyclic GMP in tissues. Life Sci. 18: 529-541.

Fray, J.C.S. (1977) Stimulation of renin release in perfused kidney by low calcium and high magnesium. Am. J. Physiol. 232: F377-F382.

Fray, J.C.S. (1980) Stimulus-secretion coupling of renin. Role of hemodynamic and other factors. Circ. Res. 47: 485-492.

Fray, J.C.S., and C.S. Park (1979) Influence of potassium, sodium, perfusion pressure, and isoprenaline on renin release induced by calcium deprivation. J. Physiol. (London) 292: 363-372.

Fynn, M., N. Onomakpome, and W.S. Peart (1977) The effects of ionophores (A23187 and RO2-2985) on renin secretion and renal vasoconstriction. Proc. R. Soc. Lond. [Biol] 199: 199-212.

Garcia-Perez, A., and W.L. Smith (1983) Use of monoclonal antibodies to isolate cortical collecting tubule cells: AVP induces PGE release. Am. J. Physiol. 244: C211-C220.

Garcia-Perez, A., and W.L. Smith (1984) Apical-basolateral membrane asymmetry in canine cortical collecting tubule cells: bradykinin, AVP, PGE2 interrelationships. J. Clin. Invest. 74: 63-74.

Gerber, K.H., C.B. Higgins, Y.S. Yuh, and J.A. Koziol (1982) Regional myocardial hemodynamics and metabolic effects of ionic and nonionic contrast media in normal and ischemic states. Circulation 65: 1307-1314.

Gerkens, J.F., H.T. Heidemann, E.K. Jackson, and R.A. Branch (1983) Aminophylline inhibits renal vasoconstriction produced by intrarenal hypertonic saline. J. Pharm. Exper. Therap. 225: 611-615.

Gmaj, P., A. Hoppe, S. Angielski, and J. Rogulski (1973) Effects of maleate and arsenite on renal reabsorption of sodium and bicarbonate. Am. J. Physiol. 225: 90-04.

Gougoux, A., G. Lemieux, and N. Lavoie (1976) Maleate-induced bicarbonaturia in the dog: a carbonic anhydrase-independent effect. Am. J. Physiol. 231: 1010-1017.

Grasl, M., and K. Turnheim (1984) Stimulation of electrolyte secretion in rabbit colon by adenosine. J. Physiol. 346: 93-110.

Grenier, F.C., T.E. Rollins, and W.L. Smith (1981) Kinin-induced prostaglandin synthesis by renal papillary collecting tubule cells in culture. Am. J. Physiol. 241: F94-F104.

Grynkiewicz, G., M. Poenie, and R.Y. Tsien (1985) A new generation of calcium indicators with greatly improved fluorescence properties. J. Biol. Chem. 260: 3440-3450.

Gunther, R., S. Silbernegl, and P. Deetjen (1979) Maleic acid-induced aminoaciduria, studied by free flow micropuncture and continuous microperfusion. Pfluegers Arch. 382: 109-114.

Guyton, A.C., J.B. Langston, and L.G. Navar (1964) Theory for renal autoregulation by feedback of juxtaglomerular apparatus. Circ. Res. 15: I187-I197.

Haddy, F.J., and J.B. Scott (1968) Metabolically linked vasoactive chemicals in local regulation of blood flow. Physiol. Rev. 48: 688-707.

Hall, J.E., and J.P. Granger (1986) Adenosine alters glomerular filtration control by angiotensin II. Am. J. Physiol. 250: F917-F923.

Hall, J.E., J.P. Granger, and R.L. Hester (1985) Interactions between adenosine and angiotensin II in controlling glomerular filtration. Am. J. Physiol. 248: F340-F346.

Harrison, H.E., and H.C. Harrison (1954) Experimental production of renal glycosuria, phosphaturia, and aminoaciduria by injection of maleic acid. Science 120: 606-608.

Harvey, P.B. (1960) Vascular resistance changes produced by hypertonic solutions. Am. J. Physiol. 199: 31-34.

Hashimoto, K., and K. Kumakura (1965) The pharmacological features of the coronary, renal, mesenteric, and femoral arteries. Jpn. J. Physiol. 15: 540-551.

Haslam, R.J., and J.A. Lynham (1972) Activation and inhibition of blood platelet adenylate cyclase by adenosine or by 2-chloroadenosine. Life Sci. 11: 1143-1154.

Hayslett, J. P., M. Kashgarian (1979) A micropuncture study of the renal handling of lithium. Pfluegers Arch. 380: 159-163.

Hedqvist, P., and B.B. Fredholm (1976) Effects of adenosine on adrenergic neurotransmission: Prejunctional inhibition and post-junctional enhancement. Naunyn-Schmeiderbergs Arch. Pharmakol. 293: 217-223.

Hedqvist, P., B.B. Fredholm, and S. Olundh (1978) Antagonistic effects of theophylline and adenosine on adrenergic neuroeffector transmission in the rabbit kidney. Circ. Res. 43: 592-598.

Hjemdahl, P., and B.B. Fredholm (1976) Cyclic AMP-dependent and independent inhibition of lipolysis by adenosine and decreased pH. Acta. Physiol. Scand. 96: 170-179.

Huang, M., and G.I. Drummond (1976) Effect of adenosine on cyclic AMP accumulation in ventricular myocardium. Biochemical Pharmacology 25: 2713-2719.

Ismail, N.A., E.S.M. ElDenshary, and W. Montague (1977) Adenosine and the regulation of insulin secretion by isolated rat islets of Langerhans. Biochem. J. 164: 409-413.

Itoh, S., O.A. Carretero, and R.D. Murray (1985) Possible role of adenosine in the macula densa mechanism of renin release in rabbits. J. Clin. Invest. 76: 1412-1417.

Katada, T., G.M. Bokoch, J.K. Northrup, M. Ui, and A.G. Gilman (1984) The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. Properties and function of the purified protein. J. Biol. Chem. 259: 3568-3577.

Katzberg, R.W., T.W. Morris, F.A. Burgener, D.E. Kamm, and H.W. Fischer (1977) Renal renin and hemodynamic responses to selctive renal artery catheterization and angiography. Invest. Radiol. 12: 381-388.

Katzberg, R.W., G. Schulman, L.G. Meggs, W.J.H. Caldicott, M.M. Damieno, and N.K. Hollenberg (1983) Mechanism of renal response to contrast media in dogs: decrease in renal function due to hypertonicity. Invest. Radiol. 18: 74-80.

Kelley, G.G., H. Barron, K. Andreoni, W.S. Curtis, and J.N. Forrest, Jr. $A_1$ adenosine receptors inhibit chloride transport in the shark rectal gland by $N_i$-coupled cyclic AMP dependent and cyclic AMP independent mechanisms. Kidney Int. 31: 274 (Abstr.).

Kelley, G.G., W.S. Curtis, A.M. Nuland, and J.N. Forrest (1985) Endogenous adenosine inhibits chloride secretion in the shark rectal gland via an $A_1$ adenosine receptor coupled to the inhibitory nucleotide regulatory protein. Bull. Mt. Desert Isl. Biol. Lab. 25: 108-110.

Kelley, G.G., D.R. Gifford, F. Wang, and J.N. Forrest (1984) Stimulation and inhibition of adenylate cyclase in the rectal gland. Bull. Mt. Desert Isl. Biol. Lab. 24: 102-104.

Kleinman, L.I., and E.P. Radford (1964) Ventilation standards for small mammals. J. Appl. Physiol. 19: 360-362.

Kolassa, N., and K. Pfleger (1975) Adenosine uptake by erythrocytes of man, rat, and guinea pig and its inhibition by hexobendine and dipyridamole. Biochem. Pharmacol. 24: 154-156.

Kowal, J., and R.P. Fiedler (1969) Adrenal cells in tissue culture.II: Steroidogenic responses to nucleosides and nucleotides. Endocrinology 84: 1113-1117.

Kramer, H.J., and H.C. Gonick (1970) Experimental Fanconi syndrome. I. Effect of maleic acid on renal cortical Na-K-ATPase activity and ATP levels. J. Lab. Clin. Med. 76: 799-808.

Kramer, H.J., and H.C. Gonick (1973) Effect of maleic acid on sodium-linked tubular transport in experimental Fanconi syndrome. Nephron 10: 306-319.

Kraupp, O. (1969) Zum wirkungsmechanismus der coronardilatoren. Arch. Exp. Pathol. Pharmakol. 263: 144-155.

Kubler, W., and H.J. Bretschneider (1963) Die permeation von adenosin durch die erythrocyten membran des hundes. Pflugers Arch. 277: 141-149.

Kurtz, A., R. Della Bruna, J. Pfeilschifter, and C. Bauer (1987) Role of cGMP as second messenger of adenosine in the inhibition of renin release from renal juxtaglomerular cells. Kidney Int. In Press.

Lang, M.A., A.S. Preston, J.S. Handler, and J.N. Forrest (1985) Adenosine stimulated sodium transport in kidney A6 epithelia in culture. Am. J. Physiol. 249: C330-C336.

Larson, T.S., K. Hudson, J.I. Mertz, J.C. Romero, and F.G. Knox (1983) Renal vasoconstrictive response to contrast medium: the role of sodium balance and the renin-angiotensin system. J. Lab. Clin. Med. 101: 385-391.

Londos, C., D.M.F. Cooper, W. Schlegel, and M. Rodbell (1978) Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process: basis for actions of adenosine and methylxanthines on cyclic AMP production and lipolysis. Proc. Natl. Acad. Sci. USA 75: 5362-5366.

Londos, C., D.M.F. Cooper, and J. Wolff (1980) Subclasses of external adenosine receptors. Proc. Natl. Acad. Sci. USA 77: 2551-2554.

Londos, C., and J. Wolff (1977) Two distinct adenosine-sensitive sites on adenylate cyclase. Proc. Natl. Acad. Sci. [USA] 74: 5482-5486.

Lund, P., N.W. Cornell, and H.A. Krebs (1975) Effect of adenosine on the adenine nucleotide content and metabolism of hepatocytes. Biochem. J. 152: 593-599.

Maesaka, J.K., and M. McCaffery (1980) Evidence for renal tubular leakage in maleic acid-induced Fanconi syndrome. Am. J. Physiol. 239: F507-F513.

Markwell, M.A.K., S.M. Haas, L.L. Bieber, and N.E. Tolbert (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. 87: 206-210.

McDonald, F.D., T. Thiel, D.R. Wilson, G.F. DiBona, and D.E. Oken (1969) The prevention of acute renal failure in the rat by long-term saline loading: a possible role of the renin-angiotensin axis. Proc. Soc. Exp. Biol. Med. 131: 610-614.

McKenzie, S.G., and H.P. Bar (1973) On the mechanism of adenyl cyclase inhibition by adenosine. Can. J. Physiol. Pharmacol. 51: 190-196.

Miller, W., R.A. Thomas, R.M. Berne, and R. Rubio (1978) Adenosine production in the ischemic kidney. Circ. Res. 43: 390-397.

Molski, T.F.P., P.H. Naccache, M.L. Marsh, J. Kermode, E.L. Becker, and R.I. Sha'afi (1984) Pertussis toxin inhibits the rise in the intracellular concentration of free calcium that is induced by chemotactic factors in rabbit neutrophils: possible role of "G proteins" in calcium mobilization. Biochem. Biophys. Res. Commun. 124: 644-650.

Moss, J., S.J. Stanley, D.L. Burns, J.A. Hsia, D.A. Yost, G.A. Myers, and E.L. Hewlett (1983) Activation by thiol of the latent NAD glycohydrolase and ADP-ribosyltransferase activities of Bordetella pertussis toxin. J. Biol. Chem. 258: 11879-11882.

Murray, R.D., and P.C. Churchill (1984) The effects of adenosine receptor agonists in the isolated perfused rat kidney. Am. J. Physiol. 247: H343-H348.

Nakamura, T., and M. Ui (1985) Simultaneous inhibitions of inositol phospholipid breakdown, arachidonic acid release, and histamine secretion in mast cells by islet-activating protein, pertussis toxin. J. Biol. Chem. 260: 3584-3593.

Newman, M.E., and A. Levitski (1983) Desensitization of normal rat kidney cells to adenosine. Biochemical Pharmacology 32: 137-140.

Osswald, H. (1975) Renal effects of adenosine and their inhibition by theophylline. Naunyn-Schmeidebergs Arch. Pharmacol. 288: 79-86.

Osswald, H. (1984) The role of adenosine in the regulation of glomerular filtration rate and renin secretion. Trends Pharmacol. Sci. 5:94-97.

Osswald, H., B. Evers, R. Massmann, and G. Nabakowski (1980) Tissue content of adenine nucleotides and adenosine in the rat kidney after administration of maleate. Kidney Int. 17: 413 (Abstract).

Osswald, H., H.H. Hermes, and G. Nabakowski (1982) Role of adenosine in signal transmission of tubuloglomerular feedback. Kidney Int. 22: S136-S142.

Osswald, H., G. Nabakowski, and H. Hermes (1980) Adenosine as a possible mediator of metabolic control of glomerular filtration rate. Int. J. Biochem. 12: 263-267.

Osswald, H., H.J. Schmitz, and O. Heidenreich (1975) Adenosine response of the rat kidney after saline loading, sodium restriction, and hemorrhagia. Pfluegers Arch. 357: 323-333.

Osswald, H., H.J. Schmitz, and R. Kemper (1977) Tissue content of adenosine, inosine, and hypoxanthine in the rat kidney after ischemia and postischemic recirculation. Pfluegers Arch. 371: 45-50.

Osswald, H., H.J. Schmitz, and R. Kemper (1978) Renal action of adenosine: effect on renin secretion in the rat. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. 303: 95-99.

Osswald, H., W.S. Spielman, and F.G. Knox (1978) Mechanisms of adenosine- mediated decreases in glomerular filtration rate in dogs. Circ Res 43: 465-469.

Palmer, G.C. (1971) Characteristics of the hormonal induced cyclic adenosine 3',5'-monophosphate response in the rat and guinea pig lung in vitro. Biochem. Biophys. Acta 252: 561-566.

Paterson, A.R.P. (1979) Adenosine transport. In H.P. Baer and G.I. Drummond (eds): Physiological and regulatory functions of adenosine and adenine nucleotides. New York: Raven Press, pp. 305-313.

Paterson, A.R.P., S.C. Kim, O. Bernard, and C.E. Cass (1975) Transport of nucleotides. Ann. NY Acad. Sci. 255: 402-410.

Pearson, J.D., J.S. Carleton, A. Hutchings, and J.L. Gordon (1978) Uptake and metabolism of adenosine by pig aortic endothelial and smooth-muscle cells in culture. Biochem. J. 170: 265-271.

Phillis, J.W., and J.P. Edstrom (1976) Effects of adenosine analogs on rat cerebral cortical neurons. Life Sci. 19: 1041-1053.

Plagemann, P.G.W. (1971) Nucleoside transport by Novikoff rat hepatoma cells growing in suspension culture. Specificity and mechanism of transport reactions and relationship to nucleoside incorporation into nucleic acids. Biochem. Biophys. Acta. 233: 688-701.

Plagemann, P.G.W., and D.P. Richey (1974) Transport of nucleosides, nucleic acid bases, choline and glucose by animal cells in culture. Biochem. Biophys. Acta. 344: 263-305.

Plagemann, P.G.W., and R.M. Wohlhueter (1980) Permeation of nucleosides, nucleic acid bases, and nucleotides in animal cells. In F. Bronner and A. Kleinzeller (eds): Current topics in membranes and transport. New York: Academic Press, pp. 225-330.

Poeschla, E., G. Kelly, and J.N. Forrest (1982) Evidence for an inhibitory adenosine receptor in the rectal gland of Squalus acanthias. Bull. Mt. Desert Isl. Biol. Lab. 22: S19-S23.

Poeschla, E., G.G. Kelly, D. Gifford, and J.N. Forrest (1985) Identification of adenosine receptor subtypes in the shark rectal gland. Clin. Res. 33: 587A (Abstr.).

Reed, J.R., R.H. Williams, and R.G. Luke (1983) The renal hemodynamic response to diatrizoate in normal and diabetic rats. Invest. Radiol. 18: 536-540.

Rodbell, M. (1980) The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 284: 17-22.

Rogulski, J., and A. Pacanis (1978) Effects of maleate on CoA metabolism in rat kidney. In W. Guder and U. Schmidt (eds): Nephrological Biochemistry. Huber: Bern, pp. 406-415.

Romero, J.C., and C.G. Strong (1977) The effect of indomethacin blockade of prostaglandin synthesis on blood pressure of normal rabbits and rabbits with renovascular hypertension. Circ. Res. 40: 35-41.

Roy, C. (1984) Regulation by adenosine of the vasopressin-sensitive adenylate cyclase in pig kidney cells (LLC-$PK_{1}$) grown in defined media. Eur. J. Biochem. 143: 243-250.

Sakai, K., J. Aono, and K. Haruta (1981) Species differences in renal vascular effects of dipyridamole and in the potentials of adenosine action by dipyridamole. J. Cardiovasc. Pharmacol. 3: 420-430.

Sattin, A., and T.W. Rall (1970) The effect of adenosine and adenine nucleotides on the cyclic adenosine 3',5'-phosphate content of guinea pig cerebral cortex slices. Mol. Pharmacol. 6: 13-23.

Schatz, R.A., C.R. Vannam, and O.Z. Sellinger (1977) Species and tissue differences in the catabolism of S-adenosyl homocysteine: a quantitative chromatographic study. Life Sci. 20: 375-383.

Schnermann, J., H. Osswald, and M. Hermle (1979) Inhibitory effect of methylxanthines on feedback control of GFR in the rat kidney. Pfluegers Arch. 369: 39-48.

Schnermann, J., F.S. Wright, J.M. Davis, W. VonStackelberg, and G. Grill (1970) Regulation of superficial nephron filtration rate by tubuloglomerular feedback. Pfluegers Arch. 318: 147-175.

Schrader, J., W. Schutz, and H. Bardenheuer (1981) Role of S-adenosylhomocysteine hydrolase in adenosine metabolism in mammalian heart. Biochem. J. 198: 65-70.

Schwabe, U., R. Ebert, and H.C. Erbler (1973) Adenosine release from isolated fat cells and its significance for the effects of hormones on cyclic 3',5'-AMP levels and lipolysis. Arch. Pharmacol. 276: 133-148.

Scott, J.B., R.M. Daugherty, Jr., and F.J. Haddy (1965) Role of chemical factors in regulation of flow through the kidney, hindlimb, and heart. Am. J. Physiol. 208: 813-824.

Shimizu, H., and J.W. Daly (1970) Formation of adenosine 3',5'- monophosphate from adenosine in brain slices. Biochem. Biophys. Acta. 222: 465-473.

Silinsky, E.M. (1981) On the calcium receptor that mediates depolarization-secretion coupling at cholinergic motor nerve terminals. Br. J. Pharmacol. 73: 413-429.

Sistare, F.D., R.A. Picking, and R.C. Haynes (1985) Sensitivity of the response of cytosolic calcium in quin-2-loaded rat hepatocytes to glucagon, adenine nucleosides, and adenine nucleotides. J. Biol. Chem. 260: 12744-12747.

Sixma, J.J., J.P.M. Lips, A.M.C. Trieschnigg, and H. Holmsen (1976) Transport and metabolism of adenosine in human blood platelets. Biochem. Biophys. Acta. 443: 33-48.

Smith, C.D., C.C. Cox, and R. Snyderman (1986) Receptor coupled activation of phosphoinositide-specific phosphlipase C by an N protein. Science 232: 97-100.

Smith, W.L., and A. Garcia-Perez (1985) Immunodissection: use of monoclonal antibodies to isolate specific types of renal cells. Am. J. Physiol. 248: F1-F7.

Souness, J.E., and V. Chagoya de Sanchez (1981) The stimulation of [1-$^{14}$C] glucose oxidation in isolated fat cells by $N^6$-methyladenosine: An effect independent of cAMP. FEBS Letts. 125: 249-252.

Spielman, W.S. (1984) Antagonistic effect of theophylline on the adenosine-induced decrease in renin release. Am. J. Physiol. 247: F246-F251.

Spielman, W.S., and H. Osswald (1978) Characterization of the post-occlusive response of renal blood flow in the cat. Am. J. Physiol. 235: F286-F290.

Spielman, W.S., and H. Osswald (1979) Blockade of postocclusive renal vasoconstriction by an angiotensin II antagonist: evidence for an angiotensin-adenosine interaction. Am J. Physiol. 237: F463-F467.

Spielman, W.S., and C.I. Thompson (1982) A proposed role for adenosine in the regulation of renal hemodynamics and renin release. Am. J. Physiol. 242: F423-F435.

Spielman, W.S., S.L. Britton, and M.J. Fiksen-Olsen (1980) Effect of adenosine on the distribution of renal blood flow in dogs. Circ Res 46: 449-456.

Spielman, W.S., W.K. Sonnenburg, M.L. Allen, L.J. Arend, K. Gerozissis, and W.L. Smith (1986) Immunodissection and culture of rabbit cortical collecting tubule cells. Am.J. Physiol. 251: F348-F357.

Spinowitz, B.S., and J.A. Zadunaisky (1979) Action of adenosine on chloride active transport of isolated frog cornea. Am. J. Physiol. 237: F121-F127.

Stoff, J.S., R. Rosa, R. Hallac, P. Silva, and F.H. Epstein (1979) Hormonal regulation of active chloride transport in the dogfish rectal gland. Am. J. Physiol. 237: F138-F144.

Sullivan, T.J., and C.W. Parker (1976) Pharmacological modulation of inflammatory mediator release by rat mast cells. Am. J. Pathol. 85: 437-464.

Szczepanska, M., and S. Angielski (1980) Prevention of maleate-induced tubular dysfunction by acetoacetate. Am. J. Phsyiol. 239: F50-F56.

Tagawa, H., and A.J. Vander (1970) Effects of adenosine compounds on renal function and renin secretion in dogs. Circ Res 26: 327-338.

Talner, L.B., and A.J. Davidson (1968) Renal hemodynamic effects of contrast media. Invest. Radiol. 3: 310-317.

Teitelbaum, I., and T. Berl (1986) Effects of calcium on vasopressin-mediated cyclic adenosine monophosphate formation in cultured rat inner medullary collecting tubule cells. Evidence for the role of intracellular calcium. J. Clin. Invest. 77: 1574-1583.

Thompson, C.I., H.V. Sparks, and W.S. Spielman (1985) Renal handling and production of plasma and urinary adenosine. Am. J. Physiol. 248: F545-F551.

Thurau, K. (1964) Renal hemodynamics. Am. J. Med. 36: 698-719.

Trams, E.G., and C.J. Lauter (1974) On the sidedness of plasma membrane enzymes. Biochem. Biophys. Acta. 345: 180-197.

Trimble, M.E., and R. Coulson (1984) Adenine transport in perfused rat kidney and renal cortical membrane vesicles. Am. J. Physiol. 15: F794-F803.

VanCalker, D., M. Muller, and B. Hamprecht (1979) Adenosine regulates via two different types of receptors, the accumulation of cAMP in cultured brain cells. J. Neurosci. 33: 999-1005.

Vandogen, R., and W.S. Peart (1979) Calcium dependence of the inhibitory effect of angiotensin on renin secretion in the isolated perfused kidney of the rat. Br. J. Pharmacol. 50: 125-129.

Verghese, M., R.J. Uhing, and R. Snyderman (1986) A pertussis/ choleratoxin-sensitive N protein may mediate chemoattractant receptor signal transduction. Biochem. Biophys. Res. Commun. 138: 887-894.

Walter, P., and E. Bassenge (1968) Wirkung von ATP, A-3,5-MP, adenosine, und dipyridamol um streifenpraparaten der a. coronari, a. renalis, und der v. portal. Pfluegers Arch. 299: 52-65.

Weir, G.C., S.D. Knowlton, and D.B. Martin (1975) Nucleotide and nucleoside stimulation of glucagon secretion. Endocrinology 97: 932-936.

Wolberg, G., T.P. Zimmerman, K. Hiemstra, M. Winston, and L.C. Chu (1975) Adenosine inhibition of lymphocyte-mediated cytolysis: possible role of cyclic adenosine monophosphate. Science 187: 957-959.

Wolff, J., and G.H. Cook (1977) Activation of steroidogenesis and adenylate cyclase by adenosine adrenal and Leydig tumor cells. J. Biol. Chem. 252: 687-693.

Woodcock, E.A., R. Loxley, E. Leung, and C.I. Johnston (1984) Demonstration of $R_a$ adenosine receptors in rat renal papillae. Biochem. Biophys. Res. Commun. 121: 434-440.

Wysocki, L.J., and G.L. Sato (1978) "Panning" for lymphoyctes: a method of cell selection. Proc. Natl. Acad. Sci. USA 75: 2844-2848.