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ADENOSINE: A Physiological Brake on Renin Release

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INTRODUCTION: OBJECTIVE OF REVIEW

In 1970 Tagawa & Vander (1) reported that adenosine infused directly into the renal artery of anesthetized, salt-depleted dogs inhibited basal renin release. For some years, this significant observation seemed not to excite the imagination of renal physiologists. Then, beginning in the late 1970s and continuing to the present, a flurry of studies have described the effects of adenosine on renin release and suggested various roles for adenosine in renal physiology and pathophysiology. The main objective of this review is to describe what is now known about the effects of adenosine on renin release and to integrate this information into a broader perspective by developing the *adenosine-brake hypothesis*. To achieve this objective, I first construct—and provide experimental evidence supporting—a model of renin release that incorporates the major physiological pathways controlling the secretion of renin. Second, I explain how an adenosine-mediated brake on the secretory activities of the juxtaglomerular cell can be incorporated into this model of renin release. Third, I review what is known about the actions of exogenous and endogenous adenosine on renin release and indicate how this information is consistent with the adenosine-brake hypothesis. Fourth, I explain the relation of the adenosine-brake hypothesis to clinical medicine. In this regard, I discuss implications of the adenosine-brake hypothesis for patients with renovascular hypertension. Finally, I consider possible sources of adenosine

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involved in the regulation of renin release. This last consideration allows me to introduce a speculative hypothesis involving adenosine, i.e. the *transmembrane negative feedback loop hypothesis*.

PHYSIOLOGICAL STIMULATION OF RENIN RELEASE: THE ADENYLATE CYCLASE MODEL

An integrated view of the major physiological systems controlling renin release, along with an appreciation of the intracellular signalling pathways activated by these systems, is prerequisite to understanding the role of adenosine as a modulator of renin secretion. However, presentation of such a model is not without risk, since an accurate and complete understanding of the control of renin release is hampered by two realities. First, the secretory activity of the juxtaglomerular cell is affected by many endocrine, paracrine and, possibly, autocrine factors. Because these hormonal interactions involve various organ systems outside the kidney, as well as several cell types within the kidney, much of the physiology of renin release cannot be investigated using a reductionistic approach. Rather, in many cases the system must be deduced from either in vitro or in vivo experiments involving the whole kidney, and interpretations of such experiments are always fraught with difficulties. Second, where a reductionistic approach is appropriate, lack of suitable cell culture models has retarded progress in our understanding of the basic cell biology of renin release. Despite these problems, numerous investigators are gradually elucidating the control of juxtaglomerular function, although there is still no clear consensus in their findings.

The working model that I employ is depicted in Figure 1, to which the reader should refer throughout this review. However, this model is not intended to illustrate *all* possible factors that can impinge on the juxtaglomerular cell; rather, its purpose is to highlight those mechanisms that may play a prominent *physiological* role in stimulating or inhibiting renin release. The mechanisms by which other agents affect renin release can usually be deduced by inserting these factors into this "core" model. For example, the effects of glucagon or dopamine on renin release can be deduced by recalling that glucagon and dopamine, like beta-adrenoceptor agonists, can stimulate adenylate cyclase.

Although many factors can cause the juxtaglomerular cell to secrete renin (see ref. 2 for comprehensive review), physiological stimulation of renin release occurs through three major pathways: (*a*) the intrarenal beta-adrenoceptor mechanism; (*b*) the intrarenal baroreceptor mechanism; and (*c*) the macula densa mechanism. The importance of these three pathways to renin release is underscored by the observation that blocking all three mechanisms renders the kidney unable to secrete renin in response to hypotensive hemorrhage (3), a complex and severe stimulus. That these three pathways

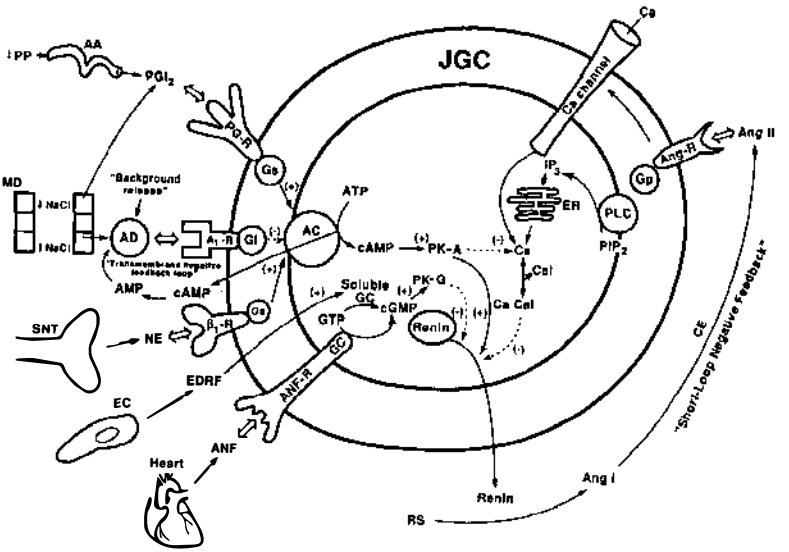

Figure 1 An integrated perspective on the physiological control of renin release and how adenosine may participate as a brake on the juxtaglomerular cell (JGC). PP = perfusion pressure; AA = afferent arteriole; PGI_2 = prostacyclin; PG-R = prostacyclin receptor; NaCl = sodium chloride; MD = macula densa; A_1-R = adenosine receptor subtype coupled to inhibition of adenylate cyclase; AD = adenosine; AMP = adenosine monophosphate; cAMP = cyclic adenosine monophosphate; ATP = adenosine triphosphate; SNT = sympathetic nerve terminal; NE = norepinephrine; β_1-R = beta$_1$-adrenoceptor; EC = endothelial cell; EDRF = endothelial derived relaxing factor; ANF = atrial natriuretic factor; ANF-R = atrial natriuretic factor receptor; AC = adenylate cyclase; GC = guanylate cyclase; G_i, G_s, and G_p = GTP-binding proteins that transduce inhibitory signals to adenylate cyclase, stimulatory signals to adenylate cyclase, and stimulatory signals to phospholipase C (PLC), respectively; GTP = guanosine triphosphate; cGMP = cyclic guanosine monophosphate; PK-A = cAMP-dependent protein kinase; PK-G = cGMP-dependent protein kinase; Ca = calcium; RS = renin substrate; Ang I = angiotensin I; Ang II = angiotensin II; CE = angiotensin I converting enzyme; Ang-R = angiotensin II receptor; IP_3 = inositol trisphosphate; PIP_2 = phosphoatidylinositol bisphosphate; ER = endoplasmic reticulum; Cal = calmodulin.

are predominantly responsible for the renin release response to most physiological stimuli has bearing on the major thesis of this review, i.e. the adenosine-brake hypothesis.

Regarding the intrarenal beta-adrenoceptor mechanism, juxtaglomerular cells are equipped with beta$_1$-adrenoceptors that are activated either by norepinephrine released from renal sympathetic nerves or by catecholamines secreted from the adrenal medulla and delivered to the kidney via the renal circulation. Activation of beta$_1$-adrenoceptors on the juxtaglomerular cell increases the secretion of renin (4–11).

Much evidence supports the contention that cAMP, via activation of

cAMP-dependent protein kinase, is the proximal intracellular mediator of beta-adrenoceptor-induced renin release. First, agonism of beta-adrenoceptors stimulates adenylate cyclase and increases cAMP concentrations in numerous cell types including cultured juxtaglomerular cells (12, 13). That the guanine nucleotide-binding protein, G_s, couples beta-adrenoceptors to adenylate cyclase is, at this point, dogma (reviewed in ref. 14, 15). Second, other maneuvers known to increase intracellular concentrations of cAMP, e.g. forskolin (12, 13, 16–18), dopamine (19–24), histamine (13, 18, 25), glucagon (21, 23, 26), calcitonin gene-related peptide (27), bradykinin (13, 28), prostacyclin (17, 29–34), pertussis toxin (35), and phosphodiesterase inhibitors (36, 37), also stimulate renin release. Third, lipophilic cAMP analogues increase renin release (18, 23, 36, 38–42). Fourth, stimulation of renin release by agonism of beta-adrenoceptors is blocked by the adenylate cyclase inhibitor, 2',5'-dideoxyadenosine (17).

Unlike most other known exocytotic mechanisms, renin secretion is stimulated by decreases in intracellular calcium and inhibited by increases in intracellular calcium, and this action of calcium is most likely mediated by calmodulin. The evidence that calcium is reciprocally linked to renin release has been recently summarized (43). Note, however, that angiotensin II inhibits renin release by increasing intracellular calcium levels (43) and that this action of angiotensin gives rise to the "short-loop" negative feedback system depicted in Figure 1. Also, as illustrated in Figure 1, angiotensin II, like many other hormones, increases intracellular calcium concentrations both by releasing calcium from intracellular stores ($G_p \rightarrow$ phospholipase C $\rightarrow$ inositol trisphosphate $\rightarrow$ release of calcium from intracellular storage sites) and by direct and/or indirect effects on calcium channels (reviewed in ref. 44–47).

Since calcium inhibits renin release and cAMP decreases intracellular concentrations of calcium by various mechanisms, including stimulation of sodium-potassium ATPase and calcium ATPase (reviewed in ref. 48, 49), beta-adrenoceptor activation could logically increase renin release in part by reducing intracellular concentrations of calcium. This hypothesis is supported by the observations of Churchill & Churchill (50) and Henrich & Campbell (17) that cAMP-mediated renin release is either blocked or greatly attenuated by manipulations that increase intracellular calcium. Also, Kurtz & Penner (51) measured calcium levels with the fura-2 method in single mouse renal juxtaglomerular cells and found that intracellular application of cAMP inhibited angiotensin II-induced increases in intracellular calcium. In contrast, Park et al (52) were unable to block renin secretion induced with high concentrations of forskolin by increasing intracellular calcium. For the present, it is reasonable to conclude that most effects of cAMP on renin release are mediated by changes in intracellular calcium concentrations, but that other noncalcium-mediated mechanisms may exist as well.

Two other important pathways mediating renin release are the intrarenal baroreceptor system and the macula densa mechanism. Physiologically, the intrarenal baroreceptor pathway is activated and inhibited by decreases and increases in renal perfusion pressure, respectively. The hydrostatic pressure signal acting upon the afferent arteriole probably is somehow transduced into chemical messengers that are released from endothelial, smooth muscle, and/or juxtaglomerular cells. These *intercellular* messengers—acting in a classical autocrine or paracrine fashion—then diffuse to nearby juxtaglomerular cells and affect renin release. As discussed below, prostacyclin is most likely one of the more important intercellular mediators of baroreceptor-induced renin release. In addition to chemical messengers, the intrarenal baroreceptor mechanism also may involve stretch-induced changes in the transmembrane potential of juxtaglomerular cells. Such changes in transmembrane potential could influence renin release by modulating voltage-dependent ion channels (53, reviewed in ref. 54).

Like the intrarenal baroreceptor mechanism, activation or inhibition of renin release via the macula densa pathway also depends on cell-cell communication. With this mechanism, decreases and increases in the luminal concentration of sodium chloride at the macula densa increase and decrease renin release, respectively (54a). Most likely, changes in sodium chloride concentration at the macula densa result in parallel changes in the flux of ions through the $1Na^+$-$1K^+$-$2Cl^-$ cotransporter, which, in turn, cause macula densa cells to release chemical messengers to the nearby juxtaglomerular cells. In this regard, the chloride ion is more important than the sodium ion (54b) because under physiological conditions the activity of the cotransporter is primarily modulated by the luminal concentration of chloride, rather than sodium. Two intercellular chemical messengers, prostacyclin and adenosine, may participate in the macula densa pathway with prostacyclin mediating stimulatory responses to decreasing concentrations of sodium chloride and adenosine mediating inhibitory responses to increasing sodium chloride concentrations. A discussion of the role of adenosine in the macula densa pathway is included later in this review in the context of the adenosine-brake hypothesis.

A considerable body of evidence links prostaglandins to the baroreceptor and macula densa pathways of renin release: (*a*) In beta-adrenoceptor-blocked animals, inhibition of prostaglandin biosynthesis decreases the renin release response to both a reduction in renal perfusion pressure and/or a decrease in sodium chloride delivery to the macula densa (55–59). These observations recently have been confirmed in humans with unilateral renovascular hypertension (60). (*b*) Prostacyclin, 6-keto-prostaglandin E_1 (an active metabolite of prostacyclin), and prostaglandin E_2 potently stimulate renin release, presumably by acting directly on juxtaglomerular cells (29–34). (*c*)

Arachidonic acid, the precursor of prostaglandins, also is a renin secretagogue, and the effects of arachidonic acid on renin release are blocked by cyclooxygenase inhibitors (32, 34, 55, 61–66). (*d*) The renal cortex synthesizes prostacyclin, 6-keto-prostaglandin E_1, and prostaglandin E_2 (67–70), and in dogs a reduction in renal perfusion pressure increases the renal secretion of 6-keto-prostaglandin $F_{1\alpha}$ (71), an inactive metabolite of prostacyclin. Since prostacyclin and 6-keto-prostaglandin E_1 are more potent renin secretagogues than prostaglandin E_2 (31, 33, 34), and selective blockade of prostacyclin synthase attenuates the renin response to arachidonic acid (31, 72) while enhancing the biosynthesis of prostaglandin E_2 (31), prostacyclin and/or its active metabolite probably is more important than prostaglandin E_2 in mediating the renin release response to activation of the baroreceptor or macula densa.

Like beta-adrenoceptor agonists, prostacyclin stimulates adenylate cyclase activity in numerous tissues, including renal cortical slices (17), and inhibition of adenylate cyclase with 2',5'-dideoxyadenosine prevents prostacyclin-induced renin release (17). Thus, prostacyclin, like beta-adrenoceptor agonists, probably stimulates renin release via cAMP. If this hypothesis is correct, then, as shown in Figure 1, all three major mechanisms mediating physiological stimulation of renin release share a common transmembrane and intracellular signalling pathway, i.e. the activation of adenylate cyclase. As discussed in the next section, the possibility that adenylate cyclase may function as the common transmembrane signalling pathway mediating physiological stimulation of renin release has important and interesting implications for the role of adenosine in renin release.

In contrast to the role of cAMP as the proximal intracellular mediator of the three most important physiological stimuli of renin release, current evidence indicates that cGMP is the proximal intracellular mediator of several inhibitors of renin release, including atrial natriuretic factor (ANF) and endothelial-dependent relaxing factor (EDRF). This finding is somewhat unexpected since cGMP lowers intracellular calcium in smooth muscle cells (73), and intracellular calcium is a well-established inhibitor of renin release (43). Thus, activation of cGMP-dependent proteins (kinase?) within the juxtaglomerular cell must inhibit renin release via a pathway not involving intracellular calcium.

The conclusion that cGMP inhibits renin release rests on the following supporting evidence: (*a*) Both ANF (74–76) and EDRF (77) inhibit renin release, at least in vitro; (*b*) ANF and EDRF activate particulate and soluble guanylate cyclase, respectively (reviewed in ref. 78); (*c*) A lipophilic analogue of cGMP, 8-bromo-cGMP, inhibits renin release (76); (*d*) Inhibition of guanylate cyclase blocks ANF-induced (74, 76) and nitroprusside-induced (76) suppression of renin release. (The experiment with nitroprusside has

bearing on the mechanism of EDRF-induced suppression of renin release since nitroprusside releases nitric oxide and EDRF most likely is NO (reviewed in ref. 79)). Whether endothelial-dependent relaxing factor and atrial natriuretic factor have a physiological role as inhibitors of renin release remains to be determined. Also, atrial natriuretic factor may indirectly inhibit renin release in vivo by increasing sodium chloride delivery to the macula densa (80, 81).

IMPLICATIONS OF THE ADENYLATE CYCLASE MODEL OF RENIN RELEASE FOR THE REGULATION OF RENIN RELEASE BY ADENOSINE

The central role for adenylate cyclase in mediating physiological stimulation of renin release provides the first suggestion of the importance of adenosine as a modulator of renin release. Adenosine interacts with biological systems primarily, although not exclusively, via two adenosine receptor subtypes, A_1 and A_2 receptors. A_1 receptors have a high affinity for adenosine (nanomolar range) and when activated cause inhibition of adenylate cyclase via the GTP-binding protein, G_i. Conversely, A_2 receptors have a low affinity for adenosine (micromolar range) and when activated cause stimulation of adenylate cyclase via G_s (See ref. 82). Because A_1 receptors are activated by very low concentrations of adenosine, this receptor subtype most likely provides the predominant target site for physiological concentrations of adenosine. If juxtaglomerular cells are in fact equipped with A_1 receptors, then adenosine would be strategically positioned to serve a physiological role as a "molecular brake" on the renin release response to all physiological stimuli by reducing the responsiveness of adenylate cyclase to the intercellular messengers, prostacyclin and norepinephrine (Figure 1). In retrospect, that juxtaglomerular cells are equipped with A1 receptors was suggested as long ago as the early 1970s by a report that adenosine inhibited rat renal cortical adenylate cyclase activity (83).

EVIDENCE SUPPORTING THE ADENOSINE-BRAKE HYPOTHESIS

Effects of Exogenous Adenosine on Renin Release

That exogenous adenosine can inhibit renin release is not in dispute. Adenosine can lower base-line renin release in vivo in both sodium-depleted dogs (1, 84–87) and sodium-depleted rats (88). However, adenosine also alters renal blood flow (biphasic response characterized by an initial, large, short-lived decrease followed by a sustained, slight increase) and decreases glomerular filtration rate, filtration fraction, and water and electrolyte excretion (89). Therefore, before concluding that adenosine inhibits renin release by a

direct action on juxtaglomerular cells, the possibility must be excluded that these hemodynamic and tubular actions of adenosine mediate its effect on renin release. In this regard, the significant study by Arend et al (85) clearly dissociated the renin-inhibitory effects of adenosine from its effects on renal hemodynamics and renal excretory function. This was accomplished by inhibiting either the renal vascular effects of adenosine with a calcium channel blocker or preventing adenosine-mediated alterations in electrolyte excretion by rendering the kidney nonfiltering. In both situations, adenosine inhibited renin release. Additional evidence that adenosine inhibits renin release by a direct action on juxtaglomerular cells is that adenosine reduces renin release in several in vitro experimental models in which hemodynamic and tubular effects are not operational. For instance, adenosine reduces renin release in rat kidney slices (90), isolated rat glomeruli (90), rabbit afferent arterioles (91), rabbit renal cortical slices (92), and isolated rat juxtaglomerular cells (93).

Both subtypes of adenosine receptors can alter renin release from juxtaglomerular cells. Studies by Churchill and colleagues demonstrate that in rat renal cortical slices adenosine analogues can either inhibit or stimulate renin release, depending on the concentration of the adenosine agonist and its selectivity for A_1 versus A_2 receptors (94). Submicromolar concentrations of adenosine agonists inhibit renin release with an order of potency consistent with an A_1 receptor-mediated effect. In contrast, micromolar concentrations of adenosine agonists stimulate renin release with an order of potency consistent with an A_2 receptor-mediated action. Similarly, in both isolated perfused rat kidneys (95, 96) and intact rats (97), activation of A_1 receptors decreases renin release and stimulation of A_2 receptors increases renin release. These data suggest that juxtaglomerular cells are equipped with both A_1 and A_2 receptors, an inference corroborated by a recent study with adenosine analogues in cultured rat juxtaglomerular cells (93).

Although clearly demonstrating that adenosine can inhibit basal renin release, these studies do not determine whether adenosine attenuates the renin release response to activation of the three major physiological pathways that normally drive the secretion of renin. To address the adenosine-brake hypothesis, we investigated the effects of intrarenal infusions of adenosine on the renin release response to well-defined stimuli in the dog. In our first study, we examined the effects of exogenous adenosine on beta-adrenoceptor-induced renin release (98). Beta-adrenoceptor-mediated renin release was induced either by electrically stimulating the renal nerves in filtering kidneys or by intrarenal infusions of norepinephrine in nonfiltering kidneys. In both sets of experiments, any actions of norepinephrine via alpha-adrenoceptors were obviated with an intrarenal infusion of phentolamine. The nerve stimulation protocol permitted activation of those physiologically relevant intrarenal beta-adrenoceptors that are ordinarily innervated in a normal filtering kidney.

However, this approach made interpretation problematical since adenosine could have attenuated renin release by inhibiting norepinephrine release and/or by altering renal excretory function. The norepinephrine infusion protocol, although less physiological, allowed activation of intrarenal beta-adrenoceptors by the naturally occurring neurotransmitter, norepinephrine, yet circumvented the difficulties of prejunctional and tubular effects of adenosine. Importantly, low-dose intrarenal infusions of adenosine greatly attenuated the renin release response to renal nerve stimulation and abolished the renin release response to intrarenal infusion of norepinephrine. Consistent with the model that adenosine attenuates renin release responses by inhibiting adenylate cyclase (Figure 1), adenosine did not attenuate the renin release response to intrarenal infusions of dibutyryl-cAMP in nonfiltering, beta-adrenoceptor blocked kidneys.

In a subsequent experimental series (99), we examined the ability of adenosine to attenuate the renin release response to simultaneous activation of the intrarenal baroreceptor and macula densa pathways. Again, two approaches were employed. In the first set of experiments, these dual pathways were activated through the use of the physiological stimulus of renal artery hypotension in the filtering kidney and in the second, by intrarenal infusions of a putative mediator of these pathways, prostacyclin (Figure 1). These latter experiments, however, were performed in nonfiltering kidneys to avoid the renal excretory effects of adenosine. Both sets of experiments were performed in dogs receiving an intravenous infusion of propranolol to block the adrenergic pathway to renin release. Low-dose, intrarenal infusions of adenosine greatly attenuated the renin release response to both renal artery hypotension and intrarenal infusions of prostacyclin. Taken together, our data indicate that exogenous adenosine can attenuate renin release induced by the three major physiological pathways, and that adenosine most likely exerts this effect by intercepting signals being fed into the adenylate cyclase system.

Concurrent with our studies, Barchowsky et al (92) investigated the effects of adenosine and adenosine analogues on the renin release response to beta-adrenoceptor agonists and prostaglandin E_1 in rabbit cortical slices. This study provides a timely in vitro correlate of our whole animal work in that stimulation of renin release by prostaglandins in vitro corresponds to activation of renin release via the intrarenal baroreceptor and macula densa pathways in vivo and treatment with beta-adrenoceptor agonists in vitro corresponds to stimulation of renal nerves in vivo. Importantly, these investigators found that adenosine and adenosine analogues attenuated the renin release response to both beta-adrenoceptor agonists and to prostaglandin E_1.

As postulated by the adenosine-brake hypothesis, adenosine most likely attenuates renin release by inhibiting adenylate cyclase activity. This conclusion is based on several observations: (*a*) Since A_1 receptors mediate adeno-

sine-induced inhibition of renin release and are negatively coupled to adenylate cyclase in most tissues, and cAMP stimulates renin release, it is logical to conclude that adenosine attenuates renin release, in part at least by inhibiting adenylate cyclase. (*b*) Adenosine inhibits the renin release response to stimuli known to activate adenylate cyclase, e.g. beta-adrenoceptor agonists and prostaglandins, yet does not inhibit renin release induced by dibutyryl-cAMP. (*c*) Uncoupling adenosine receptors from G_i with pertussis toxin reverses adenosine receptor-mediated inhibition of renin release in rat renal cortical slices (100).

Nonetheless, Kurtz et al (93) found that in cultured, but unstimulated, juxtaglomerular cells adenosine did not decrease intracellular concentrations of cAMP but did increase intracellular concentrations of cGMP. These investigators concluded that cGMP mediates the inhibitory effects of adenosine on renin release. One explanation for these unanticipated results is that the mode of adenosine's action may depend on whether renin release is stimulated by a hormone acting through adenylate cyclase. In other words, adenosine may inhibit basal renin release by increasing cGMP and hormone-stimulated renin release by preventing activation of adenylate cyclase. However, it remains to be explained how adenosine increases cGMP since all well-studied A_1 receptors are coupled to G-proteins, and to date all receptor systems known to *directly* stimulate guanylate cyclase have guanylate cyclase as a part of their cytoplasmic domains, i.e. G-proteins are not known to couple receptors to this enzyme.

Effects of Adenosine Receptor Antagonists on Renin Release

Theophylline competitively antagonizes both A_1 and A_2 receptors (101). Therefore, it is not surprising that this methylxanthine attenuates the renin inhibitory effects of adenosine and adenosine analogues. For instance, theophylline blocks adenosine-induced inhibition of renin release in cultured rat juxtaglomerular cells (93) and intact canine kidneys (84). Other, more potent, xanthine derivatives also interfere with the effects of adenosine on renin release. For example, 8-phenyltheophylline blocks the effects of adenosine on renin release in rabbit cortical slices (92), and XAC, an amine congener of 1,3-dipropylxanthine and a potent adenosine receptor antagonist, blocks the effects of an adenosine analogue, cyclohexyladenosine, on renin release in rat cortical slices (102). Given the availability of compounds proven to block the renin inhibitory effects of adenosine, it should be possible to test the adenosine-brake hypothesis in vivo using these pharmacological probes.

If the adenosine-brake hypothesis is valid, then administration of adenosine receptor antagonists should augment the renin release response to activation of one or more of the three physiological pathways to renin release. In the early 1970s, the effects of theophylline on the renin release response were

examined in two separate studies. Consistent with the adenosine-brake hypothesis, Reid and colleagues (103) concluded that in dogs theophylline enhanced the renin release response to circulating epinephrine. In contrast, Johns & Singer (104) reported that theophylline did not potentiate the renin release response to furosemide in the cat. Since furosemide blocks ion transport in the thick limb of Henle's loop, furosemide stimulates renin release primarily by deceiving the macula densa about the concentration of ions in the adjacent lumen (2). Therefore, if the adenosine-brake hypothesis is correct, furosemide-induced renin release should be potentiated by adenosine receptor antagonists.

Conflicting data were also reported in the mid 1980s. In one study, theophylline was found to potentiate the renin release response to renal artery hypotension and isoproterenol—but not to dibutyryl cAMP—in anesthetized dogs (105). On the other hand, Premem et al (106) reported that in the dog theophylline did not alter the increase in plasma renin activity associated with reductions in renal artery pressure.

We hypothesized that the reason for disparity in previously published studies was that theophylline-induced natriuresis confounded the situation. Thus, even though blockade of adenosine receptors with theophylline may have disinhibited the adenosine brake on renin release, increased delivery of sodium chloride to the macula densa tended to suppress renin release. Consequently, the overall effect of theophylline on the renin release response depended on the natriuretic response to theophylline, which in turn was a function of the physiological state of the animal.

To test this hypothesis, we examined the effects of theophylline on the renin release response to renal artery hypotension in both filtering and nonfiltering canine kidneys (107). In the filtering kidney, intrarenal infusions of theophylline greatly increased the urinary excretion of sodium, but did not increase the renin release response to renal artery hypotension. However, in kidneys rendered nonfiltering (so that changes in sodium chloride delivery to the macula densa were impossible), theophylline markedly enhanced the renin release response to renal artery hypotension. Interestingly, the natriuretic response to an intrarenal infusion of the adenosine receptor antagonist, caffeine, was much less than the natriuretic response to theophylline, and caffeine enhanced the renin release response even in filtering kidneys. These results support the conclusion that endogenous adenosine limits the renin release response to a decrease in renal perfusion pressure.

Although two adenosine receptor blockers, theophylline and caffeine, augment the renin response to renal artery hypotension, as pharmacological probes for the role of endogenous adenosine, these drugs have serious drawbacks. These methylxanthines easily penetrate cell membranes, and once inside the cell, can inhibit phosphodiesterase (108) and cause release of

calcium from intracellular stores (109). Given the importance of cAMP and calcium in signalling pathways within the juxtaglomerular cell, these effects of caffeine and theophylline are particularly worrisome.

To circumvent the problem of "nonspecific" intracellular effects of adenosine receptor antagonists, we examined the effects of another adenosine receptor blocker, 1,3-dipropyl-8-(p-sulfophenyl)xanthine (DPSPX) (110), on the renin release response to various stimuli. We reasoned that since DPSPX is negatively charged at physiological pH, this adenosine receptor antagonist would not penetrate cell membranes and, therefore, would not exert intracellular nonspecific effects.

To test this assumption (110a), a precursor of DPSPX was synthesized and tritiated. The resulting radiolabelled DPSPX was purified by high-performance liquid chromatography and added to unlabelled DPSPX. This radiolabelled material was infused into rats using the same protocol (infusion rate, dose, time, route, etc) employed in our renin release studies with DPSPX (see below). Only trace amounts of radioactivity were found in red blood cells or in the brain of rats receiving radiolabelled DPSPX, indicating that DPSPX did not significantly penetrate either the erythrocyte cell membrane or the membranes that compose the blood-brain barrier. In addition, plasma samples were analyzed for radiolabelled and unlabelled (UV absorption) DPSPX by high-performance liquid chromatography. The only radioactive peak in the chromatograms was unchanged DPSPX. Further, when plasma levels of DPSPX were calculated based on the assumption that all of the radioactivity in plasma was unchanged DPSPX, these results gave concentrations of DPSPX that were not significantly different from the concentrations determined by UV analysis of unlabelled material. Thus, we concluded that metabolism of DPSPX by the liver was trivial. This conclusion suggested that DPSPX had great difficulty penetrating membrane barriers in the liver and gaining assess to microsomal enzymes. The overall lesson of this study is that DPSPX is—for all practical purposes—restricted to the extracellular space.

Although our previous renin release studies with adenosine, theophylline, and caffeine were conducted in dogs, subsequent studies were conducted in rats because of the large amounts of DPSPX required for in vivo work. In our first study with DPSPX (111), either normal or sodium-depleted rats were prepared for in situ perfusion of their left kidneys using the method originally described by Fink & Brody (112). Briefly, an extracorporeal shunt was established between the carotid artery and lower abdominal aorta, and the aorta was ligated above the left renal artery. This created an aortic pouch so that flow through the shunt was equivalent to renal blood flow, which was measured with a blood flow probe. The left ureter was cannulated for collection of urine, and a needle was placed in the renal vein for collection of renal venous blood.

DPSPX significantly increased arterial and renal venous plasma renin activity in both normal-sodium and low-sodium animals; however, DPSPX increased plasma renin activity more in low-sodium rats than normal-sodium rats. In low-sodium, but not normal-sodium, animals, DPSPX significantly increased the venoarterial difference of renin activity across the kidney and increased the calculated net renal secretion of renin. In this study, the effects of DPSPX could not be accounted for by changes in renal hemodynamics or renal excretory function. In an additional set of studies in low-sodium rats, we observed that the effects of DPSPX on renin release were not altered by blocking beta-adrenoceptors with propranolol. This indicated that DPSPX did not enhance renin release by potentiating sympathetic neurotransmission within the kidney. Since DPSPX augmented the renin release response to a low-sodium diet, and since chronic salt depletion activated all three physiological pathways that stimulate renin release, these results strongly supported the adenosine-brake hypothesis.

In a subsequent study (113), we extended these initial observations by examining the effects of DPSPX on renin release induced by other stimuli, both in the in situ perfused rat kidney and in the conscious, unrestrained rat. In both experimental models, DPSPX enhanced the renin release response to hydralazine-induced hypotension and to chronic renal artery stenosis (2-kidney, 1-clip renovascular hypertensive rats). Again, the effects of DPSPX in these experiments could not be explained by DPSPX-induced changes in renal hemodynamics or renal excretory function. Also, DPSPX did not interfere with the renin assay and did not alter the rate of elimination of renin from the circulation. In yet another study (114), we demonstrated that DPSPX augmented the renin release response to furosemide without altering the renal excretory response or hemodynamic response to furosemide. Thus, all of our studies with this cell membrane-impermeable adenosine receptor antagonist were consistent with the adenosine-brake hypothesis.

Effects of Adenosine Deaminase on Renin Release

Even though DPSPX apparently does not penetrate cell membranes to any significant degree, this compound may exert nonspecific effects extracellularly that could affect renin release responses. Therefore, it would be reassuring if other strategies for altering the activity of endogenous adenosine affected renin release responses in a manner consistent with the adenosine-brake hypothesis.

One method to alter the concentrations of endogenous adenosine is to infuse the enzyme, adenosine deaminase. If sufficient enzyme can be delivered to the interstitial space, degradation of adenosine to inosine would be accelerated so that the interstitial concentrations of adenosine would decrease. This strategy has been employed successfully to study the role of adenosine in other physiological and pathophysiological processes (115).

To further test the adenosine-brake hypothesis, the effects of adenosine deaminase—infused into the arterial circulation—on hydralazine-induced renin release were investigated in conscious, unrestrained rats (113). Adenosine deaminase, in a dose-related fashion, enhanced hydralazine-induced renin release. Further, the slope of the relationship between the hydralazine-induced decrease in arterial blood pressure and the plasma renin activity was markedly steeper in animals treated with adenosine deaminase than in control rats. Our studies with adenosine deaminase were entirely consistent with the adenosine-brake hypothesis.

Effects of Adenosine Deaminase Inhibition on Renin Release

Rather than decrease endogenous concentrations of adenosine with exogenous adenosine deaminase, the reverse strategy, i.e. administration of an adenosine deaminase inhibitor, can also be employed to assess the in vivo significance of endogenous adenosine as a modulator of renin release. Such a strategy should result in an increase of endogenous adenosine. Therefore, provided that endogenous adenosine is not exerting maximal inhibition of renin release in the absence of an inhibitor of adenosine deaminase, if the adenosine-brake hypothesis is correct, inhibition of adenosine deaminase should attenuate the renin release response.

We tested this prediction in conscious, unrestrained rats treated with hydralazine to stimulate renin release (113). In this study, the potent adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), was infused intravenously into rats treated with hydralazine. Plasma renin activity tended to be lower in EHNA-treated rats than in control animals, although this effect was not statistically significant. However, a trend analysis on plasma renin activity in EHNA-treated rats versus control rats versus adenosine deaminase-treated rats indicated that plasma renin activity increased in the order: EHNA-treated < control < adenosine deaminase-treated. In control and adenosine deaminase-treated rats, a significant linear relationship was observed between plasma renin activity and the hypotensive response to hydralazine. It is significant that this relationship was abolished in rats treated with EHNA. Although the results of the studies with EHNA were consistent with the adenosine-brake hypothesis, they were not convincing, perhaps because endogenous adenosine was causing a near maximal effect even before EHNA was administered.

IMPLICATIONS OF THE ADENOSINE-BRAKE HYPOTHESIS FOR RENOVASCULAR HYPERTENSION

Caffeine is both an effective adenosine receptor antagonist and the most widely ingested psychotropic drug in the world. These facts render as obvious

the significance of the adenosine-brake hypothesis to public health. If adenosine functions to restrain the renin release response to physiological and pathophysiological stimuli, then caffeine ingestion may amplify any given renin release response by disabling the adenosine brake on the juxtaglomerular cell. This possible interaction between caffeine and the renin-angiotensin system may be particularly important in patients with high-renin renovascular hypertension. Since over-secretion of renin initiates and maintains hypertension in this disease, caffeine-induced potentiation of renin release could worsen the hypertension.

To test both the role of the adenosine-brake hypothesis in high-renin renovascular hypertension and the significance of disabling this putative restraining mechanism with caffeine, we investigated (116) the effects of chronic caffeine administration on the blood pressure response to unilateral renal artery clipping in rats, i.e. 2-kidney, 1-clip renovascular hypertensive rats (an experimental model of high-renin renovascular hypertension). In this study, rats were given either normal drinking water or drinking water containing 0.1% caffeine. This dose of caffeine provided pharmacologically relevant plasma concentrations of approximately 10 μg/ml (determined by high-performance liquid chromatography) and caused a significant, parallel, rightward shift of the adenosine dose-response curve (with respect to adenosine-induced reductions in arterial blood pressure). As shown in Figure 2, chronic caffeine administration markedly exacerbated the rise in systolic blood pressure in 2-kidney, 1-clip renovascular hypertensive rats. This effect of caffeine was observed when caffeine treatment was initiated at the time of renal artery clipping as well as three weeks after clipping the renal artery. Further, caffeine caused a sevenfold increase in plasma renin activity in 2-kidney, 1-clip rats. Importantly, chronic treatment with caffeine did not alter arterial blood pressure or plasma renin activity in sham-operated rats (Figure 2). In a subsequent study, we also demonstrated that caffeine augmented the hypertensive and hyperreninemic response to ligation of the suprarenal aorta, i.e. another experimental model of high-renin renovascular hypertension (117).

To test the hypothesis that caffeine exacerbated 2-kidney, 1-clip renovascular hypertension by activating the renin-angiotensin system, the effects of caffeine on arterial blood pressure were also examined in rats treated with the angiotensin converting enzyme inhibitor, enalapril (116). As long as enalapril was administered, arterial blood pressure remained low in 2-kidney, 1-clip rats regardless of whether the animals were treated with caffeine (Figure 3). However, when enalapril was withdrawn, arterial blood pressure rose much more rapidly in caffeine-treated, 2-kidney, 1-clip rats compared to 2-kidney, 1-clip rats not treated with caffeine. These results demonstrated that caffeine potentiated 2-kidney, 1-clip renovascular hypertension because of an interaction with the renin-angiotensin system.

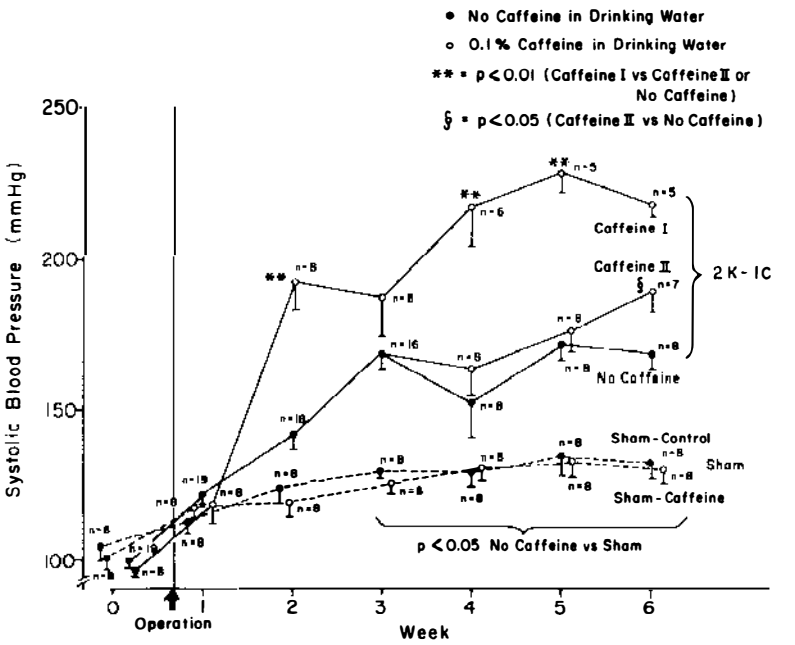

Figure 2 Chronic administration of caffeine in drinking water (0.1%) exacerbated the development of hypertension in 2-kidney, 1-clip renovascular hypertensive rats (2K-1C) regardless of whether caffeine treatment was begun at the time of clipping the left renal artery (Caffeine I group) or 3 weeks after clipping the left renal artery (Caffeine II group). Chronic caffeine treatment did not elevate systolic blood pressure in sham-operated (sham) rats. "Operation" indicates time when renal artery was clipped or rat underwent a sham procedure. Two-factor analysis of variance indicated a significant difference in the time-pressure relationship for No Caffeine group versus: Caffeine I group, Caffeine II group, Sham-Control group, and Sham-Caffeine group. ● = no caffeine in drinking water; o = 0.1% caffeine in drinking water; ** = $P < 0.01$ for caffeine I versus no caffeine by unpaired Student's t test; § = $P < 0.05$ for caffeine II versus no caffeine by unpaired Student's t test. (Reproduced from 116, with permission.)

If caffeine exacerbates hypertension and hyperreninemia in high-renin renovascular hypertension by disabling the adenosine brake on renin release, then caffeine ought not to increase arterial blood pressure or plasma renin activity in experimental models of hypertension in which the juxtaglomerular cells are not stimulated to release renin. To test this prediction, we examined the effects of chronic caffeine on the development of hypertension in spontaneously hypertensive and 1-kidney, 1-clip renovascular hypertensive rats (117). Both these models of high blood pressure are characterized by normal plasma levels of renin. As expected, caffeine did not increase either blood pressure or plasma renin levels in either experimental model (Figure 4).

Clearly, the effects of caffeine on renin release explain in part the ability of

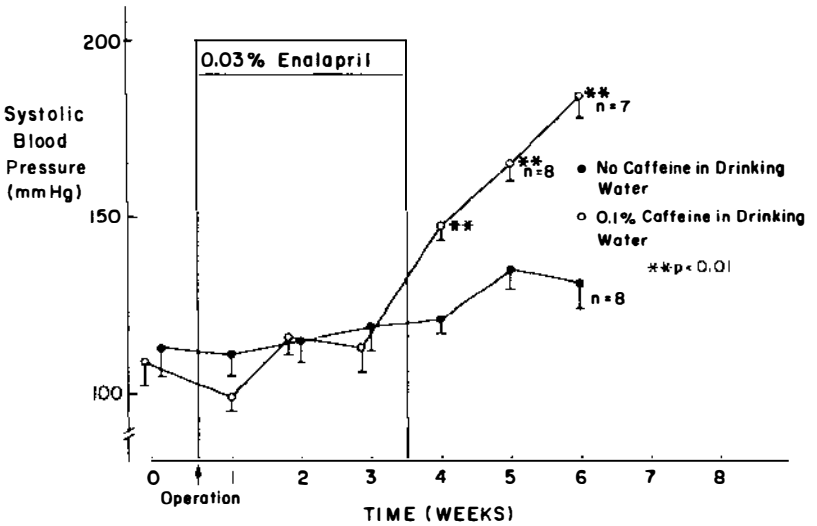

Figure 3 Treatment with enalapril (an angiotensin I converting enzyme inhibitor) prevented chronic administration of caffeine from exacerbating 2-kidney, 1-clip renovascular hypertension. "Operation" indicates time when left renal artery was clipped. Enalapril and caffeine were administered in drinking water (0.03% and 0.1%, respectively). When enalapril was withdrawn, systolic blood pressure rose more rapidly in the caffeine-treated rats than in rats not treated with caffeine (2-factor analysis of variance). ** = $P < 0.01$ for caffeine group versus no caffeine group by unpaired Student's t test. (Reproduced from 116, with permission.)

caffeine to increase arterial blood pressure in rats with high-renin hypertension. However, this may not be the entire explanation. Caffeine may increase arterial blood pressure in high-renin hypertension in part by enhancing the pressor activity of angiotensin II. We recently tested this possibility by examining the effects of chronic caffeine treatment on both the rapid-pressor response to acute intravenous infusions of angiotensin II and the slow-pressor response to long-term infusions of angiotensin II (118). Although caffeine did not alter the rapid-pressor response to angiotensin II in conscious, unrestrained rats, caffeine markedly exacerbated the slow-pressor response to angiotensin II.

Caffeine did not, however, enhance the slow-pressor response to angiotensin II in rats sympathectomized with 6-hydroxydopamine. These results indicate that caffeine enhances the slow-pressor response to angiotensin II by a mechanism involving the sympathetic nervous system. Several such mechanisms could be envisioned given the known pharmacology of angiotensin II and adenosine. For instance, blood-borne angiotensin II can increase sympathetic tone and arterial blood pressure via effects on periventricular nuclei

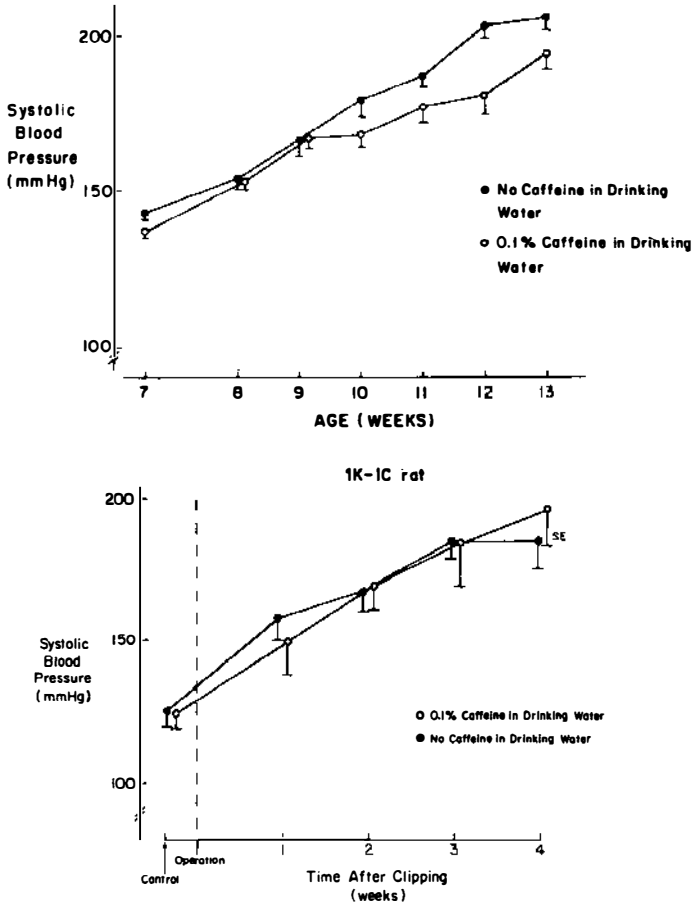

Figure 4 Chronic administration of caffeine did not elevate systolic blood pressure in either spontaneously hypertensive rats (top panel) or rats with 1-kidney, 1-clip renovascular hypertension (bottom panel). In the top panel, x-axis refers to age of rats, and in the bottom panel, x-axis indicates time after clipping left renal artery and removing right kidney. Number of animals in each group is 7 or 8. (Top panel reproduced from 116, with permission; bottom panel reproduced from 117, with permission.)

that lack a blood-brain barrier (119). Also, angiotensin II potentiates sympathetic neurotransmission by facilitating norepinephrine release, decreasing norepinephrine uptake, and enhancing the postjunctional effects of norepinephrine (120), and these actions of angiotensin II may contribute to its slow-pressor effect. On the other hand, central administration of adenosine inhibits sympathetic tone (121, 122), and peripheral sympathetic

neurotransmission is inhibited by adenosine (123). It is possible, therefore, that adenosine restrains the central and/or peripheral effects of angiotensin II on the sympathetic nervous system. If so, caffeine—by blocking these actions of adenosine—could increase the slow-pressor response to angiotensin II by enhancing the ability of angiotensin II to increase sympathetic tone and/or to facilitate neurotransmission.

Recently, we tested the hypothesis that adenosine modulates the effect of angiotensin II on peripheral noradrenergic neurotransmission (124). In this study, the effects of exogenous adenosine and DPSPX on vascular responses to sympathetic nerve stimulation were investigated in the in situ blood-perfused rat mesentery. Adenosine did not attenuate and DPSPX did not enhance the ability of angiotensin II to facilitate sympathetic neurotransmission. These data indicate that adenosine does not attenuate angiotensin II-induced facilitation of neurotransmission.

We have not yet investigated whether adenosine modulates the ability of angiotensin II to increase sympathetic tone via angiotensin receptors in periventricular nuclei. However, we have examined the effects of intravenous infusions of DPSPX on the bradycardic response to intravenous infusions of angiotensin II in conscious, unrestrained rats (125). Interestingly, bradycardic responses to intravenous infusions of angiotensin II were attenuated in animals receiving DPSPX. On the other hand, bradycardic responses to intravenous infusions of angiotensin II were increased by rendering animals hypersensitive to adenosine. Hypersensitivity to adenosine was induced by subjecting rats to one week of caffeine treatment (0.1% in drinking water) followed by abrupt withdrawal of caffeine 18 hours before the experiment.

Subsequently, we examined the effects of both intravenous infusions of DPSPX and caffeine withdrawal on the cardiovascular response to microinjections of angiotensin II into the area postrema (a blood-brain barrier deficient structure). In this study (E.K.J., manuscript in preparation), microinjections of angiotensin II into the area postrema actually decreased heart rate and blood pressure, and these responses were attenuated by DPSPX and enhanced by caffeine withdrawal. These results suggest that angiotensin receptors in the area postrema may decrease heart rate and blood pressure by a mechanism involving adenosine. Caffeine, by blocking adenosine receptors, could prevent these effects of angiotensin II and thereby increase the slow-pressure response to angiotensin II. Finally, a recent study by Mosqueda-Garcia et al (126) demonstrates that blockade of adenosine receptors in the nucleus of the solitary tract attenuates baroreceptor-mediated bradycardia, which indicates another possible site where caffeine could alter angiotensin II-induced hypertension.

To summarize, chronic blockade of adenosine receptors with caffeine

exacerbates high-renin renovascular hypertension by two mechanisms. First, caffeine increases renin release. This action of caffeine is due in part to disabling of the adenosine brake on juxtaglomerular cells; however, it is possible that caffeine increases plasma renin activity by additional mechanisms as well. Second, caffeine increases the slow-pressor response to angiotensin II. This action of caffeine is mediated via the sympathetic nervous system by an undefined mechanism.

Does caffeine worsen high-renin renovascular hypertension in humans? To my knowledge, this question has not been systematically addressed. Epidemiological studies involving large numbers of people reveal only a very slight influence of caffeine on arterial blood pressure (127). However, humans with renovascular hypertension are rare. Therefore, even if caffeine exacerbates high-renin renovascular hypertension in humans, this phenomenon could easily go unnoticed.

One case in which caffeine may have exacerbated renovascular hypertension was seen at the Vanderbilt University Medical Center Hypertension Clinic (J. A. Oates, personal communication). A patient who was diagnosed to have unilateral renovascular hypertension and whose blood pressure was monitored at home noticed that her hypertension was worse when she drank coffee and, consequently, was advised by her physician not to drink it. She heeded this advice and her blood pressure normalized without medication. However, when she became complacent and resumed drinking coffee, a hypertensive crisis occurred shortly thereafter. Although this anecdote does not establish a detrimental effect of caffeine in human renovascular hypertension, it does point out the need to investigate the adenosine-brake hypothesis in humans.

Clinical implications of the adenosine-brake hypothesis may extend beyond the infrequent setting of renovascular hypertension. Antihypertensive treatments such as salt restriction, diuretics, and vasodilators stimulate renin release, and activation of the renin-angiotensin axis by these therapies attenuates their antihypertensive potential and contributes to variability in blood pressure response to these therapies. If the adenosine-brake hypothesis is correct, dietary caffeine intake, by disabling the adenosine brake on renin release, may augment the renin release response to salt restriction, diuretics, and vasodilators. Thus, even though dietary caffeine intake may not elevate blood pressure in patients with essential hypertension, caffeine ingestion may both limit and cause variability in the therapeutic response to salt restriction, diuretics, and vasodilators. Similarly, caffeine could worsen the cardiovascular status or limit the therapeutic response to salt restriction and diuretics in patients with congestive heart failure or liver cirrhosis by augmenting renin release.

POSSIBLE SOURCES OF THE ADENOSINE THAT REGULATES RENIN RELEASE

Release of Adenosine from the Macula Densa

If adenosine acts to restrain renin release responses to physiological stimuli, the question arises as to the source of the adenosine that subserves this function. One likely source of adenosine is the macula densa cells adjacent to the renin-secreting juxtaglomerular cells.

The hypothesis that the macula densa releases adenosine to adjacent cells was first proposed to explain the phenomenon known as tubuloglomerular feedback. An increased concentration of sodium chloride at the macula densa causes a reduction in single nephron plasma flow and single nephron glomerular filtration rate (reviewed in ref. 128), and this glomerular response to changes in distal tubular sodium chloride concentration is termed tubuloglomerular feedback (TGF). TGF serves to protect the organism from excessive loss of water and electrolytes by limiting the ultrafiltrate load of each individual nephron to that which can be efficiently processed by that nephron unit. Osswald and coworkers (129) hypothesize that when the sodium chloride concentration at the macula densa is increased, the metabolic energy consumed by macula densa cells to reabsorb the additional solute in the ultrafiltrate would be increased proportionately. This in turn would deplete cellular stores of ATP and cause a corresponding increase in cellular levels of adenosine. Adenosine within the cell could then escape to the extracellular space via the adenosine transporter (reviewed in ref. 130), diffuse to the nearby afferent arteriole, and cause vasoconstriction of this preglomerular resistance vessel. An increase in preglomerular vascular resistance would reduce single nephron plasma flow and single nephron glomerular filtration rate. In addition, adenosine released from the macula densa could inhibit renin release from juxtaglomerular cells located in the wall of the afferent arteriole.

This hypothesis has substantial experimental support. For instance, TGF responses (reductions in stop flow pressure or single nephron glomerular filtration induced by increases in distal tubular perfusion) are attenuated by methylxanthines that block adenosine receptors. Although initial experiments were performed with theophylline (131, 132), Schnermann et al (133) recently demonstrated that a highly selective A_1 receptor antagonist completely abolished TGF responses.

The whole-kidney analogue of the TGF response is the reduction in whole-kidney renal blood flow, glomerular filtration rate, and renin release that occur when hypertonic saline is infused into the renal artery (134). The concept here is that intrarenal infusions of hypertonic saline increase the

concentration of sodium chloride at the macula densa and this in turn triggers simultaneous TGF responses in many nephron units. Interestingly, intrarenal infusions of hypertonic saline increase tissue concentrations of adenosine (129, 131), and the reduction in renin secretion (135) and renal blood flow (136, 137) induced by intrarenal infusions of hypertonic saline are blocked by theophylline. Also, we demonstrated that a very potent nonxanthine adenosine receptor antagonist (CGS 15943A) blocked hypertonic saline-induced reductions in renal blood flow in the dog (138).

Additional evidence that TGF is mediated by adenosine is that blockade of the renin-angiotensin system (high salt diet, converting enzyme inhibitors, angiotensin antagonists) also attenuates TGF (139–141). This would be expected if adenosine mediates TGF since adenosine-induced constriction of the afferent arteriole requires conditioning of the afferent arteriole with angiotensin II (142–145). Indeed, we recently showed that whole kidney renal blood flow responses to hypertonic saline were: (*a*) attenuated by theophylline; (*b*) attenuated by captopril; (*c*) restored by angiotensin II in captopril-pretreated animals; and (*d*) attenuated by theophylline in animals whose responses had been attenuated by captopril and then restored by angiotensin II (146).

Perhaps the most convincing evidence for release of adenosine from the macula densa was published by Itoh et al (91). These investigators found that renin release from afferent arterioles without an attached macula densa was greater than the renin release from afferent arterioles with an attached macula densa. Exogenous adenosine inhibited renin release from afferent arterioles without an attached macula densa, but did not inhibit renin release from afferent arterioles with an attached macula densa. Conversely, theophylline increased renin release from afferent arterioles with an attached macula densa, but did not affect renin release from afferent arterioles without an attached macula densa. These data strongly support the hypothesis that macula densa cells release adenosine to the adjacent juxtaglomerular cells and that this adenosine serves to inhibit renin release. This hypothesis is further strengthened by the recent observation by Weihprecht et al (147) that specific blockade of A_1 receptors with 8-cyclopentyl-1,3-dipropylxanthine attenuates the decrease in renin release induced by high concentrations of sodium chloride in the single isolated rabbit juxtaglomerular apparatus with an intact macula densa.

Although much evidence indicates that macula densa cells feed adenosine to the adjacent afferent arteriole and juxtaglomerular cells, this cannot be the only, or perhaps even the primary, adenosine input to the juxtaglomerular cells. If the adenosine that brakes renin release is derived only from the macula densa, then blockade of adenosine receptors ought not to affect renin release in situations where solute delivery to the macula densa is decreased or transport activity of the macula densa is suppressed. However, our studies

clearly indicate a greater role for endogenous adenosine (in restraining renin release) when either solute delivery to the macula densa is decreased (nonfiltering kidney (107), salt depletion (111), arterial hypotension (113), and renal artery clipping (113, 116)) or transport activity of the macula densa is decreased with furosemide (114). In each study, blockade of adenosine receptors with either theophylline, caffeine or DPSPX increased renin release more when animals were subjected to the aforementioned maneuvers. How else then is adenosine presented to the juxtaglomerular cells?

Release of Adenosine by Renal Ischemia

In general, the production rate of adenosine by any tissue increases when energy demand exceeds energy supply (148). Therefore, it is not surprising that total renal ischemia for one minute or longer increases tissue and urinary levels of adenosine in cats, dogs, and rats (149, 150). Most likely, accumulation of adenosine during complete renal artery occlusion is responsible for the reactive ischemic response that is unique to the kidneys (other organs display a reactive hyperemia in response to arterial occlusion) since the reactive ischemic response is blocked by theophylline (151).

Although extreme renal ischemia, i.e. complete occlusion of the renal artery, increases renal tissue concentrations of adenosine, more moderate changes in renal perfusion pressure do not (152). Because the kidney receives far more blood than is necessary to provide an adequate supply of nutrients and oxygen, a priori it is unlikely that moderate changes in renal blood flow would cause energy demand to outstrip energy supply. Therefore, under most physiological and pathophysiological conditions, it is improbable that the adenosine that regulates renin release is produced secondary to an inadequate renal blood flow. This point is cogently made by the observation that DPSPX increases renin release in rats with a normal renal blood flow (111, 113).

Angiotensin-Induced Adenosine Release

Fredholm & Hedqvist (153) reported in 1978 that angiotensin II releases radiolabelled adenosine from isolated, perfused rabbit kidneys that were preloaded with radiolabelled adenine. Therefore, endogenous angiotensin II may possibly stimulate adenosine release from tissues. If so, the following negative feedback loop would be operative: increased rate of renin release → increased concentrations of angiotensin II → increased concentrations of adenosine → decreased rate of renin release.

As a preliminary check on the hypothesis that angiotensin-induced adenosine release may be physiologically significant, we measured plasma adenosine concentrations in normal rats, spontaneously hypertensive rats, rats with 2-kidney, 1-clip renovascular hypertension, and rats with renovascular

hypertension induced by ligation of the suprarenal aorta (117, 154, 155). Note that these initial measurements were performed in animals that were anesthetized and had undergone a laparotomy. Interestingly, under these conditions, plasma adenosine concentrations were elevated several-fold higher in rats with renovascular hypertension than in normotensive rats or rats with genetic hypertension. However, the extent to which plasma adenosine concentrations were elevated in renovascular hypertensive rats depended greatly on the time after inducing renal artery ischemia. Plasma concentrations of adenosine were elevated more in animals with recently induced renovascular hypertension than in animals that had been hypertensive for longer periods of time. Also, adenosine concentrations in renovascular hypertensive rats were higher in the arterial plasma than in renal venous plasma. Taken together, these results suggested that endogenous angiotensin II may have induced adenosine release from the lungs and perhaps other tissues also.

As an initial test of this hypothesis, we investigated the effects of bolus injections of angiotensin II, as well as other vasoactive agents, on radiolabelled adenosine release from the in situ, Kreb's solution-perfused rat lung preparation (156). In this study, we found that: (*a*) angiotensin II potently released adenosine from the rat lung; (*b*) tachyphylaxis to this effect was induced by higher doses of angiotensin II; (*c*) angiotensin-induced adenosine release was receptor mediated and was not related to changes in pulmonary vascular resistance; and (*d*) angiotensin II was more efficacious as an adenosine releaser than norepinephrine, bradykinin, or vasopressin.

Encouraged by these findings and by our previous in vivo observations in renovascular hypertensive rats, we then examined the effects of acute intravenous infusions of angiotensin II on plasma adenosine levels in anesthetized rats (157). The results of these studies were entirely consistent with our observations in the in situ lung model. Intravenous infusion of angiotensin II increased pulmonary venous (i.e. left ventricular) levels of adenosine, as well as and as much as systemic venous concentrations of adenosine. Also, as in the in situ lung model, higher doses of angiotensin II caused tolerance to angiotensin-induced adenosine release.

However, in all these studies, angiotensin-induced adenosine release was measured under nonphysiological conditions (i.e. renal kidney slices, lungs perfused with Kreb's solution, and anesthetized, surgically traumatized animals) with acute exposure to relatively high doses of angiotensin II. To determine whether angiotensin-induced adenosine release might be important under more physiological conditions, we measured arterial concentrations of adenosine in conscious, unrestrained rats exposed chronically (several days) to physiologically relevant concentrations of exogenous angiotensin II (low-dose infusions of angiotensin II with implanted osmotic minipumps) or

endogenous angiotensin II (2-kidney, 1-clip rats and rats with aortic ligation). In these studies, neither exogenous nor endogenous angiotensin II increased arterial plasma concentrations of adenosine (157).

Taken together, the data support the following conclusions regarding angiotensin-induced adenosine-release: (*a*) angiotensin II releases adenosine from isolated kidneys and lungs; (*b*) in anesthetized, surgically traumatized rats, acute exposure to high levels of either exogenous or endogenous angiotensin II induces adenosine release from the lungs and perhaps other tissues as well; (*c*) in conscious, unrestrained animals, chronic exposure to physiologically relevant concentrations of exogenous and endogenous angiotensin II does not increase circulating concentrations of adenosine. These conclusions indicate that in vivo when normal conditions are operative, angiotensin-induced adenosine release from the lungs or other tissues is not pronounced enough to significantly alter circulating adenosine concentrations. However, local production of adenosine by angiotensin II may still increase under normal in vivo conditions within the kidneys, lungs, or other tissues. This hypothesis will have to be tested by measuring interstitial concentrations of adenosine with dialysis probes in conscious animals.

Transmembrane Negative Feedback Loop

In 1963, Davoren & Sutherland (158) reported that stimulation of adenylate cyclase caused egress of cAMP from pigeon erythrocytes. It is now appreciated that egress of cAMP out of cells following stimulation of adenylate cyclase is ubiquitously associated with hormonal stimulation of the enzyme (159). Also, cAMP egress is both unidirectional (160) and energy dependent (161). Recently, Krupinski et al (162) cloned and sequenced the cDNA for adenylate cyclase isolated from a bovine brain library. The deduced amino acid sequence of adenylate cyclase suggests that this enzyme has the topography of a transporter. Therefore, the enzyme itself may be responsible for actively pumping cAMP out of the cell onto the extracellular face of the cell membrane.

As already mentioned Tagawa & Vander (1) were the first to report that adenosine inhibits renin release. In that same study, they also examined the effects of AMP and cAMP on renin release. Intrarenal infusions of AMP were nearly as effective as adenosine in inhibiting renin release. It is well-known that a rich supply of ecto-5'-nucleotidase (the enzyme that converts AMP to adenosine) is anchored, perhaps via a glycophospholipid attachment (163), to the cell membrane of endothelial (164–166) and vascular smooth muscle cells (164, 167, 168), with the active end of this enzyme protruding into the extracellular space. Therefore, AMP is rapidly converted to adenosine on the extracellular surface of the cell membrane, and this probably explains why

AMP inhibits renin release. Tagawa & Vander also observed that in four dogs with elevated basal secretion rates of renin, intrarenal infusions of cAMP inhibited renin release. Because lipophilic analogues of cAMP stimulate renin release, the most likely explanation for this observation is that the cAMP was converted to AMP and then to adenosine. This suggests that, at least in the kidney, a sufficient quantity of *extracellular* phosphodiesterase activity exists to convert cAMP to AMP. Extracellular conversion of cAMP to AMP and adenosine has also been observed when cAMP was presented to perfused livers, isolated liver cells, adipose tissue, or cerebral cortical cells in culture (169, 170).

The above considerations support the hypothesis that a transmembrane negative feedback loop exists that functions to limit adenylate cyclase activity via formation of adenosine in the biophase adjacent to the cell surface. Thus, whenever and however adenylate cyclase is activated, cAMP is pumped out of the cell directly onto the cell surface. On the cell surface, the recently extruded cAMP is converted to AMP and hence to adenosine, which binds to A_1 receptors and inhibits adenylate cyclase. Because these enzymatic reactions would occur near the cell surface, an autocrine system would be operative in which the adenylate cyclase activity of each individual cell would be effectively regulated. The existence of such a mechanism on juxtaglomerular cells would explain why blockade of adenosine receptors augments renin release whenever renin secretion is stimulated via the adrenergic or prostaglandin pathways (see Figure 1). Additional studies will be required to determine whether a transmembrane negative feedback loop regulating renin release actually exists.

Background Release of Adenosine

Although adenosine concentrations around juxtaglomerular cells may fluctuate due to the factors discussed above, it is also possible that adenosine concentrations surrounding juxtaglomerular cells are completely or nearly static. The concentration of adenosine that mediates the adenosine brake may simply be a constant. Initially, such a situation may not seem consistent with the observation that blocking adenosine receptors increases renin secretion more when renin release is stimulated than when it is not. However, exogenous adenosine—and by inference endogenous adenosine—inhibits renin secretion more when renin release is activated compared to when renin release is unstimulated (98, 99). Therefore, the fact that adenosine receptor antagonists increase renin release more when renin release is stimulated may only reflect an increased import of an unchanged level of adenosine when renin secretion is activated. If true, then the source of the adenosine that regulates renin release may simply be "background" adenosine production by many cell types within and outside the kidney.

AN ALTERNATIVE MODEL OF THE ROLE OF PROSTAGLANDINS IN RENIN RELEASE: IMPLICATIONS FOR THE ADENOSINE-BRAKE HYPOTHESIS

The "dual pathway hypothesis" that physiological activation of renin release involves two intercellular messengers, i.e. prostaglandins and catecholamines, was first proposed by Oates and colleagues in 1979 (171). Although this model may be an oversimplification, it has proven extremely useful and was purposefully developed in this review as *the* model of renin release because it illustrated in an easily understandable manner how adenosine could regulate all physiological stimuli impinging on the juxtaglomerular cell. Now I elaborate on the dual pathway hypothesis and offer an alternative, albeit more complicated, interpretation of the evidence supporting this hypothesis. Finally, the central role of adenosine as a regulator of renin release under this alternate interpretation of the dual pathway hypothesis is delineated.

As depicted in Figure 1, the dual pathway hypothesis is that catecholamines mediate beta-adrenoceptor-induced renin release and prostacyclin, or some other prostaglandin, mediates baroreceptor and macula densa-induced renin release. Regarding the macula densa mechanism, the critical experiment implicating a role for prostaglandins was performed by Gerber et al (59). These investigators found that blockade of prostaglandin synthesis would inhibit the renin release response to activation of the macula densa when the intrarenal baroreceptor and intrarenal beta-adrenoceptors were disabled by papaverine and propranolol, respectively. One interpretation of this study is that prostaglandins *mediate* macula densa-induced renin release (i.e. are released by reductions in salt delivery to the macula densa). An alternative, but more complicated interpretation, is the following series of events: (*a*) A reduction in sodium chloride delivery to the maculas densa reduces the release of adenosine onto adjacent juxtaglomerular cells; (*b*) The decreased presentation of adenosine to the juxtaglomerular cells relieves the adenosine brake on adenylate cyclase; (*c*) Any tonic stimulation of adenylate cyclase can be expressed and results in renin release; (*e*) If all tonic stimulation of adenylate cyclase is eliminated—for example by combined blockade of beta-adrenoceptors and inhibition of prostaglandin synthesis—then reducing adenosine concentrations does not stimulate renin release. (In other words, deactivating a brake has no effect unless something is activating the accelerator.)

A similar interpretation can be given to the data supporting a role for prostaglandins in barororeceptor-induced renin release. The critical experiment here was that inhibition of prostaglandin biosynthesis attenuated the renin release response to a reduction in renal perfusion pressure in animals

with nonfiltering kidneys (no delivery of sodium chloride to the macula densa) that were treated with propranolol (no activation of intrarenal beta-adrenoceptors) (55). One interpretation of this study is that prostaglandins mediate the intrarenal baroreceptor. An alternative interpretation is that stimulatory input to juxtaglomerular cells must be present before activation of the intrarenal baroreceptor can stimulate renin release. In animals with blocked beta-adrenoceptors, juxtaglomerular cells may depend heavily on prostaglandins for such positive input.

The model that prostaglandins mediate baroreceptor and macula densa-induced renin release is extremely difficult to distinguish from the model that some tonic stimulation of adenylate cyclase by "background" levels of intrarenal prostaglandins is prerequisite for proper functioning of the intrarenal baroreceptor and macula densa. However, functionally such distinctions are not necessary. In both models the participation of prostaglandins in baroreceptor-induced and macula densa-induced renin release is critical. Also, in both models, adenosine—by inhibiting prostaglandin-induced and catecholamine-induced stimulation of adenylate cyclase—would restrain the renin release response to all three physiological pathways to renin release.

CONCLUSION

Since the discovery over 20 years ago that adenosine inhibits renin release, our knowledge of the biochemistry, cell biology, and renal physiology of adenosine has expanded enormously. Despite this rapid accumulation of knowledge, an integrated view of the role of adenosine in renin release was lacking. It is my hope that the model of renin release presented in this review—although perhaps incorrect in some details—provides a consolidated, and therefore useful, perspective on the role of adenosine as a regulator of renin release.

ACKNOWLEDGMENTS

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