A BRIEF COMMUNICATION

Role of NO and COX Pathways in Mediation of Adenosine A1 Receptor–Induced Renal Vasoconstriction

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The mechanism of adenosine A1 receptor–induced intrarenal vasoconstriction is unclear; it depends on sodium intake and may be mediated by changing the intrarenal activity of the nitric oxide (NO) and/or cyclooxygenase (COX) pathway of arachidonic acid metabolism. The effects of 2-chloro-N^6-cyclopentyladenosine (CCPA), a selective A1 receptor agonist, on renal hemodynamics were examined in anesthetized rats maintained on high sodium (HS) or low sodium (LS) diet. Total renal (i.e., cortical) blood flow (RBF) as well as superficial cortical (CBF), outer medullary (OMBF), and inner medullary (IMBF) flows were determined by laser-Doppler. In HS rats, suprarenal aortic infusions of 8–40 nmol/kg/hr CCPA decreased IMBF (15%) and other perfusion indices (22%–27%); in LS rats, IMBF increased 3% (insignificant) and other indices decreased 13%–24%. In LS rats, pretreatment with N-nitro-L-arginine methyl ester prevented the A1 receptor–mediated decrease in RBF and CBF but not OMBF; the response in IMBF was not altered. Pretreatment with indomethacin prevented the decreases in RBF, CBF, and OMBF and did not change the response of IMBF. Thus, within the cortex the vasoconstriction that follows A1 receptor activation results both from inhibition of NO synthesis and from stimulation of vasoconstrictor products of the COX pathway. In the outer medulla, the latter products seem exclusively responsible for CCPA-induced vasoconstriction. The observation that in LS rats IMBF was not affected by stimulation of adenosine A1 receptors suggests that limiting salt intake may help protect medullary perfusion against vasoconstrictor stimuli which have the potential to disturb long-term control of arterial pressure.
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Key words: renal cortical blood flow; renal medullary blood flow; adenosine A1 receptor agonist; nitric oxide; COX

Introduction

Adenosine has been established as a potent vasodilator in most tissues and organs; however, it induces vasoconstriction in the kidney. This is mediated by adenosine A1 receptors, but the detailed mechanisms involved have not been elucidated; intrarenal vasodilatation, mediated by A2a receptors, is seen after high doses of adenosine (1, 2).

In preliminary studies designed to examine if the vasoconstriction depends on body sodium balance, we found that on high sodium (HS) intake A1 receptor stimulation using a highly selective agonist (2-chloro-N^6-cyclopentyladenosine [CCPA]) induced generalized intrarenal hypoperfusion. On the other hand, in animals on low sodium (LS) intake this response was seen in the cortex and outer medulla but not in the inner medulla. Considering the postulated crucial role of renal medullary circulation and body sodium balance in long-term control of arterial pressure (3), we attempted to elucidate some aspects of A1 receptor–mediated intrarenal vasoconstriction, particularly the mechanism of the apparent protection of the inner medulla against this influence. The working hypothesis was that the protection was related to the status of some paracrine vasomotor system, different in the inner medulla compared with the outer medulla or cortex. We examined the role of the two best-established systems: nitric oxide (NO) and cyclooxygenase (COX) pathways of arachidonic acid (AA) metabolism.

Materials and Methods

The experimental procedures were approved by the First Ethical Committee, Warsaw. Male Wistar rats were fed

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Table 1. Effects of CCPA (8–40 nmol/kg/hr) on Renal Hemodynamics and Excretion in Rats on LS or HS Diet[a]

		Control	CCPA	Recovery
MAP (mm Hg)	LS	124 ± 6	120 ± 7	116 ± 7
	HS	123 ± 5	111 ± 7*	115 ± 8
RBF (ml/min/g of kidney weight)	LS	6.9 ± 0.7	5.2 ± 0.6*	6.1 ± 0.7*
	HS	7.8 ± 1.2	5.7 ± 1.0*	7.6 ± 1.0
CBF (perfusion units)	LS	467 ± 44	413 ± 41*	448 ± 44
	HS	570 ± 30	448 ± 34*	543 ± 44
OMBF (perfusion units)	LS	151 ± 25	131 ± 22*	149 ± 30
	HS	172 ± 21	132 ± 18*	156 ± 18*
IMBF (perfusion units)	LS	142 ± 14	145 ± 14	156 ± 16
	HS	158 ± 22	131 ± 16*	149 ± 20
V (μl/min/g of kidney weight)	LS	4.9 ± 1.2	3.2 ± 1.2	10.9 ± 2.9*
	HS	10.4 ± 3.4	4.0 ± 2.0*	6.7 ± 1.8
$U_{Na}V$ (μmol/min/g of kidney weight)	LS	0.2 ± 0.0	0.1 ± 0.0*	0.8 ± 0.3
	HS	2.0 ± 0.6**	0.8 ± 0.4*	1.2 ± 0.2

[a] $n = 6$–10; mean values ± SE.
* Significantly different than pre-CCPA control. ** Significantly different than the corresponding values in LS rats.

an LS (0.15% sodium, w/w) or HS (4% sodium, w/w) diet (SSNIFF GmbH, Soest, Germany) for 3 weeks. For acute experiments, they were anesthetized with intraperitoneal thiopental (Sandoz GmbH, Kundl, Austria) 100 mg/kg. The rats' body temperatures were maintained at approximately 37°C by means of a heating pad, and 3% bovine albumin serum in Ringer solution was infused *via* the femoral vein at 2.3 ml/hr to compensate for fluid losses.

Tracheal cannulae ensured free airways. The drugs (see below) were infused *via* suprarenal aortic catheters; catheters placed in the upper aorta were used for measurement of systemic blood pressure (MAP). The left kidneys were exposed from subcostal flank incisions and placed in plastic holders similar to those used for micropuncture studies; the ureters were cannulated for timed urine collection. Cuff probes placed on the renal arteries and connected with Transonic flowmeters (T106; Transonic Systems Inc., Ithaca, NY) were used for measurement of total renal blood flow (RBF), which was also taken as an index of whole cortex perfusion. The blood perfusions of the renal superficial cortex (CBF), outer medulla (OMBF), and inner medulla (IMBF) were measured separately as laser-Doppler fluxes using the Periflux 4001 system (Perimed AB, Jarfalla, Sweden). For CBF, a PF 407 probe was placed on the kidney surface; for OMBF and IMBF, two needle probes (PF 402) were inserted into the kidney to the depth of 3 and 5 mm. After experiments the positions of the medullary probes were verified at the kidney's cross-section.

Experimental Protocols, Analyses, and Statistics. At the end of surgical preparations and after placement of laser-Doppler probes, the infusion of albumin was replaced by isotonic saline at 2.3 ml/hr. Simultaneously a suprarenal aortic infusion of isotonic saline at 1 ml/hr was started. After control urine collection and measurement periods, the saline was replaced by a suprarenal aortic infusion of CCPA (Sigma-Aldrich, St. Louis, MO), an agonist of A1 receptors of adenosine. During two 20-min experimental periods the rats received first 8 and then 40 nmol/kg/hr CCPA. Thereafter, two 30-min recovery periods followed. CCPA was administered to rats that were either untreated or pretreated with (i) 5 mg/kg body wt indomethacin (Indo; Sigma-Aldrich) given intravenously at the start of acute experiments, followed by sustaining infusion at 1.6 mg/kg/hr or (ii) N-nitro-L-arginine methyl ester (L-NAME), a nonselective inhibitor of NO synthesis, given in drinking water (5 mg/100 ml) for 3 days preceding the acute experiment. This dosage and administration regimen was found to effectively increase MAP and decrease renal hemodynamics in LS rats (4).

Urine volumes (V) were determined gravimetrically. Urine osmolality was determined by freezing point depression using a semimicro osmometer (Knauer ML, Bad Homburg, Germany), and sodium concentration ($U_{Na}V$) was determined by a flame photometer (Jenway PFP7, Essex, UK).

The significance of changes within one group over time was first evaluated by repeat measurement analysis of variance (ANOVA), followed by Student's test for dependent variables. Differences in mean values between groups were first analyzed by the classical one-way ANOVA followed by modified Student's t test for independent variables; no multiple comparisons were made. $P < 0.05$ was taken to indicate significance of differences.

Results

Baseline renal hemodynamics tended to be higher in HS compared with LS rats, but the differences were not significant. However, there were diet-dependent differences in response to CCPA: HS but not LS rats showed a significant decrease in MAP and IMBF (Table 1). In both LS and HS rats, RBF, CBF, and OMBF decreased significantly after CCPA administration.

As expected, baseline $U_{Na}V$ values were much higher in

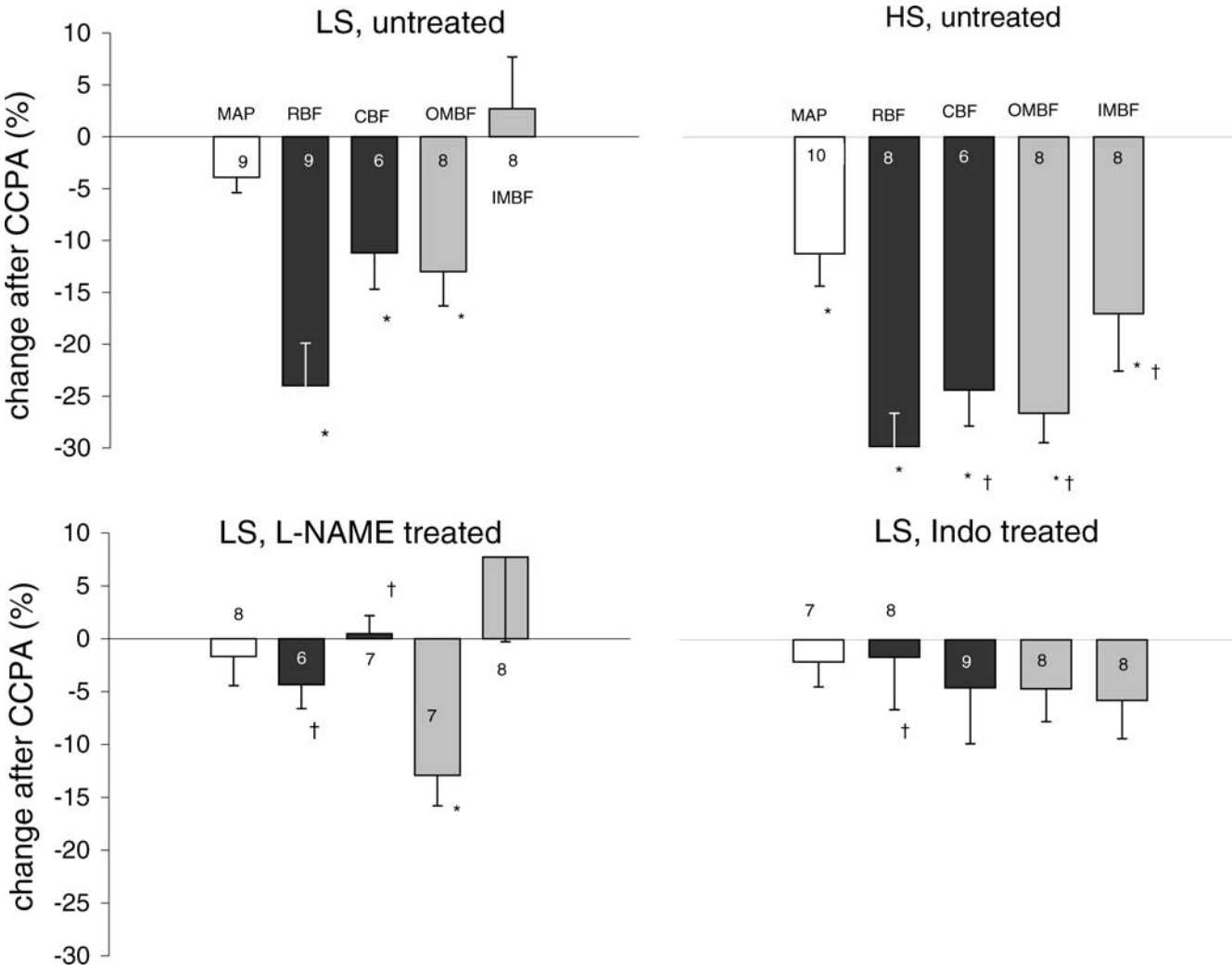

Figure 1. Effects of CCPA (8–40 nmol/kg/hr) on renal hemodynamics in rats on LS and HS (upper panel) and in LS rats pretreated with L-NAME or Indo (lower panel). Maximal stable responses are shown as mean % differences from pre-CCPA control ± SEM. *n* values are indicated on the bars, denotations as in Table 1. * = significantly different than pre-CCPA control; † = significantly different than the change in HS rats (upper panel) or than the change in untreated LS rats (lower panel).

HS compared with LS rats ($P < 0.02$). The difference was less striking for V and was not significant because of high data variability. In HS rats only, a significant antidiuresis and antinatriuresis was seen after CCPA administration (Table 1).

The diet-dependent differences in the responses of MAP and renal hemodynamic variables to CCPA are more clearly shown in Figure 1. The HS diet somewhat enhanced the decrease in MAP after CCPA administration; the baseline mean MAP values were almost identical in the LS and HS groups (Table 1). The HS diet significantly enhanced the decreases of CBF and OMBF (but not RBF) after CCPA administration. The most striking difference between the two diets was that a significant post-CCPA decrease in IMBF was seen in the HS group, roughly parallel with decreases of the other renal hemodynamic variables, but there was no change in IMBF (contrasted with decreases in RBF, CBF, and OMBF) in the LS group (Fig. 1).

The baseline renal hemodynamics in untreated and L-NAME– or Indo-pretreated rats are shown in Table 2. In the L-NAME group, MAP was higher and RBF and OMBF lower than in untreated rats; other differences were not significant. The baseline MAP and renal hemodynamic parameters did not differ between Indo-treated and untreated groups, although IMBF tended to be lower in the former.

The data in Table 2 and Figure 1 show how the responses of LS rats to CCPA were affected by pretreatment with L-NAME and Indo. L-NAME prevented the post-CCPA decreases in RBF and CBF but not in OMBF; L-NAME tended to increase IMBF more than in untreated rats, but the response was highly variable (Fig. 1). Indo treatment abolished or alleviated the post-CCPA decreases in RBF (significant difference), CBF, and OMBF. In the case of IMBF, a minor increasing tendency seen in untreated rats was reverted to a decreasing tendency (nonsignificant difference).

The baseline V and $U_{Na}V$ showed no significant dif-

Table 2. Effects of CCPA on Renal Hemodynamics in Untreated, L-NAME–Pretreated, or Indo-Pretreated LS Rats[a]

	Pretreatment	Control	CCPA max	Recovery
MAP (mm Hg)	None	124 ± 6	120 ± 7	116 ± 7
	L-NAME	148 ± 4**	146 ± 6	148 ± 6
	Indo	122 ± 6	120 ± 6	118 ± 6
RBF (ml/min/g of kidney weight)	None	6.9 ± 0.7	5.2 ± 0.6*	6.1 ± 0.7*
	L-NAME	5.5 ± 0.5**	5.4 ± 0.6	5.8 ± 0.6
	Indo	7.5 ± 0.8	7.1 ± 0.6	8.0 ± 0.7
CBF (perfusion units)	None	467 ± 44	413 ± 41*	448 ± 44
	L-NAME	463 ± 40	462 ± 41	495 ± 45*
	Indo	543 ± 44	534 ± 60	585 ± 49
OMBF (perfusion units)	None	151 ± 25	131 ± 22*	149 ± 30
	L-NAME	97 ± 10**	85 ± 9*	96 ± 12
	Indo	156 ± 18	154 ± 18	152 ± 17
IMBF (perfusion units)	None	142 ± 14	145 ± 14	156 ± 16
	L-NAME	151 ± 40	170 ± 47	162 ± 39
	Indo	126 ± 8	123 ± 11	115 ± 16

[a] $n = 6$–10; maximal values (means ± SE) within 1 hr of CCPA administration are presented.
* Significantly different than pre-CCPA control. ** Significantly different than the value for the untreated group.

ferences between untreated, L-NAME–treated, and Indo-treated LS groups. In untreated but not in L-NAME– or Indo-treated rats, CCPA decreased V and $U_{Na}V$ (data not shown).

Discussion

The stimulation of adenosine A1 receptors with a selective agonist induced intrarenal vasoconstriction, in agreement with the vast earlier evidence (1, 2). Interestingly, the effects depended on salt intake. When the intake was high, a reduction of perfusion was seen throughout the kidney, including the outer and inner medullae. For LS, the post-CCPA vasoconstriction was less pronounced and remarkably did not include the inner medulla. Considering the postulated crucial role of renal medullary circulation and body sodium balance in long-term control of arterial pressure (3), we thought it worthwhile to explore the background of the apparent protection of the inner medulla against adenosine-induced vasoconstriction.

A1 receptors mediating vasoconstriction are located in the glomerular arterioles, including those of the juxtamedullary glomeruli (5), and the outer medullary descending vasa recta (OMDVR; Ref. 6). Lesser A1 receptor–dependent vasoconstriction in LS compared with HS rats and particularly no change in the inner medulla may depend on down-regulation of A1 receptors in an LS diet, especially those mediating constriction of the OMDVR (7). However a LS intake generally up-regulates A1 receptors (8); no specific information is available on a possible diet dependence of OMDVR receptors. A parallel reduction of cortical, outer, and inner medullary perfusion after CCPA administration, as seen in rats on HS intake, can be explained by constriction of glomerular arterioles throughout the cortex, including its juxtamedullary region. Thus, medullary hypoperfusion would be a consequence of diminished inflow of blood to the medullary vessel segments; an additional constriction of OMDVR might or might not contribute. The absence of the response within the inner medulla seen in LS intake rats is difficult to explain. It may be speculated that A1 receptor–mediated constriction of OMDVR located at the periphery of the medullary vascular bundle (reflected by a decrease in OMBF), which supplies blood to the interbundle region, would divert blood to the conduit-type vasa recta located centrally in the bundle and supplying the inner medulla and papilla (3). However, there was no indication of such a "stealing" phenomenon in rats on HS intake in which the decrease in OMBF (and presumably the constriction of the peripheral OMDVR) was even greater.

The explanation for different responses to CCPA of LS versus HS rats could also be sought in different diet-dependent paracrine and humoral milieu of intrarenal vasculature. LS intake would increase intrarenal angiotensin II (Ang II), which may have been responsible for lower baseline renal hemodynamics, even though the differences were not significant in this study (Table 1). One could speculate that basal intrarenal preconstriction limits the vasoconstrictor response to A1 receptor stimulation. Ang II is known to stimulate generation of vasodilator prostaglandins, which appear to offset the hormone's vasoconstrictor effect on the inner but not outer medulla (9). It is possible that similar differentiation of the effects between the outer and inner medullae is observed also in the case of NO. We found recently that in LS rats the inner medulla, in contrast to the outer medulla, is not under tonic vasodilator influence of NO (4). Considering the established differences in the baseline activity of NO and in the functional role of prostaglandins between the two layers of the medulla, we thought that differential responses of these layers to A1 receptor stimulation could be related to these differences. Therefore, we explored the role of NO and COX pathways

of AA metabolism as potential mediators of the effects of A1 receptor activation.

We first examined if A1-dependent intrarenal vasoconstriction is mediated by a decrease in the activity of vasodilator NO. Admittedly, the majority of studies indicate that adenosine stimulates rather than inhibits the synthesis of NO; however, the synthesis is mediated by A2a receptors (1, 2). The baseline RBF was reduced by L-NAME treatment (Table 2), which indicates an NO-dependent vasodilator tonus in the cortex. In the absence of this tonus (after L-NAME treatment), no further decrease in RBF occurred after A1 receptor stimulation, which supports the hypothesis that the vasoconstriction is mediated by NO inhibition. In contrast to the effect on RBF, NO blockade did not prevent the usual decrease in OMBF (Fig. 1). This suggests that, although outer medullary perfusion was under tonic vasodilator influence of NO (Table 2), an inhibition of NO synthesis was not the mechanism of the decrease in OMBF after A1 receptor stimulation. In this study, the IMBF of LS rats was not under baseline NO tonus (Table 2), which is in agreement with our earlier study (4). Assuming the role of NO inhibition in response to A1 receptor stimulation, it is not surprising that CCPA did not affect IMBF similarly in untreated or L-NAME–treated rats.

On the whole, the data suggest that NO inhibition is a major mechanism of post-CCPA decrease in perfusion of the cortex but is not responsible for the decrease in perfusion of the outer and inner medullae. Presumably, the absence of baseline vasodilator NO tonus in the inner medulla makes it unresponsive to inhibitory stimuli, such as activation of adenosine A1 receptors. By contrast, we showed recently, also in LS rats, that a considerable NO tonus in the outer medulla makes it unresponsive to stimulation of NO after activation of ATP receptors of the P2Y type (4). Thus, in general, the difference in the baseline NO tonus may be the background of differential responses of the inner versus outer medulla to various vasomotor stimuli.

In this study with LS rats, blockade of the prostaglandin COX pathway of AA metabolism did not significantly affect basal renal hemodynamics. Inhibition of COX with Indo would have eliminated the influence of both vasodilator prostaglandin and vasoconstrictor products of this pathway, such as thromboxane A2 or prostaglandin $F_{2\alpha}$. The observation that Indo pretreatment prevented or greatly attenuated the post-CCPA decrease in perfusion of the cortex and outer medulla suggests that A1 receptor–dependent vasoconstriction is mediated by stimulation of the synthesis and action of vasoconstrictor agents generated in the COX pathway; it is known that thromboxane receptors are expressed in renal microvessels (10). This interpretation does not apply to the inner medulla where Indo pretreatment did not significantly affect the vascular response to CCPA.

Taken together, the results suggest that within the renal cortex the vasoconstriction which follows A1 receptor activation results both from inhibition of NO synthesis and from stimulation of vasoconstrictor products of the COX pathway of AA metabolism. Also, in the outer medulla, the latter products seem responsible for CCPA-induced vasoconstriction while there is no indication of a role for NO inhibition. In animals maintained on an LS diet, IMBF is not tonically influenced by NO or vasoactive products of the COX pathway and is not modified by stimulation of adenosine A1 receptors. The above response pattern is characteristic for animals on LS intake only: we saw that on HS intake the A1 receptor–mediated vasoconstriction does include the inner medulla. Unlike our LS rats, the IMBF of anesthetized rats on a standard diet has been repeatedly shown to be maintained by vasodilator prostaglandins (11), and intrarenal vasoconstriction after A1 receptor stimulation was substantially augmented by L-NAME treatment (12).

Generally speaking, LS intake appears to create a situation in which the IMBF is not critically dependent on the local activity of paracrine vasoactive systems and is relatively resistant to vasoconstrictor stimuli. Considering the postulated role of adequate renal medullary perfusion in long-term control of arterial pressure (3), this resistance may explain in part the beneficial influence of sodium restriction during the development and progress of arterial hypertension.

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