Renal Effects of Adenosine A₁-Receptor Blockade with 8-Cyclopentyl-1,3-Dipropylxanthine in Hypoxemic Newborn Rabbits

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ABSTRACT

The key role of intrarenal adenosine in mediating the hypoxemic acute renal insufficiency in newborn rabbits has been well demonstrated using the nonspecific adenosine antagonist theophylline. The present study was designed to define the role of adenosine A₁ receptors during systemic hypoxemia by using the specific A₁-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). Renal function parameters were assessed in 31 anesthetized and mechanically ventilated newborn rabbits. In normoxia, DPCPX infusion induced a significant increase in diuresis (+44%) and GFR (+19%), despite a significant decrease in renal blood flow (RBF) (−22%) and an increase in renal vascular resistance (RVR) (+37%). In hypoxemic conditions, diuresis (−19%), GFR (−26%), and RBF (−35%) were decreased, whereas RVR increased (+33%). DPCPX administration hindered the hypoxemia-induced decrease in GFR and diuresis. However, RBF was still significantly decreased (−27%), whereas RVR increased (+22%). In all groups, the filtration fraction increased significantly. The overall results support the hypothesis that, in physiologic conditions, intrarenal adenosine plays a key role in regulating glomerular filtration in the neonatal period through preferential A₁-mediated afferent

vasoconstriction. During a hypoxemic stress, the A₁-specific antagonist DPCPX only partially prevented the hypoxemia-induced changes, as illustrated by the elevated RVR and drop in RBF. These findings imply that the contribution of intrarenal adenosine to the acute adverse effects of hypoxemia might not be solely mediated *via* the A₁ receptor. (*Pediatr Res* 54: 400–405, 2003)

Abbreviations

A₁, adenosine type 1 receptor
A₂, adenosine type 2 receptor
A_{2a/b}, adenosine type 2a or type 2b receptor
DPCPX, 8-cyclopentyl-1,3-dipropylxanthine
FF, filtration fraction
MAP, mean arterial blood pressure
PAH, para-aminohippuric acid
RBF, renal blood flow
RVR, renal vascular resistance
TGF, tubuloglomerular feedback
UV, urine flow rate
UNaV, urinary excretion of sodium

Adenosine is ubiquitous in the kidney. Intrarenal adenosine vasoconstricts the preglomerular vessels *via* the A₁ receptors, while dilating the efferent arterioles *via* the A₂ receptors. It thus affects GFR as well as RBF and its distribution within the kidney. In addition to its vascular actions, intrarenal adenosine modulates renal mechanisms, including TGF, renin release, and the tubular handling of electrolytes and water. Intrarenal adenosine also appears to be an important hemodynamic me-

diator in some models of vasomotor nephropathy, such as those induced by intramuscular glycerol injection (1), contrast media (2), cisplatin (3), or hypoxemia (4).

In animal models as in humans, hypoxemia can lead to functional renal insufficiency (5–7). In the newborn rabbit, acute normocapnic hypoxemia induces renal hypoperfusion with decreased GFR and RBF (4, 7–9). The underlying mechanism of this hypoxemia-induced vasomotor nephropathy still remains controversial. The chemoreceptor-mediated reflex and/or the activation of vasoactive factors such as angiotensin II (AII) (9), endothelin (10), nitric oxide (8), or bradykinin (11) have been suggested to play a role.

We previously demonstrated that the administration of theophylline, a nonspecific antagonist to adenosine receptors, ameliorates the hypoxemia-induced renal hemodynamic changes seen in the newborn rabbit model (4). Moreover, in neonates with respiratory distress syndrome (12) or perinatal

Received July 17, 2002; accepted March 6, 2003.

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Supported by the Swiss National Science Foundation, grants numbers 3200-036574/92 and 3200-064041.00.

Presented in abstract form at the 12th Congress of the International Pediatric Nephrology Association, Seattle, WA, U.S.A., September 2001, and the 36th Annual Meeting of the European Society for Pediatric Nephrology, Bilbao, Spain, September 2002.

DOI: 10.1203/01.PDR.0000078273.08316.26

asphyxia (13), as in diuretic-dependent critically ill children (14), the use of theophylline improves diuresis and creatinine clearance. Specific adenosine receptor antagonists are now available for clinical use (15). The aim of the present study was thus to define the role of the A₁ receptor in physiologic and hypoxemic conditions, using the highly specific A₁ antagonist DPCPX (16–18).

METHODS

Animal experiments. A total of 31 newborn, 6.9 ± 0.2 (mean $\pm$ SEM) d old New Zealand White rabbits were studied. All animals, weighing 106.9 ± 2.3 g, were born in our own animal facilities by spontaneous vaginal delivery and breast-fed in their litter until the time of study. The animal studies were performed according to the guidelines of the Swiss National Research Foundation.

All methods have been described previously (9, 19). Briefly, the rabbits were anesthetized with 25 mg/kg body weight of sodium pentobarbital intraperitoneally and artificially ventilated *via* tracheostomy (respiratory rate 40 breaths/min, tidal volume adjusted for age and weight). Body temperature was kept at approximately 39°C. The femoral vessels had catheters implanted for solute infusion, arterial blood sampling, and continuous monitoring of MAP and heart rate. Urine was sampled by bladder catheterization. After completion of the surgical procedure, inulin and para-aminohippuric acid (PAH) clearances (C_{in} , C_{PAH}) were measured throughout the experiments. From this point onward, all animals received a constant infusion of a modified rabbit Ringer solution at a rate of 1 mL/h/100 g body weight (9).

Experimental protocol. The animals were randomly divided into three groups: normoxia + DPCPX (NxD, $n = 8$; age, 7.4 ± 0.4 d; weight, 108.6 ± 7.1 g); hypoxemia alone (Hx, $n = 10$; 7.3 ± 0.3 d; 106.1 ± 3.1 g); and hypoxemia + DPCPX (HxD, $n = 13$; 6.4 ± 0.3 d; 106.6 ± 2.9 g) (Fig. 1).

In all groups, the experimental protocol started, after a 90-min equilibration period, by a normoxic 60-min control period, C, consisting of two 30-min urine collections with 0.4 mL of blood withdrawn at the midpoint. Eighty microliters were used for immediate measurements of blood gases, hematocrit, and plasma protein levels. The red blood cells were reconstituted in diluted human albumin and reinfused. The remainder of the plasma was kept at -20°C for later determination of electrolytes and renal function markers.

In the two hypoxemic groups, acute hypoxemia was induced over 30 min by gradually reducing the fractional inspiratory oxygen concentration with a gas mixture of 10% O₂ and 90% N₂, to reach a partial pressure of arterial oxygen (P_{aO_2}) close to 40 mm Hg. In the NxD and HxD groups, DPCPX was then infused at a dose of $60 \mu\text{g/kg/h}$.

Two additional 30-min urine collections (experimental periods E1 and E2) were obtained from all animals, with blood withdrawn at the midpoint of each period; the red blood cells were again reconstituted and reinfused.

Analytical procedures. Urine volume was measured gravimetrically. Blood gas determinations were performed with a pH/blood gas analyzer (Blood gas system 248, Bayer Schweiz AG, Zürich, Switzerland). The automatic anthrone method of Wright and Gann (20) and the Bratton and Marshall (21) method were used for the determination of inulin and PAH

Figure 1. Experimental protocol in the three groups.

concentrations, respectively. Sodium was measured by flame photometry. The plasma protein concentration was estimated by refractometry.

Calculation of data. C_{in} and C_{PAH} , representing GFR and RPF, respectively, RBF, FF, RVR, and UNaV were calculated from standard equations as previously reported (9). The PAH extraction ratio used for the calculation of RBF was 0.55 ± 0.04 ; this is the usual value found in normoxic neonatal rabbits in our laboratory (7).

Statistical analysis. All results are given as mean $\pm$ SEM. Intergroup comparisons of the baseline control data and comparison of the percent changes (Fig. 2) were made with the nonparametric Kruskal-Wallis rank-sum test. The Fisher's paired least-significant difference test (ANOVA) was used for repeated measurements. Intragroup comparisons between the C period and periods E1 or E2 were performed on the absolute individual data with the nonparametric Friedman and Wilcoxon signed-rank tests. A $p < 0.05$ was considered statistically significant.

RESULTS

The three groups were similar for age, weight, and all baseline control data (Tables 1 and 2).

The systemic hemodynamic parameters and blood gas data are shown in Table 1 (absolute data). During the experiments, there were relatively minor, albeit statistically significant, changes in plasma protein levels and hematocrit. These are recurrent findings in the neonatal animal experiments, as a result of repeated blood sampling for P_{aO_2} monitoring. However, these changes do not interfere with the physiologic stability of the neonatal model (7).

Renal functional responses to i.v. DPCPX are described in Table 2 (absolute data), and illustrated in Figure 2 (percentage changes based on individual data).

In the normoxic group, DPCPX induced a transient increase of GFR in the first experimental period (+19% in E1, $p < 0.05$; +7% in E2, NS compared with C period). RBF fell significantly (−22% in E2), whereas RVR (+37%) and FF (+30%) increased significantly. The urine flow rate and UNaV were significantly increased by DPCPX infusion (+44% and +257%, respectively).

In the Hx group, hypoxemia significantly decreased MAP (−17%), GFR (−26%), RBF (−35%), and diuresis (−19%), whereas RVR increased by +33%. UNaV remained unchanged.

Figure 2. The percentage changes ($\Delta\%$) in MAP, GFR, RBF, RVR, FF, and UV in normoxic and hypoxemic newborn rabbits (i.v. dose of DPCPX = 60 $\mu\text{g/kg/h}$). The values presented are means $\pm$ SEM of the percentage changes of each individual for all groups, from the control period C to the experimental period E2. * $p < 0.05$; ** $p < 0.01$, when absolute values of E2 are compared with the values of the C period (absolute values presented in Tables 1 and 2). Intergroup comparison of the percentage changes: horizontal lines indicate the groups showing a significant difference.

Table 1. MAP, heart rate (HR), hematocrit (Htc), plasma protein levels (Prot), blood gases (Pao₂, Paco₂), and systemic pH in normoxemic and hypoxemic newborn rabbits (i.v. dose of DPCPX = 60 µg/kg/h)

		Normoxemia + DPCPX	Hypoxemia	Hypoxemia + DPCPX
MAP (mm Hg)	C	30.3 ± 1.0	30.4 ± 0.7	30.0 ± 0.7
	E1	30.4 ± 1.7	25.8 ± 0.9**	28.3 ± 1.1*
	E2	31.8 ± 1.6	25.2 ± 0.8**	25.6 ± 1.8*
HR (per min)	C	305 ± 10	273 ± 7	271 ± 8
	E1	305 ± 9	277 ± 6	286 ± 8
	E2	310 ± 9	280 ± 6	276 ± 17
Htc (%)	C	26.1 ± 1.2	27.3 ± 0.7	27.8 ± 1.0
	E1	24.4 ± 0.9*	25.1 ± 0.7**	26.2 ± 0.8*
	E2	20.8 ± 3.1*	25.1 ± 0.7**	23.7 ± 2.1**
Prot (g/L)	C	24.9 ± 0.6	26.2 ± 1.3	25.5 ± 0.8
	E1	23.8 ± 0.8*	24.1 ± 1.1*	23.9 ± 0.8*
	E2	20.5 ± 3.0*	24.1 ± 1.1*	22.7 ± 2.1*
Pao ₂ (mm Hg)	C	131.3 ± 4.4	126.6 ± 2.5	127.4 ± 2.2
	E1	131.1 ± 4.4	39.9 ± 0.5**	40.4 ± 0.8**
	E2	132.4 ± 3.2	39.6 ± 0.6**	40.2 ± 0.7**
Paco ₂ (mm Hg)	C	34.8 ± 0.4	35.0 ± 0.5	34.7 ± 0.5
	E1	33.9 ± 1.1	32.6 ± 0.9	31.8 ± 0.7**
	E2	33.9 ± 1.0	32.5 ± 0.8	31.5 ± 0.7**
pH	C	7.47 ± 0.01	7.49 ± 0.01	7.47 ± 0.01
	E1	7.45 ± 0.01*	7.46 ± 0.01	7.45 ± 0.01
	E2	7.44 ± 0.01	7.45 ± 0.01**	7.45 ± 0.01

C, normoxemic control period; E1, E2, experimental periods 1 and 2 (Fig. 1). Values are means ± SEM

* $p < 0.05$; ** $p < 0.01$ vs control period of the same group of animals.

Table 2. UV, UVNa, GFR, RBF, FF, and RVR in normoxemic and hypoxemic newborn rabbits (i.v. dose of DPCPX = 60 µg/kg/h)

		Normoxemia + DPCPX	Hypoxemia	Hypoxemia + DPCPX
UV (mL/kg/min)	C	0.087 ± 0.008	0.085 ± 0.006	0.079 ± 0.005
	E1	0.117 ± 0.008*	0.075 ± 0.007	0.088 ± 0.006
	E2	0.124 ± 0.013*	0.067 ± 0.007*	0.100 ± 0.009*
GFR (mL/kg/min)	C	2.07 ± 0.17	1.95 ± 0.11	1.90 ± 0.10
	E1	2.47 ± 0.23*	1.65 ± 0.15*	1.97 ± 0.18
	E2	2.28 ± 0.30	1.44 ± 0.12**	1.86 ± 0.16
RBF (mL/kg/min)	C	17.8 ± 1.0	18.0 ± 1.5	16.2 ± 0.9
	E1	17.4 ± 0.9	12.6 ± 1.2**	13.6 ± 0.7*
	E2	13.8 ± 1.0*	11.3 ± 0.9**	11.6 ± 0.9**
FF (%)	C	15.7 ± 0.7	15.8 ± 1.3	16.2 ± 0.9
	E1	18.6 ± 1.1*	17.9 ± 1.4**	19.6 ± 1.3**
	E2	20.5 ± 1.6*	17.3 ± 1.3*	20.8 ± 1.2**
RVR (mm Hg/mL/kg/min)	C	1.74 ± 0.08	1.85 ± 0.19	1.93 ± 0.13
	E1	1.77 ± 0.09	2.18 ± 0.19*	2.13 ± 0.10
	E2	2.36 ± 0.15*	2.34 ± 0.19*	2.30 ± 0.18*
UNaV (mmol/kg/min)	C	2.27 ± 0.75	2.64 ± 0.59	2.18 ± 0.49
	E1	3.43 ± 0.96	2.09 ± 0.43	2.34 ± 0.42
	E2	5.98 ± 1.44*	1.80 ± 0.30	3.84 ± 0.66**

C, normoxemic control period; E1, E2, experimental periods 1 and 2 (Fig. 1). Values are means ± SEM.

* $p < 0.05$; ** $p < 0.01$ vs control period of the same group of animals.

In the HxD group, DPCPX totally prevented the drop in GFR and diuresis seen with hypoxemia alone, although MAP was still significantly decreased (−15%). Meanwhile, RVR remained increased significantly by −22% in period E2, with a concomitant significant decrease in RBF (−27%), and an increase in FF (+28%). UNaV increased significantly (+164%).

DISCUSSION

The use of DPCPX confirms the physiologic role of adenosine A₁ receptors to maintain renal function in the neonatal period, as well as their involvement in the renal response to acute hypoxemia.

In both normoxic and hypoxemic animals, DPCPX administration increased diuresis and natriuresis without an increase in MAP, ruling out a systemic arterial pressure-related effect. Altogether, the present study provides evidence that A₁ blockade acts primarily by a direct proximal tubular effect rather than a secondary renal hemodynamic effect. Kost *et al.* (22) demonstrated that the diuretic/natriuretic response to DPCPX is probably mediated by blockade of A₁ receptors linked to tubular sodium transport through pertussis toxin-sensitive G-proteins. Using renal micropuncture techniques, Munger and Jackson (23) demonstrated that DPCPX increased diuresis, even with no significant alteration in GFR. Likewise, Zou *et al.* (24) demonstrated that infusion of DPCPX directly into the

renal medullary interstitial tissue increased water and sodium excretion without altering GFR or medullary blood flow. *In vivo*, DPCPX may remove the inhibition of vasopressin-induced cyclic AMP production by endogenous adenosine, and thus result in a decrease in sodium reabsorption in the proximal tubule (23). A second possibility might be that high levels of AII ($>10^{-8}$ M) inhibit the Na/H antiporter in the proximal tubule, thus increasing sodium excretion. High levels of AII may easily be reached, inasmuch as studies by Navar *et al.* (25) showed proximal tubular concentrations of AI and AII in the nanomolar range. Disruption of the physiologic brake of adenosine on renin release during A_1 blockade may be sufficient to increase sodium excretion in the proximal tubule by an AII-dependent mechanism (23, 25, 26).

In the normoxic group, the decrease in RBF and the increase in GFR, RVR and FF after A_1 blockade with DPCPX suggest both afferent dilation and efferent constriction. This is consistent with A_1 -dependent afferent vasoconstricting, as well as efferent vasodilating effects of adenosine in physiologic conditions (27). The renal effects of DPCPX are unlikely to be due to a systemic action, as MAP and heart rate remained unchanged.

Nishiyama *et al.* (28) recently reported that, during A_1 blockade, adenosine elicits both afferent and efferent arteriolar vasodilatation, with a slightly greater effect on afferent arterioles. These vasodilatory responses are markedly attenuated by A_{2a} blockade. These data are consistent with the hypothesis that A_{2a} -mediated vasodilatation buffers A_1 -mediated vasoconstriction in the renal microvasculature (28). Thus, in the present study, in addition to the direct inhibition of the vasoconstricting effect of A_1 receptors, A_1 antagonism with DPCPX may have unveiled the A_{2a} -mediated afferent vasodilatation of endogenous renal adenosine.

Paracrine interactions might also occur in response to endogenous adenosine, such as the release of vasodilatory prostaglandins (29) or nitric oxide (26). Adenosine may also act by interfering with the TGF mechanism: in response to increased sodium chloride presented to the distal nephron, the macula densa releases adenosine, which then induces vasoconstriction of the afferent arteriole of the same nephron by binding to the A_1 receptors (14, 30). Antagonism by DPCPX would blunt the TGF response (31) and consequently result in preservation of GFR and urine flow (14, 30), as observed in our experiments.

The significant increase in RVR and decrease in RBF after DPCPX administration suggest an efferent vasoconstriction. Under physiologic conditions, interactions of adenosine with its A_1 receptors contribute to the basal tone of the afferent and efferent arterioles, contract glomerular mesangial cells, and thereby reduce the ultrafiltration coefficient (Kf) and inhibit renin release from juxtaglomerular (JG) cells (23, 26). When A_1 receptors are blocked with DPCPX, the afferent resistance falls (GFR was indeed increased in our study), and local renin/angiotensin II levels rise, thus contributing to the fall in Kf and the increase in efferent resistance (23).

The release of endothelin by adenosine in the kidney might also be possible, as it was reported that endothelin-induced bronchoconstriction is enhanced by adenosine (32).

As previously shown in newborn rabbits (4, 7–11, 19), as well as lambs and puppies (33–35), acute normocapnic hypoxemia in the present study was associated with a significant drop in GFR, together with a concomitant decrease in RBF, MAP, and diuresis, and a significant increase in RVR. Administration of the specific A_1 antagonist DPCPX totally prevented the well-documented hypoxemia-induced fall in GFR and diuresis; both parameters were not modified from the control period to the experimental hypoxemia plus DPCPX periods, although MAP remained significantly decreased. DPCPX administration prevented the hypoxemia-induced afferent vasoconstriction, obviously mediated *via* the A_1 receptor. Another possibility is that the A_{2a} , or even the low-affinity A_{2b} receptors, were activated at high adenosine concentrations (>10 μ mol/L) and elicited a vasodilatory response (28). Microdialysis experiments performed in anesthetized adult rabbits (36) have indicated that renal interstitial adenosine concentrations normally remain in the micromolar range. Yet, recent studies have demonstrated that the medullary thick ascending loop of Henle releases adenosine in response to hypoxia (28, 37).

However, although DPCPX prevented the decrease in GFR induced by hypoxemia, an increase in RVR was still present, with a consequent decrease in RBF. The increase in FF indicates predominant efferent vasoconstriction. When related to the results of Gouyon and Guignard (4) with the same model, it appears that the nonspecific inhibition with theophylline was more likely to protect renal function in the neonatal period, as theophylline totally prevented the hypoxemia-induced vasomotor nephropathy in the newborn rabbit. Furthermore, theophylline significantly increased urine output and creatinine clearances in neonates with acute renal insufficiency due to respiratory distress syndrome (12) or perinatal asphyxia (13), as in diuretic-dependent critically ill children aged 1 wk to 17 y (14). As in the normoxic group, an increased renin release from the JG cells might have occurred after DPCPX administration, and adenosine-induced endothelin release in the kidney might also be involved. Moreover, that the renin-angiotensin system is activated in hypoxemic conditions in this model was previously demonstrated (4, 7). Angiotensin II-induced vasoconstriction therefore may have supplanted A_2 -mediated efferent dilation.

In conclusion, the present results demonstrate that, although specific antagonism of the A_1 receptors with DPCPX prevented the hypoxemia-induced decrease in GFR and diuresis, it did not blunt the increase in RVR and the consequent drop in RBF. These results are in contrast with those observed previously with nonspecific adenosine antagonists. These data may thus be clinically relevant as A_1 receptor antagonists are now being developed for clinical use.

Acknowledgments. The authors thank Mrs. M. Julita and M. Thonney Viani for skillful technical help and their valuable contributions in the laboratory.

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