Adenosine Regulates Renal Nitric Oxide Production in Hypothyroid Rats

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Abstract. In the hypothyroid kidney, exogenous adenosine (ADO) produces vasodilation and restores renal function to near-normal values. This study evaluates whether this response is mediated by nitric oxide synthesis stimulated by adenosine. GFR and urinary excretion of NO\textsubscript{2}/NO\textsubscript{3} (UNO\textsubscript{2}/NO\textsubscript{3}) were measured in normal (NL) and hypothyroid (HTX) rats under basal conditions and during infusion of: intra-aortic ADO, intravenously, 1,3-dipropyl-8p-sulfophenylxanthine (DPSPX), 8-cyclopentyl-1,3-dipropyl xanthine (DPCPX), N\textsuperscript{ω}-nitro-L-arginine methylester (L-NAME) and exogenous adenosine. L-NAME completely blocked the increase in NO\textsubscript{2}/NO\textsubscript{3} in HTX rats. L-NAME, DPSPX, and DPCPX, and intrarenal (IR) ADO or DPCPX + IR ADO. Intra-aortic ADO induced a fall in GFR and increased UNO\textsubscript{2}/NO\textsubscript{3} slightly in NL rats; in HTX rats, both GFR and UNO\textsubscript{2}/NO\textsubscript{3} increased significantly. DPSPX and DPCPX increased UNO\textsubscript{2}/NO\textsubscript{3} excretion in NL animals with minor changes in GFR; the blockers increased both GFR and UNO\textsubscript{2}/NO\textsubscript{3} in HTX rats. L-NAME completely blocked the increase in NO\textsubscript{2}/NO\textsubscript{3} induced by ADO, DPSPX, and DPCPX. The intra-aortic infusion of ADO at 1, 10, and 35 nmol/kg per min progressively decreased GFR with a slight increase in UNO\textsubscript{2}/NO\textsubscript{3} in NL rats; in the HTX, GFR increased with the highest dose and UNO\textsubscript{2}/NO\textsubscript{3} progressively increased. DPCPX prevented the fall in GFR induced by intrarenal ADO in NL rats, with no further changes in UNO\textsubscript{2}/NO\textsubscript{3}; in HTX rats, intrarenal ADO under A1 blockade further increased GFR and UNO\textsubscript{2}/NO\textsubscript{3}. Arterial and venous ADO concentrations were lower in the HTX rats. In the HTX kidney, NO production was stimulated by ADO, most likely through activation of A2 or A3 receptors, whereas A1 receptors had an inhibitory effect. Thus, ADO receptors are involved in the regulation of kidney function in pathophysiologic conditions.

Although exogenous adenosine (ADO) produces systemic vasodilation in the majority of vascular beds (1), in the kidney the nucleoside induces renal vasoconstriction (2,3). This unique vasoactive property is mediated by activation of extracellular receptors: high-affinity A1 receptors induce vasoconstriction and decrease adenylyl cyclase activity and renin secretion; in contrast, activation of low-affinity A2 receptors induces vasodilation and increases adenylyl cyclase activity and renin secretion (4). Recent evidence has suggested that in addition to direct effects on smooth muscle, the vasodilation produced by ADO might implicate nitric oxide (NO) production. In this regard, it has been demonstrated that the vasodilator action of A2 ADO analogues in coronary vasculature is mediated by NO production, since A2 receptors are expressed in the endothelium and in the smooth muscle (5). In isolated renal arteries, N\textsuperscript{ω}-cyclopentyl adenosine and 5′-N-ethylcarboxyamido adenosine induced vasodilation by activation of ADO receptors located in the endothelium and in the smooth muscle, the former involving NO release (6).

Furthermore, ADO stimulates NO production in endothelial cell culture (7). However, controversial evidence has been obtained regarding the effects of ADO on intact aortas and aortic endothelium-denuded preparations contracted with phenylephrine (8).

In the kidney, it is known that the release of NO increases, thus opposing the vasoconstrictor effects of ADO; the same occurs with various vasoconstrictor substances such as angiotensin II and norepinephrine (9). However, an interaction between ADO and renal NO production has not been completely elucidated.

Recent studies from our laboratory have demonstrated that in pathophysiologic conditions such as hypothyroidism, exogenous ADO vasodilates the kidney. The presence of low renal ADO content may induce preferential activation of A1 receptors that leads to the vasoconstriction and decreased GFR as it is generally observed in hypothyroidism (10); under these conditions, a nonselective ADO receptor blocker was able to restore renal function to normal, and exogenous ADO infused into the aorta or the renal artery produced paradoxical vasodilation and increased glomerular blood flow and GFR (11).

To understand the unknown effects of ADO in pathophysiologic conditions, we further studied whether the vasodilatory response to ADO observed in the hypothyroid kidney is mediated by NO production induced by the nucleoside. Total GFR
and urinary excretion of NO$_3^-$/NO$_2^-$ were measured in normal (NL) and hypothyroid (HTX) rats under basal conditions and in response to infusion of ADO, a nonselective ADO receptor blocker (1,3-dipropyl-8-β-sulfophenylxanthine, DPSPX), and an A1 ADO receptor blocker (8-cyclopentyl-1,3-dipropylxanthine, DPCPX), as well as an NO inhibitor (Nω-nitro-L-arginine methylester, L-NAME).

### Materials and Methods

#### Induction of Hypothyroidism

Male Wistar rats weighing 250 to 300 g underwent surgical thyroidectomy with parathyroid reimplant, as described previously (11). Briefly, under ether anesthesia, the trachea was exposed, and under stereoscopic microscope (model M5; Wild, Heerbrugg, Switzerland) the parathyroid glands were visualized, dissected from the thyroid gland, and reimplanted into the surrounding neck muscles. The thyroid gland was then carefully dissected, to avoid injury to the laryngeal nerves, and completely excised. The effectiveness of this procedure was assessed by evaluation of serum calcium, phosphate, and T4 concentrations in 10 control and 10 thyroidectomized rats using standard techniques (Ca 10.1 ± 0.7 NL versus 9.7 ± 0.7 mg/dl HTX, P = NS; phosphate 6.9 ± 0.4 NL versus 6.5 ± 1.1 mg/dl HTX, P = NS, T4 5.1 ± 0.3 NL versus 1.3 ± 0.2 μg/ml HTX, P < 0.05; Diagnostic Products Corp.). Studies were performed 15 d after thyroidectomy in six groups of rats.

#### Experimental Design

Group 1 included seven normal rats used as controls and seven hypothyroid rats. GFR and urinary excretion of NO$_2^-$/NO$_3^-$ (UNO$_2^-$/UNO$_3^-$) were evaluated in a basal period and during an intra-aortic infusion of ADO at 100 nmol/kg per min.

Group 2 included: (a) seven normal rats and seven hypothyroid rats, in which GFR and UNO$_2^-$/UNO$_3^-$ excretion were evaluated in a basal period and during the infusion of the nonselective water-soluble ADO receptor blocker DPSPX; or (b) the A1 ADO receptor blocker DPCPX. A 20 mM solution of DPSPX was infused through the jugular vein at a rate of 1.1 μl/h. We previously found that this dose is sufficient to block a 3 μM N6-cyclopentyladenosine dose (12); DPCPX was infused at a dose of 10 μg/kg per min. In preliminary studies, this dose was sufficient to effectively inhibit A1-dependent ADO responses in the normal kidney (data not shown) (13). Group 3 included seven control and seven hypothyroid rats in which GFR and UNO$_2^-$/UNO$_3^-$ were evaluated in a basal period, during the infusion of the NO blocker L-NAME (100 to 150 μg/kg per min) or l-NAME + ADO. Group 4 included seven control and seven hypothyroid rats in which GFR and UNO$_2^-$/UNO$_3^-$ were evaluated in a basal period, during the infusion of the NO blocker L-NAME, l-NAME + DPSPX, or l-NAME + DPCPX. Group 5 included: (a) Seven normal and seven hypothyroid rats. GFR and UNO$_2^-$/UNO$_3^-$ were evaluated under basal conditions and during the intraaortal infusion of ADO at 1, 10, and 35 nmol/kg per min. In this group, seven additional HTX rats were studied under basal conditions and during intrarenal infusion of vehicle (0.9% NaCl solution). (b) The ability of ADO to stimulate A2 receptors was tested in an additional seven NL and seven HTX rats; in these animals, 35 nM/kg per min ADO was infused into the renal artery during the specific blockade of A1 receptors with DPCPX (10 μg/kg per min). Group 6 included 10 NL and 10 HTX rats in which arterial and venous plasma ADO concentrations were measured.

### Experimental Protocol

For the experiments, the rats were anesthetized with pentobarbital sodium (30 mg/kg, intraperitoneally), and supplementary doses were instilled as required. The rats were placed on a thermo-regulated table, and the temperature was maintained at 37°C. Polyethylene tubing was used to catheterize the trachea (PE 240), both jugular veins, and femoral arteries (PE 50) and ureters (PE 10). In all of the experiments, the intra-aortic catheter was introduced so as to reach 3 to 4 mm beyond the left renal artery. For direct intrarenal infusion, the suprarenal artery was catheterized. In these experiments, the right kidney was studied because only the right renal artery has a suprarenal branch. Mean arterial BP (MAP) was continuously monitored with a pressure transducer (model P23DB; Gould, Hato Rey, Puerto Rico) and recorded on a polygraph (Grass Instruments, Quincy, MA). Blood samples were taken at the beginning and at the end of each urine collection, and replaced with blood from an NL or an HTX donor rat.

The rats were maintained euvoletic by infusion of isotonic rat plasma (10 ml/kg body wt) during surgery, followed by an infusion of 10% polyfructosan (Inuets, Laevosan-Gesellschaft, Austria) and 0.9% sodium saline solution, as vehicle, at 1.1 ml/h.

For the experiments with DPSPX and ADO, a 30-min equilibration period was allowed, for DPCPX a 15-min period, and for l-NAME a 45-min period, after the administration of the compounds. The samples were taken beforehand. DPCPX was solubilized with 100 μl of DMSO plus 100 μl of 1 mM NaOH and added to 2.4 ml of 2-hydroxypropyl-β-cyclodextrin. The pH was adjusted to 7.5 with 0.1N HCl, and the solution was maintained warm during the infusion period. A solution of 2-hydroxypropyl-β-cyclodextrin in 100 μl of DMSO at pH 7.5 was used as vehicle in the DPCPX groups. Polyfructosan concentrations were determined by the technique of Davidson and Sackner (14).

### Quantification of NO$_2^-$/NO$_3^-$

The stable end products of NO$_3^-$ and NO$_2^-$ were generated in the urine samples obtained during the sampling period. Samples were incubated with Escherichia coli nitrate reductase to convert the NO$_3^-$ to NO$_2^-$, as described by Bartholomew (15) and Granger (16). To prepare this enzyme, Escherichia coli were grown for 18 h under anaerobic conditions in a nitrate-rich medium, washed, resuspended in phosphate-buffered saline, and frozen at −70°C until use. The samples were incubated with the enzyme in phosphate-ammonium forma buffer, pH 7.3, for 1 h at 37°C. After incubation, total NO$_2^-$ in the samples (representing both NO$_2^-$ and reduced NO$_3^-$) was measured using the Griess reagent. Known concentrations of NaNO$_2$ and NaNO$_3$ were used as standards in each assay.

### Quantification of Plasma Adenosine

Arterial and venous ADO plasma levels were measured in separate groups of 10 NL and 10 HTX rats (group 6). Under pentobarbital anesthesia, the animals were bled from the carotid artery and from the cava vein above the renal arteries. The blood volume was replaced immediately after the arterial sample was taken with the blood of an NL or HTX donor rat. Only animals whose BP remained unchanged after the arterial sample were used. Two-milliliter blood samples were taken in a 40-μl cold mixture of 20 μl of 20 mM dipyridamol + 20 μl of 20 mM erythro-9-(2-hydroxy-3-nonil)adenine, immediately centrifuged, and the plasma was stored at −70°C until use. The samples were analyzed for ADO using reverse phase HPLC (LC-2355 HPLC system; Perkin Elmer, Norwalk, CT) according to Hammer et al. (17). Separation of the compound was achieved using a C18 octadecyl silane column and a binary gradient containing: buffer A: 30 mM...
KH$_2$PO$_4$, 7.5 mM tetrabutylammonium dihydrogen phosphate (TBA), pH 5.45; and buffer B: 30 mM KH$_2$PO$_4$, 7.5 mM TBA, pH 7.0 in acetonitrile 50% vol/vol. Adenosine was detected by an absorbance change at 255 nm, and the ADO peak was identified and quantified by comparing retention times and peak areas of known standards.

**Statistical Analyses**

The results are expressed as means ± SEM. Statistical differences between baseline and experimental condition data were assessed by $t$ test for paired samples or by repeated measures one-way ANOVA, followed by a Bonferroni test. Differences between groups were evaluated by $t$ test for nonpaired samples, or one-way ANOVA, followed by a Bonferroni test. Statistical significance was defined at $P < 0.05$.

**Results**

**Effects of Intra-Aortic Adenosine Infusion on Renal Function**

In NL rats (group 1), the infusion of ADO had profound effects on renal hemodynamics: GFR decreased by 30%, whereas urinary excretion of NO$_2^-$/NO$_3^-$ increased 2.2 times (Figure 1). In contrast, in HTX rats, ADO infusion produced the opposite effect on renal hemodynamics than that observed in NL rats: GFR increased by 36%, and the increase in UNO$_2^-$ / NO$_3^-$ was 3.6 times higher than in NL (Figure 1). No effects were observed either in MAP (125.8 ± 6 to 121 ± 11.0 mmHg NL versus 115 ± 2.8 to 115.5 ± 2.7 mmHg HTX) or sodium excretion (0.69 ± 0.19 to 0.66 ± 0.16 μEq/min NL versus 0.44 ± 0.07 to 0.46 ± 0.11 μEq/min HTX).

**Effects of Adenosine Receptor Blockade on Renal Function**

The blockade of A1 and A2 ADO receptors with DPSPX in NL rats (group 2a) did not change GFR; however, it significantly increased UNO$_2^-$/NO$_3^-$ fourfold (Figure 2). In HTX rats GFR increased by 32% and UNO$_2^-$/NO$_3^-$ increased 3.3 times (Figure 2). MAP did not change in any group (127.1 ± 1.6 to 126.8 ± 1.0 mmHg NL versus 123.5 ± 6.9 to 113.3 ± 3.8 mmHg HTX). As expected from a xanthine, DPSPX significantly increased urinary sodium excretion in both groups (0.52 ± 0.08 to 2.56 ± 0.97 μEq/min NL, $P < 0.05$ versus 0.46 ± 0.03 to 1.61 ± 0.40 μEq/min, $P < 0.05$ HTX).

The specific blockade of A1 receptors with DPCPX in NL rats (group 2b) increased GFR by 18% and caused a fourfold increase in UNO$_2^-$/NO$_3^-$ (Figure 2). In HTX rats the GFR was 40% higher than in NL, and UNO$_2^-$/NO$_3^-$ increased fourfold (Figure 2). MAP remained unchanged during DPCPX administration in both groups (127.1 ± 1.6 to 126.8 ± 1.0 mmHg NL versus 123.5 ± 6.9 to 113.3 ± 3.8 mmHg HTX), and the blocker increased urinary sodium excretion in both groups.

* $P < 0.05$ vs. BASAL

**Figure 1.** GFR and UNO$_2^-$/NO$_3^-$ in normal (NL) and hypothyroid (HTX) rats under basal conditions (BASAL) and during the infusion of intra-aortic adenosine (ADO).

**Figure 2.** GFR and UNO$_2^-$/NO$_3^-$ in NL and HTX rats under basal conditions (BASAL) and during the infusion of the adenosine receptor blockers 1,3-dipropyl-8-p-sulfophenylxanthine (DPSPX) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).
(0.49 ± 0.07 to 3.18 ± 0.54 P < 0.05 NL versus 0.48 ± 0.08 to 2.41 ± 0.37 μEq/min; P < 0.05 HTX).

**Effects of NO Inhibition on the Renal Response to Intra-Aortic Adenosine and Adenosine Blockers**

In NL rats, L-NAME alone significantly decreased GFR from 1.33 ± 0.11 to 1.5 ± 0.09 ml/min, whereas UNO$_2$/NO$_3$ did not change. When ADO was administered simultaneously with L-NAME to NL rats (group 3), GFR did not change further (Figure 3, top panel), and the increase in UNO$_2$/NO$_3$ produced by ADO was prevented (Figure 3, bottom panel). In HTX rats, GFR as well as UNO$_2$/NO$_3$ remained unchanged with the administration of L-NAME alone. The administration of L-NAME + ADO completely prevented the renal vasodilatory effects of ADO, GFR remained unchanged, and the increase in UNO$_2$/NO$_3$ produced by ADO was completely inhibited (Figure 3). L-NAME alone increased MAP by 17 mmHg in the NL rats. The addition of ADO decreased pressure slightly. In contrast, in the HTX, the increase in MAP was only 10 mmHg and the elevation was transient. MAP returned to basal values with the addition of ADO. L-NAME induced an eightfold increase in sodium excretion, which remained within similar values with L-NAME + ADO in both groups (0.51 ± 0.11 basal, to 3.32 ± 0.83 with L-NAME, and to 3.41 ± 0.52 μEq/min with L-NAME + ADO NL rats versus 0.39 basal, to 3.71 ± 0.07 with L-NAME, to 2.49 ± 0.08 μEq/min with L-NAME + ADO HTX).

When DPSPX was administered simultaneously with L-NAME to NL rats (group 4), GFR returned to normal values and L-NAME completely blocked the increase in UNO$_2$/NO$_3$ induced by DPSPX (Figure 4). In contrast, in the HTX rats, GFR remained unchanged with L-NAME + DPSPX, and the NO inhibitor completely blocked the increase in UNO$_2$/NO$_3$ induced by DPSPX (Figure 4). A similar effect was obtained when DPCPX was simultaneously infused with L-NAME. The inhibition of NO production prevented the increase in GFR produced by A1 ADO receptor blockade in both NL and HTX rats, and the NO inhibitor completely blocked the increase in NO$_2$/NO$_3$ urinary excretion induced by DPCPX in both groups (Figure 4).

In NL rats, both DPSPX and DPCPX produced a significant decrease in MAP, which remained above the basal values. In the HTX rats, MAP returned to basal values. The blockers did not further increase the natriuresis induced by L-NAME in any group (0.50 ± 0.06 basal, to 3.707 ± 0.74 with L-NAME, and to 5.48 ± 0.61 μEq/min with L-NAME + DPSPX, and 0.56 ± 0.07 basal, 4.44 ± 0.57 with L-NAME, and to 5.11 ± 0.42 μEq/min with L-NAME + DPCPX in NL rats versus 0.60 ±

![Figure 3](image3.png)

* P < 0.05 vs. BASAL

*Figure 3. GFR and UNO$_2$/NO$_3$ in NL and HTX rats under basal conditions (BASAL) and during the infusion of the nitric oxide inhibitor N$^\omega$-nitro-l-arginine methyl ester (l-NAME) + ADO.

![Figure 4](image4.png)

*Figure 4. GFR and UNO$_2$/NO$_3$ in NL and HTX rats under basal conditions (BASAL) and during the concomitant infusion of the nitric oxide inhibitor l-NAME plus the adenosine receptor blockers DPSPX or DPCPX.*
0.08 basal, to 4.28 ± 0.70 with l-NAME and to 4.50 ± 0.46 with l-NAME + DPCPX, and 0.61 ± 0.09 basal, to 4.11 ± 0.75 with l-NAME, and to 6.15 ± 0.82 μEq/min with l-NAME + DPCPX in HTX rats).

Effects of Intrarenal Adenosine on Renal Function
When ADO was infused into the renal artery (group 5a), a dose-dependent decrease in GFR was observed in NL rats, 10, 18, and 56% with 1, 10, and 35 nmol/kg per min, respectively (Figure 5A, top panel). Urinary excretion of NO₂⁻/NO₃⁻ increased progressively 1.5 times, 2.8 times, and threefold with 1, 10, and 35 nmol/kg per min, respectively (Figure 5A, bottom panel). Sodium excretion increased from 0.21 ± 0.03 basal to 0.30 ± 0.07, to 0.73 ± 0.12 (P < 0.05), and to 1.03 ± 0.16 μEq/min (P < 0.05), respectively, with the same infusion rates.

In HTX rats, ADO infused into the renal artery produced the opposite effects, and GFR did not change with 1 or 10 nmol/kg per min, but increased by 32% with 35 nmol/kg per min (Figure 5A, top panel). In a similar manner, UNO₂⁻/NO₃⁻ excretion did not change with 1 nmol/kg per min, but increased 3.5 times with 10 nmol/kg per min, and 5.5 times with 35 nmol/kg per min (Figure 5A, bottom panel). Sodium excretion remained unchanged with 1 nmol/kg per min (0.39 ± 0.07 basal versus 0.36 ± 0.07), but increased to 0.79 ± 0.14 μEq/min (P < 0.05) and to 1.24 ± 0.19 μEq/min (P < 0.05) with 10 and 35 nmol/kg per min, respectively.

When ADO was infused into the renal artery under specific blockade of A1 receptors with 10 μg/kg per min DPCPX (group 5b), the blocker induced a slight, 10% increase in GFR, and completely blocked the decrease induced by 35 nmol/kg per min in NL rats (Figure 5B, top panel). UNO₂⁻/NO₃⁻ significantly increased 2.6 times, and remained unchanged with the infusion of DPCPX + ADO (Figure 5B, bottom panel). In contrast, in the HTX rats, the blockade of A1 ADO receptors significantly increased GFR by 15%, and when DPCPX + ADO were infused, a 20% further increase in GFR was observed (Figure 5B, top panel). UNO₂⁻/NO₃⁻ increased fourfold with DPCPX, and a further increase of fivefold altogether was observed with DPCPX + ADO (Figure 5B, bottom panel). UNaV increased in a similar manner in both groups with DPCPX and DPCPX + ADO to reach fivefold (from 0.44 ± 0.10 basal to 3.4 ± 0.51 and to 2.93 ± 0.60 NL, and from 0.37 ± 0.05 to 2.38 ± 0.66 and to 2.6 ± 0.61 μEq/min HTX, respectively).

To exclude the possibility that volume expansion was responsible for the changes observed during ADO infusion, an additional group of seven HTX rats was studied with intrarenal infusion of saline solution as vehicle. No changes were observed in any of the variables estimated (Table 1).

Plasma Adenosine Concentrations
In the NL rats, ADO plasma concentrations were 3.46 ± 0.21 nmol/ml in arterial samples and 3.15 ± 0.20 nmol/ml in venous samples. In the HTX rats, the ADO plasma concentrations were significantly lower, 2.07 ± 0.21 nmol/ml in arterial samples (P < 0.05) and 1.81 ± 0.17 nmol/ml in venous samples (P < 0.05).

Discussion
Under normal conditions, exogenous ADO produces renal vasoconstriction (3,4); however, under certain pathophysiological conditions, different effects may be obtained. We have recently demonstrated that in hypothyroidism, exogenous infusion of the nucleoside induces renal vasodilatation instead of the known vasoconstrictive effect (11). In this study, we evaluated whether this renal vasodilatory response is mediated by increased NO production stimulated by ADO.

The characteristic decrease in GFR induced by ADO in NL rats was demonstrated in group 1 (3,4), whereas in HTX animals, GFR, which was significantly lower than in NL, increased during the infusion of exogenous ADO. This paradoxical response may be related to abnormal renal handling of ADO in this pathophysiologic condition. In hypothyroidism, ADO metabolism is markedly altered; increased 5'-nucleotidase and decreased adenosine kinase activities have been observed in brain cells (18); and 5'-nucleotidase and adenosine deaminase activities in brown and white adipocytes are diminished (19). Scattered information about enzymes involved in ADO metabolism in the hypothyroid kidney is available; however, the fact that renal content of ADO is extremely low suggests an alteration in its renal handling (11). Under these conditions, preferential activation of A1 receptors might contribute to renal vasoconstriction in hypothyroid rats; infusion of exogenous ADO may stimulate A2 receptors, inducing renal vasodilatation.

It has been shown that activation of A2 ADO receptors in vascular beds stimulates NO synthesis in endothelial cells. This was demonstrated in coronary vessels (5), renal artery rings (6), and endothelial cell cultures (7). Furthermore, it has been reported that A3 ADO receptor activation in RBL2H3 cells is

Figure 5. (A) GFR and urinary excretion of NO₂⁻/NO₃⁻ in NL and HTX rats under basal conditions (BASAL) and during the intrarenal infusion (IR) of different doses of ADO. (B) GFR and UNO₂⁻/NO₃⁻ in NL and HTX rats under basal conditions (BASAL) and during specific A1 blockade with DPCPX and DPCPX + IR ADO (35 nmol/kg per min).
coupled to cGMP and stimulates NO production (20). In the kidney, the role of ADO in regulating NO production has not been studied. It has been suggested that both systems interact by NO increasing its release, and thus opposing the ADO-mediated renal vasoconstriction (9). The results with group 1 demonstrated that ADO stimulated NO\textsubscript{2}/NO\textsubscript{3} production in NL and HTX kidneys, but this effect was significantly higher in HTX rats (3.6 times increase in HTX versus 2.2 times increase in NL). Urinary excretion of NO\textsubscript{2}/NO\textsubscript{3} is an accepted marker of endogenous NO production (21). Although acute changes in NO\textsubscript{2}/NO\textsubscript{3} urinary excretion might not reflect changes in NO production (22), recent studies demonstrated a direct increase in urinary NO\textsubscript{2}/NO\textsubscript{3} derived from inhaled NO, supporting that NO\textsubscript{2}/NO\textsubscript{3} provides an accurate reflection of acute systemic NO\textsubscript{2}/NO\textsubscript{3} changes (23). Controversial evidence of sodium reabsorption directly influencing NO\textsubscript{2}/NO\textsubscript{3} excretion has been obtained (21,22), since no changes in UNaV excretion were observed in NL or HTX rats, this possibility can be discarded.

Because ADO was infused into the aorta, and urinary excretion of NO\textsubscript{2}/NO\textsubscript{3} represents various sources of nitrates, the possibility that stimulatory effects of A2 ADO receptors in aortic endothelial cells may contribute to NO production cannot be ruled out; however, the increase in GFR in HTX kidneys suggests a renal effect.

To further study the effects of ADO in the HTX kidney, ADO receptors were blocked with a nonselective compound (DPSPX) and a specific A1 receptor antagonist (DPCPX). The blockade of A1 and A2 ADO receptors did not change GFR in NL animals, but the specific blockade of A1 receptors slightly increased GFR. UNO\textsubscript{2}/NO\textsubscript{3} increased in NL and HTX groups with the nonselective antagonist and to a larger extent with the A1 blocker. Under normal conditions, A1 ADO receptors are constitutively activated (24), and in hypothyroidism preferential activation of A1 receptors may be responsible for the renal vasoconstriction observed in this pathology (11). Thus, the effect of the nonselective blocker may be essentially due to blockade of A1 receptors. Certainly, this was supported in a direct manner by the specific blockade of A1 receptors. The increases in GFR and NO in the HTX rats induced with both blockers indicate that activation of A1 ADO receptors contributes to the reduction of GFR in this pathophysiologic condition, and that these receptors exert an inhibitory effect on renal NO production. In addition, the higher increase of GFR in NL and HTX groups, as well as the higher increase in UNO\textsubscript{2}/NO\textsubscript{3} obtained with the specific blockade of A1 receptors, suggest a role for A2 receptors in the increased GFR and UNO\textsubscript{2}/NO\textsubscript{3} in NL and HTX rats, since A2 receptors were blocked when DPSPX was used. Nevertheless, the dependency of GFR on NO production seems to be very important in hypothyroidism. The blockade of ADO receptors induced a significant natriuresis in NL and HTX animals, which is in agreement with previous reports (25).

To support the evidence that the increased UNO\textsubscript{2}/NO\textsubscript{3} induced by ADO and ADO blockers was actually due to changes in NO production, NO synthase was inhibited with L-NAME. L-NAME decreased GFR without changing UNO\textsubscript{2}/NO\textsubscript{3} in NL animals, as has been described (21,26,27). In HTX rats, high doses of L-NAME did not change either GFR or UNO\textsubscript{2}/NO\textsubscript{3} excretion. The lack of effects of L-NAME on GFR in the HTX group may be related to the diminished response of smooth muscle to vasoconstrictors in hypothyroidism, as observed in the aorta and the renal artery (28,29). When ADO, DPSPX, or DPCPX was infused simultaneously with L-NAME, the most remarkable finding was that the blockade of the NO synthesis completely prevented the stimulatory effect of ADO and ADO blockers on NO production in NL or HTX rats. Furthermore, the administration of L-NAME + ADO prevented the rise in GFR induced by the nucleoside in the HTX rats. L-NAME also prevented the rise in GFR induced by DPSPX and DPCPX in HTX, as well as the rise induced by the latter in NL rats. These data suggest that ADO directly modulates renal NO production. It should be mentioned that in NL rats ADO + L-NAME did not further decrease GFR below the level obtained with L-NAME alone. Thus, the previously reported potentiation of ADO effects with L-NAME (9,30) was not observed. We attributed this discrepancy to the high L-NAME dose used before ADO administration; if a maximal L-NAME-induced decrease in GFR is achieved, it may impair an additional reduction.

To further define the role of renal NO synthesis in the response to exogenous ADO, ADO was infused into the renal artery. In NL rats, ADO produced only a slight increase in UNO\textsubscript{2}/NO\textsubscript{3} excretion associated with progressive decrease in GFR. This can be explained by stimulation of NO in response to the renal ADO-mediated vasoconstriction as has been proposed (9). In contrast, in HTX rats, the infusion of ADO produced a much greater increase in the UNO\textsubscript{2}/NO\textsubscript{3} (5.6

| Table 1. Time control of GFR, urinary excretion of nitrates (UNO\textsubscript{2}/NO\textsubscript{3}), urinary sodium excretion (UNaV), and mean arterial pressure (MAP) under basal and three consecutive 70- to 90-min clearance periods with infusion of vehicle (VEH) in hypothyroid rats (n = 7) |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | Basal           | VEH 1           | VEH 2           | VEH 3           |
| GFR (ml/min)                   | 0.81 ± 0.05     | 0.79 ± 0.06     | 0.76 ± 0.07     | 0.82 ± 0.06     |
| UNO\textsubscript{2}/NO\textsubscript{3} (nmol/min per g kidney wt) | 7.40 ± 0.77     | 8.49 ± 0.92     | 8.69 ± 1.17     | 8.12 ± 0.89     |
| UNaV (μEq/min)                 | 0.5057 ± 0.0804 | 0.4297 ± 0.1186 | 0.4088 ± 0.0909 | 0.4245 ± 0.1207 |
| MAP (mmHg)                     | 106.0 ± 1.7     | 103.4 ± 2.4     | 106.8 ± 1.6     | 101.0 ± 1.2     |
versus 3.0 times), which was associated with an increase in GFR instead of the fall observed in NL animals. The dose-related ADO stimulation of NO production in the HTX kidney suggests that NO mediates the renal vasodilatory response. This notion was further supported by the data obtained with DPCPX and intrarenal ADO. The A1 receptor blockade increased UNO\textsuperscript{2}/NO\textsubscript{3} excretion in NL rats, which remained unchanged with further addition of ADO, and prevented the fall in GFR observed with the nucleoside. In HTX rats, the rise of UNO\textsuperscript{2}/NO\textsubscript{3} and GFR induced by DPCPCX further increased with the addition of ADO, suggesting stimulation of A2 or A3 receptors. Upregulation of ADO A2 or A3 receptors, changes in sensitivity to ADO, as well as changes in the coupling with G proteins or in intracellular signals could explain the response observed in the HTX kidney. However, little is known about the regulation of ADO receptors and the mechanisms involved in A2 or A3 receptor regulation in pathophysiologic conditions (31).

It should be mentioned that intrarenal ADO increased UNaV in NL and HTX rats to the same extent. These results, in addition to the lack of changes in UNaV obtained with the intra-aortic ADO, support the notion that the increase of UNO\textsuperscript{2}/NO\textsubscript{3} is due to renal production, since it can be clearly dissociated from urinary sodium excretion. These results are in agreement with previous studies (32).

Finally, since the arterial ADO concentrations that reach the kidney contribute to the renal effects of the nucleoside, i.e., regulation of glomerular blood flow, renal resistance, renin secretion, and in some cases GFR, we measured plasma ADO concentrations in arterial and venous samples. Indeed, both arterial and venous ADO concentrations were significantly lower in HTX rats than in NL (40 and 42% fall, respectively). However, arterial ADO plasma concentrations are not the only source of ADO, which is also formed in cells from the diverse renal structures such as tubules, mesangium, and glomerular tuft. Thus, interstitial concentrations are relevant for the paracrine action of ADO, since it would exert its vascular effects from the adventitial side (33). These results, in addition to the decreased renal content of ADO reported previously (11), support the possibility that alterations in ADO concentration may modify ADO receptor expression and/or sensitivity in the hypothyroid kidney, as has been shown in adipocytes from HTX rats (34,35). This mechanism may be responsible for the paradoxical renal vasodilation induced by ADO in this model. However, the contribution of other receptor-mediated mechanisms such as coupling with G proteins and changes in intracellular signals cannot be ruled out.

In summary, in the hypothyroid kidney A1 ADO receptors have an inhibitory effect, whereas A2 or A3 receptors have a stimulatory effect of NO production. This is a novel role for ADO regulating NO production in physiopathologic conditions. Additional studies will be required to establish whether A2 and A3 ADO receptors or changes in membrane or intracellular signals are responsible for the increased NO production in the hypothyroid kidney.

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References

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