Nitric oxide inhibits superoxide-stimulated urea permeability in the rat inner medullary collecting duct

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Zimpelmann, Joseph, Ningjun Li, and Kevin D. Burns. Nitric oxide inhibits superoxide-stimulated urea permeability in the rat inner medullary collecting duct. Am J Physiol Renal Physiol 285: F1160–F1167, 2003. First published September 9, 2003; 10.1152/ajprenal.00077.2003.—The inner medullary collecting duct (IMCD) contains relatively high nitric oxide (NO) synthetic capacity, but the effect of NO on IMCD transport remains unclear. We determined the effect of NO on basal and vasopressin (AVP)-stimulated urea (P_{area}) and water (P_{f}) permeabilities in isolated, perfused rat IMCD. The NO donor S-nitroso-N-acetylpenicillamine (SNAP) increased cGMP production in IMCD, but neither SNAP (10^{-4} M) nor 8-BrcGMP (10^{-4} M), the cell-permeable analog of cGMP, affected basal or AVP-stimulated P_{area}. The free radical superoxide is produced by oxidases in the kidney and can interact with NO. To determine the effect of superoxide generation on transport, IMCDs were incubated with diethyldithiocarbamate (DETC; 10^{-5} M), the inhibitor of superoxide dismutase (SOD). DETC significantly increased basal and AVP-stimulated P_{area} (control: 28.7 ± 4.5 vs. DETC: 40.9 ± 6.2 × 10^{-5} cm/s; P < 0.001; n = 9). Preincubation of IMCD with SNAP or the SOD mimetic tempol completely inhibited DETC-stimulated P_{area}. DETC caused a significant increase in superoxide generation by IMCD, and this was blocked by SNAP. Incubation of IMCD with the NO synthase (NOS) substrate L-arginine blocked the stimulatory effect of DETC on P_{area}, and this was reversed by the neuronal NOS inhibitor 7-nitroindazole. In contrast, neither basal nor AVP-stimulated P_{f} was affected by NO donors or DETC. In summary, exogenous or endogenously produced NO does not affect basal urea transport in the IMCD but inhibits superoxide-stimulated P_{area}. In the inner medulla, superoxide generation by local oxidases may contribute to the urine-concentrating mechanism. The IMCD contains the highest capacity for NO synthetism of all nephron segments (42) and expresses all three isoforms of NOS (1, 33, 40, 42). However, the function of NO in the IMCD remains unclear. Inhibition of NO production in the inner medulla has been shown to decrease urinary sodium excretion and increase blood pressure in rats (18), suggesting a role for NO in regulating vascular tone. On the other hand, NO donors have been reported to inhibit both sodium reabsorption and vasopressin (AVP)-stimulated osmotic water permeability (P_{f}) (7, 8, 39). Taken together, our results suggest that NO is an intrarenal natriuretic and diuretic factor.

NOS is expressed in the mammalian kidney, and studies indicate that the gaseous product of NOS, nitric oxide (NO), regulates transport function in several nephron segments (26). In the proximal tubule, both inhibitory and stimulatory effects of NO on apical Na^{+}/H^{+} exchange have been described (6, 32, 41), and NO appears to inhibit the basolateral Na^{+}-K^{+}-ATPase (16). In thick ascending limb, NO inhibits apical Na^{+}/H^{+} exchange and Na^{+}-K^{+}-2Cl^{-} cotransport activity (9, 25, 29). Recent studies by Ortiz and Garvin (27, 28) reveal that the bioavailability of NO produced by the thick ascending limb is reduced by endogenous production of superoxide anion (O_{2}^{-}), which itself exerts a stimulatory effect on NaCl transport in this segment. In cortical collecting duct, NO has been shown to inhibit both sodium reabsorption and vasopressin (AVP)-stimulated osmotic water permeability (P_{f}) (7, 8, 39). Taken together, these studies suggest that NO is an antinatriuretic and diuretic factor.

The present studies examined the effect of NO on basal and AVP-stimulated urea (P_{area}) and P_{f} in the rat IMCD, using the isolated, perfused tubule technique. We uncover a role for an interaction between NO and O_{2}^{-} in the regulation of urea transport in IMCD.

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MATERIALS AND METHODS

Preparation of rat IMCDs. Rat terminal IMCD segments were prepared for microperfusion, essentially as described (21). Briefly, male Sprague-Dawley rats (100–150 g), fed regular chow and with unlimited access to water, were killed by decapitation and the kidneys were rapidly removed. Coronary sections were cut and placed in a chilled hyperton solution containing (in mM) 190 NaCl, 25 NaHCO3, 5 KCl, 10 urea, 5 HEPES, 1.2 MgCl2, 15 CaCl2, and 8 glucose, as well as 0.1% albumin (solution A), previously equilibrated with 95% air-5% CO2. IMCD segments were dissected and isolated from the terminal two-thirds of the inner medulla (terminal IMCD). Once dissected, the tubule was transferred to a thermostatically controlled perfusion chamber containing bathing solution, on the stage of an inverted microscope, and mounted on glass pipettes that suspended the tubule in the bath. The temperature of the bath was maintained at 37°C. The inner pipette contained perfusion solution, and perfusion was initiated via a 1-mL syringe connected to PE-10 tubing that was mounted behind the taper at the tip of the perfusion pipette. The perfusate accumulated in the tip of the opposite pipette. Samples of collected perfusate were taken at timed intervals during each experiment. At least 15 min elapsed after each perfusate change before collections were started.

Measurement of Pfl. For experiments involving water transport, the bath solution was identical to solution A. The perfusate solution was of identical composition, except that it contained 115 mM NaCl, to ensure existence of a transepithelial osmotic gradient. All solutions were prepared weekly and were equilibrated with 95% air-5% CO2 at 37°C before each experiment, with solution pH between 7.37 and 7.43. For water transport, perfusates contained [methoxy-3H]inulin (100–500 mCi/g, New England Nuclear, Division of PerkinElmer, Boston, MA) that had been dialyzed against distilled water at 4°C for at least 48 h. The bath solution was pumped through the perfusion chamber at 1.0 mL/min. AVP (5 × 10−12 or 10−9 M), NO donors, or other agents were added to the bath in addition to AVP.

Net volume flux (Jv; nL-mm−2-mm−1) was calculated for each collection from the equation Jv = (V0 − Vl)/ML, where V0 is the perfusion rate (nl/min), and L is tubule length (mm). Vl was measured directly, and V0 was calculated from V0 = Vl/C0, where C0 and C represent molarity (mM) of collected fluid and perfusate, respectively. Tubule length was determined by eyepiece micrometer at the end of each experiment. Jv (μm/s) was determined from Jv = RL(Nw/Vw), where Nw is the partial molar volume of water, and L is the hydraulic conductivity, determined as described (5); Lp = 1/[RTSC(C0[V0 − Vl] + C0(1/C0 − 1/C0)C0C0Vl/C0C0Vl)]. l is the luminal surface area calculated from lumen diameter and assumes that the tubule is a perfect cylinder. C0 and C represent the perfusate and bath osmolalities, respectively, measured with a freezing-point osmometer (Advanced Instruments, WVR, Montreal, Quebec). This calculation of Jv assumes that an effective osmotic gradient exists along the entire length of the perfused tubule. To ensure that this was true for all experimental conditions, tubules were perfused at 17–25 nL/min. In all experiments, three to four collections were made for each experimental condition, and results were averaged to obtain a single value, which was used for statistical analysis.

Measurement of Pfl. Pfl was measured using a bath-to-lumen urea concentration difference, essentially as described (37, 38). The bath solution contained (in mM) 115 NaCl, 25 NaHCO3, 5 KCl, 5 HEPES, 1.2 MgCl2, 1.5 CaCl2, 8 glucose, and 20 urea. The perfusion solution was identical, except that it contained 20 mM raffinose, but no urea. This established a concentration gradient driving the passive transport of urea from bath to lumen. Urea transport rate was calculated as $J_{\text{urea}} = C_0 - V_0 - C_L - V_L$, where $C_0$ is the urea concentration in the perfusate, $C_L$ is the urea concentration in the collected fluid, $V_0$ is the perfusion rate per unit tubule length, and $V_L$ is the collection rate per unit tubule length. $C_0$ was zero for all experiments. $P_{\text{urea}}$ was then calculated from $J_{\text{urea}}$, as $P_{\text{urea}} = J_{\text{urea}}/(\pi D^2 \Delta C_0)$, where $\Delta C_0$ is the mean urea concentration difference along the tubule, and $D$ is the tubule inner diameter, measured by eyepiece micrometer. In all experiments involving urea transport, three to four samples were collected for each experimental condition, and results were averaged to obtain a single value that was used for statistical analysis. The tubule characteristics and solution osmolalities for experiments involving urea and water transport are presented in Table 1.

The concentrations of urea in the perfusate, bath, and collected fluid were measured by an enzymatic assay, involving spectrophotometry. In this assay, urea is first converted to ammonia in the presence of urease, and then ammonia reacts with alkaline hypochlorite, in the presence of the catalyst phenol nitroprusside to form iodophenol. Iodophenol concentration was measured by absorbance at 570 nm (Spectronic Genesys V, ESBE Scientific, St. Laurent, Quebec) and is proportional to urea concentration. All reagents were purchased as a urea nitrogen assay kit (640A, Sigma, St. Louis, MO). Standard curves were prepared for each experiment, using serial dilutions of the bath solution, which demonstrated linearity.

NO measurements. NO emission from the NO donors S-nitroso-N-acetylpenicillamine (SNAP) and SNP was measured using a carbon fiber NO electrode (ISONOP sensor, 2-mm tip diameter, World Precision Instruments, Sarasota, FL), with calibration performed in the presence of SNAP and copper sulfate, essentially as we have described (14). The incubation solution consisted of an isotonic solution of (in mM) 115 NaCl, 25 NaHCO3, 5 KCl, 5 HEPES, 1.2 MgCl2, 1.5 CaCl2, 8 glucose, and 20 urea.

Measurement of cGMP. Suspensions of rat IMCDs were isolated from renal papillae, after reconstitution in hypotonic solution, exactly as previously described (33). Tubules were then immediately washed three times in Krebs buffer and incubated at 37°C for 30 min in Krebs, supplemented with IBMX (5 × 10−4 M), in the presence or absence of agonists. After addition of ice-cold trichloroacetic acid (final concentration 10% vol/vol), samples were extracted four times with four volumes of water-saturated ether and brought to pH 7.0 with Tris. Aliquots were assayed for cGMP, using a radioligand competitive binding assay kit, containing [3H]cGMP (Amersham, Mississauga, Ontario), as we have performed.

Table 1. Isolated, perfused IMCD characteristics and solution osmolalities

<table>
<thead>
<tr>
<th>Tubule Length, mm</th>
<th>Tubule Inner Diameter, μm</th>
<th>Bath Osmolality, mosmol/kgH2O</th>
<th>Perfusion Osmolality, mosmol/kgH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{urea}}$</td>
<td>0.75 ± 0.01</td>
<td>24.1 ± 0.2</td>
<td>307 ± 1</td>
</tr>
<tr>
<td>$P_l$</td>
<td>0.84 ± 0.02</td>
<td>21.7 ± 0.3</td>
<td>439 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 48 tubules [measurements of urea permeability ($P_{\text{urea}}$)] and n = 26 tubules [measurements of water permeability ($P_l$)]. IMCD, inner medullary collecting duct.
Fig. 1. Nitric oxide (NO) stimulates cGMP production in inner medullary collecting duct (IMCD). A: IMCD segments were incubated with S-nitroso-N-acetylpenicillamine (SNAP; 10⁻⁴ M) or atrial natriuretic peptide (ANP; 10⁻⁷ M) for 30 min before assay of cGMP. *P < 0.01 vs. control (C). **P < 0.001 vs. C (n = 7). B: concentration dependence of stimulation of cGMP by the NO donor SNAP. Values are means ± SE of experiments performed in duplicate. *P < 0.01 vs. C. **P < 0.02 vs. C (n = 3).

**RESULTS**

Effect of NO donors on cGMP in IMCD. NO stimulates guanylate cyclase in many tissues, leading to generation of cGMP. Experiments were first performed to determine whether NO stimulates cGMP production in isolated IMCD segments. As shown in Fig. 1, the NO donor SNAP caused concentration-dependent increases in cGMP in IMCD, with maximal effect at 10⁻⁷ M. ANP (10⁻⁷ M) also significantly increased production of cGMP. The NO donor SNAP also stimulated cGMP production in IMCD segments [control: 11.5 ± 1.0 vs. SNP (10⁻⁴ M): 21.1 ± 2.9 fmol/μg; P < 0.04; n = 3]. In separate experiments, NO production was measured in solution, using a carbon fiber NO electrode in the presence of SNAP or SNP. SNAP (10⁻⁴ M) was associated with significantly higher NO production in solution, compared with 10⁻⁴ M SNP [SNAP (10⁻⁴ M): 1.98 ± 0.05 μM NO vs. SNP (10⁻⁴ M): 0.23 ± 0.02 μM NO; P < 0.001; n = 3]. Accordingly, further experiments predominantly utilized SNAP (10⁻⁴ M) as a NO donor.

Effect of NO and 8-Br-cGMP on Pₚ. Single-tubule microperfusion studies were performed to determine the effect of SNAP or the cell-permeable analog of cGMP 8-Br-cGMP on urea transport in IMCD. In microdissected IMCD, AVP (5 × 10⁻¹² M) caused a sig-

![Image of Fig. 2](https://via.placeholder.com/150)

**Fig. 2.** Effect of SNAP and 8-Br-cGMP on basal and AVP-stimulated urea permeability (Pₚ). A: results from 7 tubules in which Pₚ was determined at baseline, after addition of AVP (5 × 10⁻¹² M) to the bath, and after addition of SNAP (10⁻⁴ M) with AVP (5 × 10⁻¹² M) to the bath. Values are means ± SE. *P < 0.001 vs. control (C). B: results from 4 tubules in which Pₚ was measured at baseline (C), after addition of SNAP (10⁻⁴ M), and then after addition of AVP (5 × 10⁻¹² M) and SNAP (10⁻⁴ M) to the bath. Values are means ± SE. *P < 0.01 vs. C. C: results from 5 tubules in which Pₚ was measured at baseline (C), after addition of 8-Br-cGMP (10⁻⁴ M) to the bath, and then after addition of AVP (5 × 10⁻¹² M) and 8-Br-cGMP (10⁻⁴ M) to the bath. Values are means ± SE. *P < 0.001 vs. C.
significant increase in $P_{\text{urea}}$ (Fig. 2). This concentration of AVP elicited a submaximal response. In separate experiments, AVP ($10^{-9}$ M) caused a further stimulation of $P_{\text{urea}}$ [AVP ($5 \times 10^{-12}$ M): $64.7 \pm 1.7 \times 10^{-5}$ cm/s vs. AVP ($10^{-9}$ M): $103.2 \pm 4.8 \times 10^{-5}$ cm/s; $P < 0.001$; $n = 3$].

The addition of SNAP ($10^{-4}$ M) to the bath did not affect basal or AVP-stimulated $P_{\text{urea}}$, when added before or after AVP ($5 \times 10^{-12}$ M) (Fig. 2, A and B). The addition of 8-BrcGMP ($10^{-4}$ M) also had no effect on basal or AVP-stimulated $P_{\text{urea}}$ (Fig. 2C).

Effect of $O_2^*$ generation on $P_{\text{urea}}$ in IMCD. Recent studies indicate that $O_2^*$ stimulates tubular sodium transport, an effect that is diminished in the presence of NO (28, 29). In microdissected IMCD, the inhibitor of superoxide dismutase DETC ($10^{-3}$ M) (28) caused a significant stimulation of basal and AVP-stimulated $P_{\text{urea}}$ (basal $P_{\text{urea}}$: control, $28.7 \pm 4.5 \times 10^{-5}$ cm/s; DETC, $40.9 \pm 6.2 \times 10^{-5}$ cm/s; $P < 0.001$; $n = 9$) (Fig. 3).

Preincubation of tubules with SNAP ($10^{-4}$ M) completely blocked the stimulatory effect of DETC on $P_{\text{urea}}$, whereas removal of SNAP uncovered the DETC-associated increase in $P_{\text{urea}}$ (Fig. 4A). The cell-permeable mimic of superoxide dismutase 4-hydroxytetramethyl-piperidine-1-oxyl (tempol; $10^{-4}$ M) (11, 30) did not affect basal $P_{\text{urea}}$ but inhibited the stimulatory effect of DETC on $P_{\text{urea}}$, with a small but significant recovery of $P_{\text{urea}}$ when tempol was removed from the bath (Fig. 4B).

Effect of NO on DETC-induced $O_2^*$ generation in IMCD. In microdissected IMCD segments, DETC ($10^{-3}$ M) caused a significant increase in generation of $O_2^*$ (Fig. 5). The NO donor SNAP ($10^{-4}$ M) did not affect basal $O_2^*$ levels but completely inhibited DETC-stimulated $O_2^*$ production. Incubation of IMCD with cGMP ($10^{-4}$ M) or 8-BrcGMP ($10^{-4}$ M) had no effect on $O_2^*$ generation (control: $15.9 \pm 2.1$ vs. cGMP: $14.0 \pm 1.4$, vs. 8-BrcGMP: $17.0 \pm 1.1$ arbitrary units; $P = NS$ for all comparisons; $n = 3–5$).

Effect of endogenous NO production on DETC-stimulated $P_{\text{urea}}$. The IMCD has been shown to have considerable capacity to synthesize NO (42), and the neuronal isoform of nitric oxide synthase (nNOS) is highly expressed in this segment (33). To determine whether endogenous NO production might affect DETC-stimulated urea transport, microdissected IMCD were preincubated with the NOS substrate L-arginine (L-Arg, $0.25 \times 10^{-3}$ M). As shown in Fig. 6A, L-Arg had no effect on basal $P_{\text{urea}}$, but it blocked DETC-stimulated $P_{\text{urea}}$ (control: $21.5 \pm 6.2$ vs. L-Arg: $22.7 \pm 7.7$, vs. L-Arg+DETC: $24.9 \pm 7.4 \times 10^{-5}$ cm/s; $P = NS$ for all comparisons; $n = 4$). Preincubation of IMCD with the inhibitor of nNOS 7-nitroindazole (7-NI; $10^{-6}$ M) sig-

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**Fig. 3.** Effect of the superoxide dismutase inhibitor diethyldithiocarbamate (DETC) on basal and AVP-stimulated $P_{\text{urea}}$ in IMCD. A: results from 9 tubules in which $P_{\text{urea}}$ was measured at baseline (C) and then after addition of DETC ($10^{-3}$ M) to the bath. Values are means $\pm$ SE. $\ast P < 0.001$ vs. C. B: results from 3 tubules in which $P_{\text{urea}}$ was measured at baseline (C), after AVP ($5 \times 10^{-12}$ M) to the bath, and after addition of DETC ($10^{-3}$ M). Values are means $\pm$ SE. $\ast P < 0.001$ vs. C. **$\ast P < 0.005$ vs. AVP.

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**Fig. 4.** SNAP and 4-hydroxytetramethyl-piperidine-1-oxyl (tempol) inhibit DETC-stimulated $P_{\text{urea}}$ in IMCD. A: results from 6 tubules in which $P_{\text{urea}}$ was measured at baseline (C), after addition of SNAP ($10^{-4}$ M) to the bath, and then after addition of DETC ($10^{-3}$ M) to the bath. In the final part of the experiment, SNAP was removed from the bath perfusate, and DETC alone was present, which caused a significant stimulation of $P_{\text{urea}}$. Values are means $\pm$ SE. $\ast P < 0.001$ vs. C. B: results from 5 tubules in which $P_{\text{urea}}$ was measured at baseline (C), after addition of the permeable superoxide dismutase mimetic tempol ($10^{-4}$ M) to the bath, and then after addition of tempol ($10^{-4}$ M) and DETC ($10^{-3}$ M) to the bath. In the last part of the experiment, tempol was removed from the bath perfusate, and DETC alone was present. Values are means $\pm$ SE. Removal of tempol in the presence of DETC caused a significant stimulation of $P_{\text{urea}}$. $\ast P < 0.005$ vs. C.
In the present studies, we found no significant effect of endogenous or exogenous NO on basal or AVP-stimulated $P_{\text{urea}}$ or $P_f$ in the IMCD. The major positive finding is that NO completely blocked the stimulatory effect of DETC, an inhibitor of superoxide dismutase (SOD), on $P_{\text{urea}}$. This occurred with exogenous NO (SNAP) or with incubation with the NO precursor l-Arg. We utilized SNAP as a NO donor for most experiments, because it was associated with significantly higher NO release than SNP. Although SNAP may release both NO and O$_2^\cdot$ rapidly activates urea transport in IMCD, and NO may reduce the availability of O$_2^\cdot$ to form peroxynitrite. However, the possible independent effects of peroxynitrite on urea transport were not significantly reversed the inhibitory effect of l-Arg on DETC-stimulated $P_{\text{urea}}$ (Fig. 6B).

Effect of NO and DETC on basal and AVP-stimulated $P_f$. Administration of AVP to the bath caused a concentration-dependent stimulation of $P_f$, as expected [control: 64.5 ± 19.7 μm/s vs. AVP (5 × 10$^{-12}$ M): 1,025.4 ± 47.5 μm/s; $P < 0.001$ vs. control; vs. AVP (10$^{-9}$ M): 1,349.0 ± 26.9 μm/s; $P < 0.005$ vs. AVP (5 × 10$^{-12}$ M); $n = 3$]. Neither SNAP (10$^{-4}$ M) nor SNP (10$^{-4}$ M) affected basal or AVP-stimulated $P_f$ (Fig. 7A). Addition of SNAP (10$^{-4}$ M) after administration of AVP to the bath also had no significant effect on $P_f$ (Fig. 7B). Similarly, addition of 8-BrcGMP (10$^{-4}$ M) to the bath had no effect on basal or AVP-stimulated $P_f$ [control: 42.5 ± 13.9 μm/s vs. 8-BrcGMP: 34.2 ± 5.9 μm/s; $P = $NS vs. control; 8-BrcGMP+AVP (5 × 10$^{-12}$ M): 1,116.4 ± 17.2 μm/s; $P < 0.001$ vs. AVP; $n = 3$]. In contrast, as a positive control, addition of endothelin (10$^{-8}$ M) to the bath caused a significant inhibition of AVP-stimulated $P_f$, as previously described [control: 23.8 ± 9.5 μm/s vs. AVP (5 × 10$^{-12}$ M): 1,211.1 ± 102.8 μm/s; $P < 0.001$ vs. control; AVP+endothelin (10$^{-8}$ M): 574.1 ± 51.8 μm/s; $P < 0.01$ vs. AVP alone; $n = 3$] (22).
addressed in this study and require further investigation. Our data also confirm that NO stimulates cGMP production in IMCD (13) and that basal $P_{\text{urea}}$ in terminal IMCD is relatively high and increased further by AVP (37, 38).

Of all nephron segments, the capacity to generate NO is highest in the IMCD (42), and indeed all three isoforms of NOS are expressed in the IMCD (1, 33, 40, 41). A high-salt diet increases protein expression of nNOS in IMCD (33) and, in cultured mouse IMCD cells, shear stress increases NO production (3). Studies support a role for IMCD-derived NO in modulating sodium transport in IMCD. Zeidel and colleagues (43) showed that SNP inhibited oxygen consumption and sodium uptake in rabbit IMCD cells, an effect mimicked by 8-BrcGMP. Furthermore, cGMP inhibits apical membrane sodium channels in IMCD, an effect that may be involved in mediating the inhibitory effects of ANP on sodium transport in this segment (17).

The present studies focused on effects of NO on urea and water transport, rather than sodium transport. NO had no effect on basal or AVP-stimulated $P_{\text{urea}}$ or $P_t$. Recent studies indicate that tubular segments generate O$_2^-$, mainly via the NADH oxidase pathway, with the highest activity in the thick ascending limb of the loop of Henle (15). NAD(P)H oxidase has been localized to various rat nephron segments, including IMCD (4). Reactive oxygen species such as O$_2^-$ are important regulators of cell signaling and regulate vascular tone, via their interaction with NO. Inactivation of SOD activity with DETC, for example, selectively inhibits NO-induced vasorelaxation in coronary arteries (20). In rats, renal medullary infusion of DETC decreases medullary blood flow and sodium excretion, whereas the SOD mimetic tempol induces the opposite effect (46). In thick ascending limb, endogenous production of O$_2^-$ is associated with stimulation of Cl$^-$ transport and with a decrease in the bioavailability of NO (27, 28). This suggests that O$_2^-$ regulates nephron transport under physiological conditions.

The present studies demonstrate that DETC significantly increases $P_{\text{urea}}$ in IMCD, determined by imposing a bath-to-lumen urea gradient across the tubule, as described elsewhere (37). Exogenous NO blocked this effect, as did incubation of IMCDs with tempol (Fig. 4). The effect of endogenous production of NO in IMCD was also examined. L-Arg had no effect on basal $P_{\text{urea}}$, but it blocked DETC-stimulated $P_{\text{urea}}$ (Fig. 6). Furthermore, 7-NI, the inhibitor of nNOS, blocked this inhibitory effect of L-Arg but had no effect on basal $P_{\text{urea}}$. These data suggest that, under basal conditions, O$_2^-$ levels in IMCD are low and do not affect urea transport. The lack of effect of exogenous NO or the SOD mimetic tempol on basal $P_{\text{urea}}$ supports this possibility, because any further decrease in O$_2^-$ levels induced by these maneuvers would not be expected to affect urea transport. However, stimulation of O$_2^-$ increases $P_{\text{urea}}$, an effect prevented by NO generation. Accordingly, the

![Fig. 7. Effect of NO on basal and AVP-stimulated water permeability ($P_t$). A: IMCD tubules were incubated with or without SNAP or SNP (10$^{-4}$ M) in the bath, followed by AVP (5 × 10$^{-12}$ M) in the bath in the presence or absence of SNAP or SNP. Values in parentheses above the bars indicate nos. of tubules. *$P < 0.001$ vs. C. B: osmotic $P_t$ was determined in IMCD tubules (n = 4) at baseline (C), after the addition of AVP (5 × 10$^{-12}$ M), and then after the addition of SNAP (10$^{-4}$ M) with AVP to the bath. Values are means ± SE at each point. *$P < 0.001$ vs. C.](http://ajprenal.physiology.org/)

![Fig. 8. Effect of DETC on basal and AVP-stimulated osmotic $P_t$ in IMCD. A: results from 4 tubules (results from 2 tubules are superimposed) in which $P_t$ was measured at baseline (C), after addition of DETC (10$^{-3}$ M) to the bath, and then after addition of AVP (5 × 10$^{-12}$ M) to the bath. Values are means ± SE. *$P < 0.001$ vs. C. B: results from 4 tubules in which $P_t$ was measured at baseline (C), after addition of AVP (5 × 10$^{-12}$ M) to the bath, and then after addition of DETC (10$^{-3}$ M) to the bath. Values are means ± SE. *$P < 0.001$ vs. C.](http://ajprenal.physiology.org/)
data suggest that SOD activity in the inner medulla plays an important role in preventing accumulation of O$_2^\cdot$ to levels required to stimulate urea transport. In this regard, it is noteworthy that renal medullary SOD activity is markedly reduced in salt-fed Dahl salt-sensitive rats, associated with increased renal oxidative stress (19), and it is conceivable that a reduction in medullary SOD activity in pathophysiological states might induce O$_2^\cdot$-mediated stimulation of urea transport. On the other hand, endogenous nNOS activity in IMCD may regulate $P_{\text{urea}}$ by decreasing the availability of locally generated O$_2^\cdot$. It is also of interest that angiotensin II stimulates $P_{\text{urea}}$ in IMCD (12) and increases O$_2^\cdot$-production by thick limb segments (15). The mechanism whereby O$_2^\cdot$ stimulates $P_{\text{urea}}$ requires further study, including examination of the phosphorylation pattern of UT-A transporters.

ANP stimulates cGMP production in terminal IMCD and inhibits AVP-stimulated osmotic $P_f$ (23, 24). In contrast, Nonoguchi et al. (24) found no effect of ANP on $P_{\text{urea}}$ in the rat terminal IMCD (24). In the present studies, we observed no effect of NO on basal or AVP-stimulated $P_f$ (Fig. 7). Experiments utilized submaximal doses of AVP, and, as a positive control, a significant inhibition of AVP-stimulated $P_f$ occurred with endothelin. Our data contrast with reports of inhibitory effects of NO donors on $P_f$ in the rat cortical collecting duct, where activation of guanylate cyclase was proposed to mediate the inhibitory response (7, 8). However, using the excised patch-clamp technique in rat cortical collecting duct, Hirsch et al. (10) found no effect of SNP on cGMP levels or on basal or AVP-stimulated $P_f$. Furthermore, in our studies addition of 8-BrcGMP, the cell-permeable analog of cGMP, had no effect on $P_f$. Forssmann et al. (23) found that the inhibitory effect of ANP on AVP-stimulated $P_f$ in the terminal IMCD was mimicked by the natural form of cGMP, but not by 8-BrcGMP, each at $10^{-4}$ M. They suggested that 8-BrcGMP might induce stimulation of protein kinase A at high concentrations, negating its possible inhibitory effects on $P_f$ (24). Rocha and Kudo (31) found no effect of exogenous cGMP on basal water transport in rat cortical collecting duct, where activation of guanylate cyclase was proposed to mediate the inhibitory response (7, 8). It is difficult to reconcile these conflicting data, but our results suggest no net effect of NO on $P_f$ in the rat IMCD. Furthermore, we showed that neither cGMP nor 8-BrcGMP had any effect on production of O$_2^\cdot$ in IMCD, suggesting that this second messenger pathway is not involved in the inhibitory effect of NO on O$_2^\cdot$ generation. However, because ANP ($10^{-7}$ M) caused higher levels of cGMP generation in IMCD compared with SNAP, we cannot rule out the possibility that the lack of effect of SNAP on $P_f$ was due to insufficient stimulation of cGMP.

In summary, enhanced generation of O$_2^\cdot$ is associated with a stimulation of urea transport but not $P_f$ in the IMCD. While NO does not affect basal $P_{\text{urea}}$ or $P_f$, endogenous or exogenous NO prevents the stimulatory effect of increased O$_2^\cdot$ levels on $P_{\text{urea}}$. This suggests that nNOS-derived NO in the IMCD may dampen stimulation of urea transport in states of high O$_2^\cdot$ generation.

DISCLOSURES

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REFERENCES


