Low [NaCl]-induced neuronal nitric oxide synthase (nNOS) expression and NO generation are regulated by intracellular pH in a mouse macula densa cell line (NE-MD)

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Abstract Changes in the luminal NaCl concentration ([NaCl]) at the macula densa (MD) modulate the tubulo-glomerular feedback (TGF) responses via an affect on the release of nitric oxide (NO). This study was performed in a newly established mouse macula densa cell line (NE-MD) to investigate the effects of lowering [NaCl] on the neuronal NO synthase (nNOS) protein expression and l-arginine (Arg)-induced NO release. Expression of nNOS protein and release of NO were evaluated by Western blot analysis and an NO-sensitive electrode, respectively. Intracellular pH (pH i) was monitored by the BCECF assay. Although there was weak staining of the nNOS protein expression, L-Arg-induced NO generation was negligible in normal (140 mM NaCl) solution. Both were significantly (P < 0.05) increased either in the presence of furosemide (12 μM), an inhibitor of the Na–K–2Cl-cotransporter, or in a low (23 mM) Cl− solution. Furosemide- and low Cl−-induced NO generation was completely inhibited by 50 μM 7-nitroindasole (7-NI), a nNOS inhibitor. Moreover, these increases were significantly (P < 0.05) inhibited by the addition of 100 μM amiloride, an inhibitor of the Na+/H+ exchanger, or by its analogue 5-(N)-ethyl-N-isopropyl amiloride (EIPA), and also at a lower pH of 7.1. Furthermore, nNOS expression and NO release were not stimulated in as low as 19 mM Na+ solution. In conclusion, low [Cl−], but not low [Na+], in the lumen at the MD, increased nNOS protein expression and NO generation. Changes in the luminal [NaCl] may modulate the TGF system via an effect on the NO generation from the MD.

Keywords Neuronal nitric oxide synthase · Macula densa · Intracellular pH · Na+/H+ exchanger · Tubuloglomerular feedback

Introduction

Kidney macula densa (MD), a small group of distal tubule cells next to the cortical thick ascending limb of Henle’s loop (TAL), is faced to the same glomerulus from which it originated, and is located between the afferent and efferent arterioles [1]. The MD cells uniquely express neuronal nitric oxide synthase (nNOS), a brain-type of NOS, in the kidney, and generate NO in the presence of l-Arg [2, 3]. Several lines of evidence suggest that the expression of nNOS protein and mRNA in MD cells is regulated by low salt intake [5–7] and stimulated by the addition of furosemide (a loop diuretic), an inhibitor of the Na+/K+–2Cl− cotransporter [7, 8]. The NO generated in the kidney results in natriuresis and diuresis [9].

Our previous study showed that the nNOS protein expressed in a newly established mouse macula densa cell line (NE-MD) was increased time-dependently in the presence of 12 μM furosemide [10]. This finding, i.e., furosemide-induced expression of nNOS protein, is consistent with the view that the nNOS in MD cells plays an important role in these changes, with high distal flow...
examined whether nNOS protein expression and L-Arg-
[NaCl]-dependent NO generation in the MD, we have
term saline infusion [16] as well as long-term (2 weeks)
NO metabolites, such as NO 2 and NO 3. These apparent
as reflected by increased plasma levels or excretion of the
[25].
membrane of MD significantly enhanced the TGF response
luminal NHE. In contrast, blocking NHE in the luminal
membrane [22]. Part of the Na
\[\text{?}\] mediated via the Na
\[\text{?}\] –2Cl
\[\text{-}\] cotransporter and the Na
\[\text{?}\] –H
\[\text{?}\] exchanger with rela-
tively high water permeability at the luminal membrane
[18–21], although the latter is not consistent with the
finding of low water permeability of the MD luminal
membrane [22]. Part of the Na
\[\text{?}\] entry into MD cells is
mediated via the Na
\[\text{?}\] –K
\[\text{?}\] –2Cl
\[\text{-}\] cotransporter (NKCC2) (80%) and the Na
\[\text{?}\]/H
\[\text{?}\] exchanger (NHE2) (20%). Thus,
changes in luminal [NaCl] may cause changes in both
luminal NaCl entry and intracellular pH (pHi) in the MD.
Recently, in isolated and perfused rabbit kidney tubules,
pH\text{\textsubscript{i}} was increased upon either the addition of furosemide
[23] or lowering of the luminal [Cl
\[\text{-}\]] [24] via activation of
luminal NHE. In contrast, blocking NHE in the luminal
membrane of MD significantly enhanced the TGF response
[25].

In this study, to resolve the apparent discrepancies of
[NaCl]-dependent NO generation in the MD, we have
examined whether nNOS protein expression and L-Arg-
induced NO generation in NE-MD cells are (1) furosemide-
dependent, (2) pH-sensitive, (3) [Na
\[\text{?}\]]-dependent, and/or
(4) [Cl
\[\text{-}\]]-dependent.

Materials and methods

Cell culture

A kidney tubule cell line of macula densa, NE-MD, was
recently established in our laboratory [10] and was used for
the present studies at passages of 5–20. Briefly, NE-MD
 cells were derived from the cultured kidney cells of SV40
transgenic mice with an nNOS-EGFP transgene. When
NE-MD cells became confluent (typically in 7–10 days),
they were maintained by subculture. NE-MD cells were
grown in RITC80-7 medium (Iwaki, Tokyo) supplemented
with 5% FBS (Invitrogen, Carlsbad, CA), 10 µg/ml trans-
ferrin (Roche Diagnostics, Indianapolis, IN), 0.08 U/ml
insulin (Novo Nordisk, Copenhagen, Denmark), 10 ng/ml
recombinant human EGF (Wakunaga pharmaceutical Co.
Ltd, Osaka), and 50 U/ml penicillin G/50 µg/ml strepto-
mycin (Invitrogen). Cells were maintained at a permissive
temperature (33°C) for the expression of SV40 tempera-
ture-sensitive large T-antigen gene, in humidified 5% CO
atmosphere.

Reagents

Furosemide and 2′,7′-bis-(2-carboxyethyl)-5-carboxyfluoro-
rescein diacetoxymethyl ester (BCECF-AM) were
purchased from Sanofi Aventis (Tokyo) and Molecular
Probes (Eugene, OR), respectively. Tris (hydroxymethyl)
aminomethane [2-amino-2-hydroxymethyl-1,3-propane-
diol] was from Wako Pure Chemical Industries (Osaka).
All other reagents, such as 4-(2-hydroxyethyl)-1-piperazi-
neethanesulfonic acid (HEPES), amiloride hydrochloride,
5-(N-ethyl-N-isopropyl)-amiloride (EIPA), dimethyl sul-
oxide (DMSO), and S-Nitroso-N-acetyl-d,l-penicillamine
(SNAP) were purchased from Sigma–Aldrich Inc (St
Louis, MO).

 Experimental solutions

A normal solution (Na
\[\text{?}\] Ringer) was composed of (in
mM): 140 NaCl, 5.0 KCl, 1.0 CaCl
\[\text{2}\], 1.0 MgCl
\[\text{2}\], 5.5
\[\text{?}\] glucose, 1.0 Na pyruvate, and 10 HEPES (pH 7.4
with NaOH) (292 mOsm/kgH\textsubscript{2}O). Ion composition of 0 Na
\[\text{?}\] solution was the same as a normal solution except for Na
\[\text{?}\] (NaCl was totally replaced with choline Cl). Isosmotic
low salt media were made by mixing appropriate volumes
of normal saline with free salt medium. The mixing ratios for
low (1/4 and 1/10) salt solutions were 1:3 and 1:9 (normal :
free salt solution), respectively. Ionic compositions of
various extracellular solutions are shown in Table 1. In
some experiments, 12 µM furosemide and/or 100 µM
amiloride (or EIPA) was added to the medium throughout
the pre-incubation and NO-measurement. Osmolality was
measured by a freezing point osmometry (Fiske’s one-ten
osmometer, Fiske Associates, Norwood, MA).

Measurement of nitric oxide (NO)

Culture medium was replaced and pre-incubated with
various types of ionic solutions (Table 1) with or without
amiloride or its analogue for 2–5 h until the NO-meas-
urement. The release of L-Arg-induced NO from the
NE-MD cells was directly measured using an NO meter
with an associated NO sensor [ISO-NO nitric oxide sensor,
The size of the electrode (ISO-NOP200) was 200 μm diameter. The ISO-NO sensors were amperometric. This redox current is proportional to the concentration of NO in the sample. In solution, this sensor can measure NO concentrations as low as 1 nM.

For calibration, SNAP was used in combination with a catalyst, copper sulfate, to generate a known quantity of NO in solution. The signal slowly declines in this method because the generated NO is quickly oxidized to nitrite and nitrate, which are no longer detected by the probe. The calibration curve can be simply constructed by plotting the magnitude of the signal in picoamperes versus the final concentration of SNAP for that particular signal. A fresh stock solution of SNAP was prepared at the beginning of every day. In this study, the bathing solution always contained 1 mM D-arginine to avoid an unexpected shift of the baseline upon addition of the concentrated L-arginine solutions.

Table 1 Ionic composition

| Component | Na⁺ Ringer | Low Na⁺ | Low Cl⁻ |
|-----------|------------|---------|---------|
| Na⁺       | 144.5      | 18.5    | 39.5    | 144.5   | 144.5   |
| Choline⁺   | –          | 126     | 105     | –       | –       |
| K⁺        | 5          | 5       | 5       | 5       | 5       |
| Cl⁻       | 149        | 149     | 149     | 23      | 44      |
| Gluconate⁻| –          | –       | –       | 126     | 105     |
| Mg²⁺      | 1          | 1       | 1       | 1       | 1       |
| Ca²⁺      | 1          | 1       | 1       | 1       | 1       |
| HEPES     | 10         | 10      | 10      | 10      | 10      |
| Osmolality| 293        | 292     | 293     | 291     | 292     |

Solutions are expressed in mM. pH was adjusted to 7.4 by the addition of NaOH.

Western blot analysis

Using methods similar to those described previously [10], NE-MD cells were lysed in a TBS buffer containing 24 mM Tris, 136 mM NaCl, 2.6 mM KCl, and 2 mM leupeptin (Sigma–Aldrich) adjusted to pH 7.4. Large tissue debris and nuclear fragments were removed by centrifugation (800 rpm) for 5 min at 4°C. The supernatant was isolated and then diluted in sodium dodecyl sulfate (SDS) sample buffer with 6% β-mercaptoethanol. Proteins were denatured in boiling water for 10 min, separated by polyacrylamide gel electrophoresis, and transferred to a polyvinylidene difluoride membrane (PVDF) (Bio-Rad Laboratories, Hercules, CA). The blots were blocked for 30 min with 5% nonfat dry milk in Tris-buffered saline with 0.05% Tween 20 (TBS/T), followed by incubation overnight at 4°C with the rabbit polyclonal anti-human nNOS antibody (1:1000, Cayman Chemical Co, Ann Arbor, MI) or the mouse monoclonal anti-rabbit GAPDH antibody (1:5,000, Abcam Inc., Cambridge, MA). After washing with TBS/T, blots were incubated with a horse-radish peroxidase-conjugated goat anti-rabbit secondary antibody (GE Healthcare, Buckinghamshire, UK) or goat anti-mouse secondary antibody (GE Healthcare) for 2 h at room temperature. The blots were then washed with TBS/T and subjected to visualization using ECL kits (GE Healthcare).

Statistical analysis

Results are presented as the means ± SE of the experiments. Significance was determined by Student’s t-test. Significance was set at P < 0.05.

Results

NO generation in cultured NE-MD cells

L-Arg-induced NO generation was measured by using an NO sensing-electrode in NE-MD cells. NO generation was negligible when the cells were incubated in a normal (140 mM NaCl) solution without furosemide (Fig. 1a, top). In contrast, it was highly increased when the cells were incubated with furosemide (12 μM) for 2 h (Fig. 1a, middle). The NO value was expressed as the mean of the 6-min rise after the addition of L-Arg to the bath. Furosemide-induced NO generation was completely abolished in the presence of 50 μM 7-nitroindasole (7-NI), a relatively specific nNOS inhibitor (Fig. 1a, bottom). These results are summarized in Fig. 1b. Further, NO generation was negligible even in the furosemide-treated NE-MD cells when d-arginine (n = 1), L-lysine (n = 1), or D-arginine (n = 1) was used.
L-glutamate ($n = 1$) was added to the bath solution (data not shown). In the next series of experiments, L-Arg-induced NO generation was measured in NE-MD cells pre-incubated for 5 h with furosemide (12 $\mu$M) (Fig. 2a). NO generation was significantly ($P < 0.02$) and dramatically increased at 1.0 mM L-Arg, but not at 0.2 mM. Secondly, L-Arg (1 mM)-induced NO generation was measured in NE-MD cells pre-incubated with furosemide for 0, 2, and 5 h (Fig. 2b). It increased time-dependently through the period of measurement. In summary, L-Arg-induced NO generation increased concentration-dependently and time-dependently. In this study hereafter, NO generation was examined in the NE-MD cells pre-treated for 2 h under the various conditions.

Effects of low [Cl\textsuperscript{-}] and low [Na\textsuperscript{+}]

L-Arg (1 mM)-induced NO generation was significantly increased when NE-MD cells were incubated in a low
[Cl\(^-\)] solution. The increase was partially inhibited in the presence of 100 μM amiloride, a Na\(^+\)/H\(^+\) exchanger inhibitor, and is completely inhibited by the addition of 50 μM 7-NI. These results are summarized in Fig. 3, suggesting that l-Arg-induced NO generation may be pH-sensitive.

On the other hand, l-Arg-induced NO generation was slight when the cells were incubated in a low [Na\(^+\)] solution (Fig. 4a, top). No further decrease was observed in the presence of 50 μM 7-NI (Fig. 4a, bottom). These data are summarized in Fig. 4b. The results suggest that NO generation from the low [Na\(^+\)] pretreated NE-MD cells may be inhibited by the intracellular acidification caused by the inhibition of Na\(^+\)/H\(^+\) exchanger because of a lowering of the [Na\(^+\)] in the bath.

NO generation is pH-sensitive

Acute and chronic effects of pH on l-Arg-induced NO generation were examined in the furosemide-treated NE-MD cells with or without EIPA, an analogue of amiloride (a Na\(^+\)/H\(^+\) exchanger inhibitor). l-Arg (1 mM)-induced NO generation was significantly decreased in the presence of EIPA. In contrast, it was acutely (approximately 20 min) recovered after the removal of EIPA (Fig. 5).

In the next series of experiments, l-Arg-induced NO generation was measured under different pH conditions. Compared with NO generation of control at pH 7.4 (Fig. 6a, middle), it was significantly decreased at pH 7.1 (Fig. 6a, bottom) and was unchanged at pH 7.6 (Fig. 6a, top). These results are summarized in Fig. 6b.

Intracellular pH measurement

Intracellular pH (pH\(_i\)) was measured in the NE-MD cells pre-loaded with BCECF-AM by using an Argus Hisca device (Methods). In this study, there was no significant difference in the initial pH\(_i\) of NE-MD cells with or without furosemide. However, pH\(_i\) was decreased in response to a stepwise decrease in extracellular [Na\(^+\)] (Fig. 7a). Values of decreased pH\(_i\) were estimated to be approximately 7.0 and 7.1, respectively, at Na\(^+\) concentrations of 17.5 and 35 mM (Fig. 7b). Further, pH\(_i\) was quickly decreased upon the addition of EIPA to the normal (140 mM Na\(^+\)) solution (Fig. 8a). The half-decreased dose (apparent EC\(_{50}\)) was 0.9 μM (Fig. 8b).

Western blotting

To determine if intracellular acidification was responsible for the inhibitory effect on the furosemide-induced nNOS protein expression, confluent NE-MD cells were incubated in normal salt, normal salt plus furosemide (12 μM), and normal salt containing furosemide plus EIPA or DMSO for 2 h. Compared with normal salt (Na\(^+\) Ringer), incubation with furosemide (2 h) increased the level of nNOS protein expression (+12 μM furosemide). Interestingly, the level was decreased in the presence of EIPA, but was unchanged in the presence of the vehicle (DMSO) (Fig. 9a, left four blots). Moreover, we determined whether a reduction in Na\(^+\) or Cl\(^-\) was responsible for the low [NaCl] effect on nNOS protein expression. NE-MD cells were incubated in various types of low salt media for 2 h (Table 1). Low Cl\(^-\)
media stimulated the level of nNOS protein expression slightly (at 1/4 [Cl\textsuperscript{−}]) and significantly (at 1/10 [Cl\textsuperscript{−}]). In contrast, the level of nNOS protein expression was unchanged in low Na\textsuperscript{+} media (Fig. 9a, right four blots). Although nNOS protein expression was significantly increased in the presence of furosemide and at 1/10 [Cl\textsuperscript{−}], it was not in low Na\textsuperscript{+} media. Further, furosemide-induced nNOS protein expression was significantly ($P < 0.02$) inhibited in the presence of EIPA. These results are summarized in Fig. 9b.

**Discussion**

In this study, it was demonstrated in a newly established mouse macula densa cell line (NE-MD) [10] that furose-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig5.png}
\caption{Effects of EIPA on NO production in furosemide-pretreated NE-MD cells. The NO released is $50.9 \pm 7.4$ (+Furo, $n = 6$), $12.6 \pm 5.4$ (+Furo+EIPA, $n = 3$), and $57.1 \pm 6.4$ nM (wash-out, $n = 3$). **$P < 0.02$, ns not significant (vs. +Furo)}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig6.png}
\caption{\textit{l}-Arg-induced NO release at different pH levels in furosemide-pretreated NE-MD cells. \textit{a} Representative traces of NO release at pH 7.6 (top), 7.4 (middle), and 7.1 (bottom). Arrows and arrowheads indicate the same meanings shown in Fig. 1. \textit{b} NOs released are $12.1 \pm 1.1$ (pH 7.1), $51.1 \pm 10.0$ (pH 7.4), and $45.5 \pm 13.6$ nM (pH 7.6) in the presence of 1 mM \textit{l}-Arg ($n = 3$, each column). Statistical significance was obtained by comparison with control values (pH 7.4).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig7.png}
\caption{The effects of Na\textsuperscript{+} removal on pH\textsubscript{i} in NE-MD cells. \textit{a} Representative tracing of the pH\textsubscript{i} changes induced by the reduction of Na\textsuperscript{+} from the bathing solution and its calibration at the end of the trace. Concentrations of the remaining [Na\textsuperscript{+}] in the bathing solution are indicated at the top (left). Values at the top (right) show pH of the extracellular solutions for the calibrations. \textit{b} pH\textsubscript{i}-[Na\textsuperscript{+}] curve. Bars indicate the standard errors. $n = 7$ each}
\end{figure}
mide (an inhibitor of Na\(^{+}\)-K\(^{+}\)–2Cl\(^{-}\) cotransporter) and low [Cl\(^{-}\)], but not low [Na\(^{+}\)], induced increases in nNOS protein expression and L-Arg-induced NO generation. These results strongly suggest that (1) inhibition of NaCl entry at the luminal membrane of MD cells increases nNOS protein expression and its activity, and (2) the intracellular acidification induced by the inhibition of Na\(^{+}\)/H\(^{+}\) exchanger because of either amiloride or the low luminal [Na\(^{+}\)] failed to increase nNOS protein expression and activity. Changes in luminal [NaCl] have at least two different and independent effects on the NO generation in MD. First, low [NaCl] in the lumen may stimulate nNOS isoform [28]. Second, low [Na\(^{+}\)] may cancel or inhibit the intracellular signals of the nNOS protein expression process by decreasing the pH\(_{i}\). Thus, the overall signals of low [NaCl] at MD to stimulate nNOS protein expression appear to be negligible. These findings and the possible intracellular mechanisms are consistent with a previous report that inhibition of luminal Na\(^{+}\)/H\(^{+}\) exchangers (NHE) on MD cells augments the TGF system [25]. On the other hand, acute increase in NO generation after the removal of EIPA (Fig. 5) is, from another angle, inconsistent with the finding of the Western blotting analysis (Fig. 9). It is speculated that instantaneous alkalization (or normalization) in the pH\(_{i}\) may stimulate NO generation, although nNOS protein expression was never induced under the pre-treated conditions of either furosemide plus EIPA or low Na\(^{+}\) media. Thus, the present study may provide several valuable leads to help solve the apparent discrepancies in [NaCl]-dependent nNOS expression and NO generation among different researchers [5, 7, 11, 13–16].

In this study, pH\(_{i}\) in NE-MD cells was measured using the fluorescent probe BCECF-AM and was calibrated by a unique method [27]. Initial measurements of pH\(_{i}\) (control) were conducted in the medium of a normal solution without EIPA, an inhibitor of the Na\(^{+}\)/H\(^{+}\) exchanger. The slope of the relationship between pH\(_{i}\) in a log scale and extracellular [Na\(^{+}\)] in [mM] was almost linear in the range of 40–145 mM, but was steeper in the range of 10–40 mM. The pH\(_{i}\) obtained in NE-MD cells was 7.0–7.2 in 10–70 mM [Na\(^{+}\)] and was approximately 7.2–7.4 in 70–145 mM [Na\(^{+}\)]. These values are similar to those obtained in a variety of epithelial cells, including MD [23]. According to previous studies, luminal [NaCl] at the MD varied between 25 and 75 mM [29, 30]. These observations suggest that pH\(_{i}\) in MD cells may quickly vary up and down in response to normal physiological changes in [NaCl] [23]. It should be noted that NOS activity is strongly pH-sensitive [23, 28, 31].

We speculate that pH\(_{i}\) in NE-MD cells may be controlled by the functional activity and the expression level of NHE isoforms in the cell membrane [19, 32]. Interestingly, after adding EIPA to the medium, pH\(_{i}\) was dose-dependently decreased. The inhibitory potency (K\(_{i}\)) of EIPA was determined to be approximately 1 \(\mu\)M. This is consistent with the previous finding that luminal NHE isoform in the macula densa was NHE2 [19]. Moreover, there was no significant difference in the initial pH\(_{i}\) when NE-MD cells were incubated with or without furosemide. This suggests that NHE2 may not be functional under the control conditions (140 mM NaCl). However, when NE-MD cells were pre-incubated in low Cl\(^{-}\) with 140 mM Na\(^{+}\), L-Arg-induced NO generation was significantly increased, and this was reversed in the presence of amiloride. This may suggest that decreases in intracellular [Na\(^{+}\)] and [Cl\(^{-}\)] due to an inhibitory effect on Na\(^{+}\)-K\(^{+}\)–2Cl\(^{-}\) cotransport, which presumably increases proton efflux through the activation of NHE and stimulates the nNOS protein expression. Overall effects of low [Cl\(^{-}\)] stimulate the L-Arg-induced NO generation in MD cells.
In the kidney, NO has many physiological roles in the regulation of renal hemodynamics, including the glomerular filtration rate and medullary blood circulation via relaxation of vascular tone [7, 11, 20]. The net effect of NO generated in the kidney results in natriuresis and diuresis [9]. A part of the function of the kidney macula densa (MD), control of the body fluid volume in response to acute and chronic alterations in salt balance [7, 11], may depend on the [NaCl]-dependent NO release from the MD. The present study suggests that L-Arg-induced NO generation in MD cells can be influenced by at least two processes in response to changes in luminal [NaCl]. First, MD cell pH varies along with changes in the extracellular [NaCl]. A low flow rate along the distal tubule, including the MD region, is associated with low luminal [Na⁺] and probably acidified MD cells, while high flow rates result in high luminal [Na⁺] and alkalinized MD cells. Second, nNOS protein expression was stimulated by inhibition of NaCl entry through Na⁺-K⁺-2Cl⁻ cotransport in the presence of furosemide. However, under physiological conditions (25–75 mM NaCl, [29, 30]), an increase in nNOS protein expression did not occur, and NO generation was negligible in the present study. This is probably due to intracellular acidification, while a higher luminal [Na⁺] in proportion to the high flow rates alkalinizes the MD cells. At this point, NO generation would be instantaneously stimulated upon changes in pH_i, if the nNOS protein expression were sufficient. The net effect of increased luminal [NaCl] is alkalinization of the pH_i in MD via activation of the luminal Na⁺/H⁺ exchanger. Thus, higher [NaCl]-dependent NO release from the macula densa is induced by the acute normalization of pH_i. The low luminal [Na⁺] failed to induce the nNOS protein expression probably because of the accompanying acidification in the cell. It is still uncertain whether prolonged low [NaCl] conditions may induce the nNOS protein expression or not.

In conclusion, low [Cl⁻] as well as addition of furosemide in the lumen at the MD increased nNOS protein expression and L-Arg induced NO generation. Changes in the luminal [NaCl] may modulate the TGF system via an effect on the NO generation from the MD.

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