Renal handling of circulating nitrates in anesthetized dogs

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Godfrey, Murrell, and Dewan S. A. Majid. Renal handling of circulating nitrates in anesthetized dogs. Am. J. Physiol. 275 (Renal Physiol. 44): F68–F73, 1998.—Nitric oxide (NO) is rapidly oxidized to nitrite (NO2−) and then to nitrate (NO3−) in biological tissues. Although urinary excretion rates of NO3− are often used as an index of NO production in the body, very little is known regarding the kidney’s ability to excrete circulating NO3−. We have evaluated the renal responses to systemic administration of sodium nitrate (NaNO3) in eight anesthetized dogs treated with the NO synthase inhibitor, nitro-L-arginine (NLA; 50 µg·kg−1·min−1), intrarenally to minimize renal production of NO. Urinary and plasma concentrations of NO3−/NO2− (NOX) were determined by the Greiss reaction after enzymatic reduction of NO3− to NO2−. NLA treatment alone resulted in reductions in urinary NOX excretion rates (U NOXV, 1.13 ± 0.7 to 0.53 ± 0.1 nmol·min−1·g−1) and an increase in fractional reabsorption of NOX (FR NOX, 93.8 ± 3.5 to 97 ± 0.6%) without changes in arterial plasma concentrations (A NOX, 18.7 ± 1.4 to 21.2 ± 3.7 µM). Administration of NaNO3 (10, 20, 30, and 40 µg·kg−1·min−1) resulted in dose-dependent increases in A NOX (34.5 ± 8.0, 46.4 ± 7.3, 60.7 ± 6.3, and 78.1 ± 6.3 µM), U NOXV (1.8 ± 0.7, 4.2 ± 1.8, 7.0 ± 2.0, and 11.4 ± 3.3 nmol·min−1·g−1), and decreases in FR NOX (93.8 ± 2.3, 90.3 ± 3.5, 88.6 ± 3.2, and 84.6 ± 3.5%). Absolute net tubular reabsorption of NO3− showed a linear relationship with filtered loads, with no evidence of a transport maximum. These data show that, in the absence of additions from intrarenal sources, urinary excretion rates of nitrate increases progressively in response to increases in its circulating levels without exhibiting a functional reabsorption.

nitric oxide; plasma nitrate; tubular reabsorption; nitrate excretion

IT HAS BEEN KNOWN for many years that extracellular body fluids contain the stable metabolite, nitrate (NO3−), which is derived from both dietary sources and endogenous production. Until recently, its endogenous origin remained uncertain (8, 18), but it is now appreciated that nitric oxide (NO), a small relatively unstable diatomic free radical formed and released by endothelial cells, as well as many other cell types, is the major precursor of endogenous NO3− (12, 16, 18, 19). NO has a very short half-life and is oxidized within seconds of its release to various nitrogen oxides including nitrite (NO2−), which interacts with hemoglobin to yield NO3− (8, 9). These nitrogen oxides (NO3− and NO2−) are present in circulating blood and are excreted into the urine (3, 5, 8). It has been suggested that (NO3−/NO2−) levels in plasma and their urinary excretion rates may be an indicator of endogenous NO activity (2, 8, 13, 18, 24). Because NO3− is readily oxidized to NO2− in the presence of hemoglobin, circulating blood mainly contains NO3− rather than NO2− (8, 9, 20). NO3− is relatively stable in plasma and urine and thus can be readily measured using the Greiss reaction technique following in vitro enzymatic reduction of NO3− back to NO2− (6, 8, 13, 24). The amount of NO3− measured at the end of the assay reflects the total NO2− and NO3− in the original samples.

In view of the increased interest that has developed recently, experiments were performed to characterize the renal reabsorption and excretion of circulating NO3− in response to increases in plasma levels of NO3− in anesthetized dogs. Increasing doses of sodium nitrate (NaNO3) were infused to achieve different levels of NO3− concentrations in plasma. It is known that the renal epithelial cells can also produce NO, which can influence urinary excretion rate of NO3−/NO2− (1, 2, 11). To minimize such possible addition of NO metabolites NO3−/NO2−, which would complicate the assessment of the relationship between filtered load and urinary excretion/tubular reabsorption rate, the renal responses to NaNO3 administration were examined during blockade of NO synthase in the kidney by intravenous infusion of nitro-L-arginine (NLA; 50 µg·kg−1·min−1) (13–15). However, continuous infusion of the dose of NLA in the renal artery may cause some degree of systemic effect, such as slight increases in arterial pressure due to spillover in the systemic circulation as observed previously (14, 15). To minimize any possible influence of altered sympathetic activity on renal hemodynamics and renal function due to changes in systemic arterial pressure following NLA infusion, these experiments were conducted in the denervated kidney.

METHODS

Experiments were conducted in eight mongrel dogs (18.8 ± 1.5 kg body wt). These dogs were given supplemental amounts of sodium chloride (1.5 g·kg body wt−1·day−1 for 3 days) added to the normal laboratory diet, so that they achieved a sodium-replete state. To reduce the dietary contribution to the NO3− levels in plasma, these dogs were fasted for 16–20 h prior to the start of experiment. On the day of experiment, pentobarbital sodium was administered intravenously at 30 mg/kg body wt for induction of anesthesia and was supplemented throughout the experiment as needed. Auffed endotracheal tube was inserted and connected to an artificial ventilator set at a rate of 18 strokes/min with a stroke volume of 15 ml/kg body wt. A radial telethermometer was used to monitor body temperature, which was maintained within a range of 99–101°F with an electric heating pad. Systemic arterial pressure (SAP) was monitored via a catheter inserted into the right femoral artery and connected to a Statham pressure transducer (P23 DC) and recorded on a polygraph (model 7D, Grass Instruments). The left femoral artery was cannulated for the collection of blood samples. The jugular vein was cannulated for the administration of inulin, and the...
left and right femoral veins were cannulated for systemic infusion of NaNO₃ (10, 20, 30, and 40 µg·kg⁻¹·min⁻¹) and isotonic saline (30 ml/h), respectively.

The left kidney was exposed retroperitoneally and denervated by cutting all the renal nerves projecting to the kidney from the aorticorenal ganglion. Renal blood flow (RBF) was measured by placing an electromagnetic flow probe (Carolina Medical Electronics) around the renal artery, which was isolated from surrounding tissue. A curved 23-gauge needle cannula was inserted into the renal artery and connected to a pressure transducer for measurement of renal arterial pressure. Additional catheters were connected to the needle cannula for continuous infusion of heparinized saline (0.4 ml/min), as well as to prevent clotting in the cannula tip and for the administration of NLA. Urine was collected from a catheter placed in the left ureter. After completion of surgical procedures, a dose (1.6 ml/kg) of 2.5% solution of inulin in normal saline was administered into the jugular vein at least 45 min before the initiation of the experimental protocol followed by a continuous infusion (0.03 ml·kg⁻¹·min⁻¹) for whole experimental period.

The experimental protocol began with two consecutive 10-min urine collections with an arterial blood sample (2 ml) collected at the midpoint of each urine collection period to measure initial plasma inulin, sodium, potassium, and NO₃⁻/NO₂⁻ concentrations. A continuous infusion of NLA was initiated intrarenally (50 µg·kg⁻¹·min⁻¹) for the duration of the experimental period. Thirty minutes after the initiation of NLA infusion, two consecutive 10-min urine collections were made. Then the first dose of NaNO₃ (10 µg·kg⁻¹·min⁻¹) was administered systemically for 30 min in the presence of NLA. Ten minutes were allowed for stabilization period before two consecutive 10-min urine collections were made. The protocol was repeated using step-wise increases of the NaNO₃ doses (20, 30, and 40 µg·kg⁻¹·min⁻¹, respectively).

The calibration in situ of the electromagnetic flow probe was performed by cannulating the renal artery and collecting timed blood samples into a graduated cylinder at different flows. The kidney was removed, stripped of all surrounding tissue, blotted dry, and weighed, so that the calculated parameters could be expressed per gram of kidney mass. Sodium and potassium concentrations in the urine and plasma samples were determined using the flame photometer (Instrumentation Laboratory, Lexington, MA). The anthrone calorimetric technique (Gilford, Oberlin, OH) was used to determine inulin concentrations in urine and plasma samples. Duplicate plasma and urine samples were assayed for NO₃⁻/NO₂⁻ as described previously (8, 13). Commercially available Aspergillus nitrate reductase enzymes (Boehringer-Mannheim, Indianapolis, IN) were used to reduce NO₂⁻ to NO₂ during a 30-min incubation period. The Greiss reagent (equivalents of 0.2% naphthylenediamine dihydrochloride + 2% sulfamylonamide in 5% phosphoric acid) was then added to the resultant solution to yield a purple azo derivative that can be measured spectrophotometrically at an absorbance of 543 nm. It is observed that the presence of heparin in plasma samples usually produce precipitation on addition of the Greiss reagent (8). Therefore, heparin in plasma samples was precipitated by addition of protamine sulphate and removed prior to the addition of the Greiss reagent (8). The amount of NO₂⁻ measured at the end of the assay reflects the total NO₂⁻ and NO₃⁻ in the original samples. Known concentrations of NaNO₃ and NaNO₂ were used as standards in each assay. This Griess reaction technique for nitrate analysis has been widely used in many laboratories and provides a simple and fairly sensitive method to determine NO₃⁻ in biological fluids (6, 8, 13, 23, 24).

Statistical comparisons were conducted using analysis of variances for repeated measures followed by Newman-Keuls test. Differences in the mean values were deemed significant at ≤0.05.

RESULTS

During control collection periods, the mean values of plasma sodium, potassium, and hematocrit were 147 ± 1.9 meq/l, 3.3 ± 0.5 meq/l, and 41.8 ± 1.4%, respectively. There were no significant changes in these parameters during the course of the experimental procedures.

Responses to NaNO₃ infusions on plasma NO₃⁻/NO₂⁻ concentration. As shown in Fig. 1, intrarenal administration of NLA (50 µg·kg⁻¹·min⁻¹) prior to the infusions of NaNO₃ doses did not cause any significant changes in plasma NO₃⁻/NO₂⁻ levels (18.7 ± 1.4 to 21.2 ± 3.7 µM). During the infusion of increasing doses of NaNO₃ (10, 20, 30, 40 µg·kg⁻¹·min⁻¹), there were significant increases in the plasma NO₃⁻/NO₂⁻ concentrations compared with that of the NLA period (from 21.2 ± 3.7 to 34.6 ± 8.0, 46.4 ± 7.3, 60.7 ± 6.3, and 78.1 ± 6.3 µM, respectively).

Responses to NaNO₃ infusions on systemic and renal hemodynamics. As shown in Table 1 and in agreement with previous reports (13–15), intrarenal administration of NLA alone prior to NaNO₃ infusions led to significant increases in SAP, renal vascular resistance (RVR), and a decrease in RBF, with no significant changes in glomerular filtration rates (GFR). Infusion of increasing doses of NaNO₃ did not cause significant changes in RVR, RBF, or GFR. There were no significant changes in SAP during infusions of 10 and 20 µg·kg⁻¹·min⁻¹ NaNO₃, but the continuous infusion of NLA led to further increases in SAP during the 30 and 40 µg·kg⁻¹·min⁻¹ NaNO₃ doses in these dogs.

## Table 1

<table>
<thead>
<tr>
<th>NaNO₃ (µg·kg⁻¹·min⁻¹)</th>
<th>No NLA</th>
<th>NLA (50 µg·kg⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>20.3 ± 0.3</td>
<td>21.2 ± 3.7</td>
</tr>
<tr>
<td>20</td>
<td>20.3 ± 0.3</td>
<td>21.2 ± 3.7</td>
</tr>
<tr>
<td>30</td>
<td>20.3 ± 0.3</td>
<td>21.2 ± 3.7</td>
</tr>
<tr>
<td>40</td>
<td>20.3 ± 0.3</td>
<td>21.2 ± 3.7</td>
</tr>
</tbody>
</table>

Fig. 1. Plasma concentrations of nitrate/nitrite (NO₃⁻/NO₂⁻) in arterial blood, achieved during intravenous infusions of sodium nitrate (NaNO₃) doses in dogs treated with nitro-L-arginine (NLA) intrarenally (n = 8). *P < 0.05 vs. period of NLA administration alone.
Infusion of increasing doses of NaNO$_3$ in these NLA-treated dogs resulted in dose-dependent increases in urinary NO$_3$ excretion (Table 1). As reported earlier (13–15), these results are summarized in Table 1 and illustrated in Figs. 2, 3, and 4. As reported earlier (13–15), NLA administration prior to NaNO$_3$ infusions resulted in significant decreases in urine flow, urinary sodium excretion, and fractional excretion of sodium with no changes in urinary potassium excretion (Table 1). All the renal values are expressed per gram of kidney mass. There were also significant decreases in urinary NO$_3$/NO$_2$ excretion rates (Fig. 2), and fractional excretion of NO$_3$/NO$_2$ (6.2 ± 0.62 to 3.1 ± 0.62% Fig. 4), without significant changes in the filtered load of NO$_3$/NO$_2$ (16.7 ± 1.7 to 17.8 ± 3.1 nmol·min$^{-1}$·g$^{-1}$; Fig. 3). Fractional tubular reabsorption of NO$_3$/NO$_2$ showed progressive decreases (from 97.0 ± 1.8 to 93.8 ± 6.4, 90.3 ± 9.9, 88.6 ± 8.9, and 84.6 ± 9.8% Fig. 4) in response to infusions of incremental doses of NaNO$_3$. There were no significant changes in urine flow, urinary sodium excretion, fractional excretion of sodium, and urinary potassium excretion during administration of NaNO$_3$ doses (Table 1). Plasma levels of NO$_3$/NO$_2$ during infusions of NaNO$_3$ solutions were linearly related to net tubular reabsorption rates (r = 0.96, P < 0.001; Fig. 5A) and the urinary excretion rates of NO$_3$/NO$_2$ (r = 0.57, P < 0.001; Fig. 5B). Filtered loads of NO$_3$/NO$_2$ also showed strong positive correlation with net tubular reabsorption rates (r = 0.95, P < 0.001) and with plasma NO$_3$/NO$_2$ levels (r = 0.69, P < 0.001) observed during infusions of doses of NaNO$_3$ in these NLA-treated dogs.

### Table 1. Responses to NaNO$_3$ administrations in dogs pretreated with NLA

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NaNO$_3$, 0 µg·kg$^{-1}$·min$^{-1}$</th>
<th>NaNO$_3$, 10 µg·kg$^{-1}$·min$^{-1}$</th>
<th>NaNO$_3$, 20 µg·kg$^{-1}$·min$^{-1}$</th>
<th>NaNO$_3$, 30 µg·kg$^{-1}$·min$^{-1}$</th>
<th>NaNO$_3$, 40 µg·kg$^{-1}$·min$^{-1}$</th>
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</thead>
<tbody>
<tr>
<td>SAP, mmHg</td>
<td>139±12</td>
<td>151±7.0*</td>
<td>160±10</td>
<td>159±12</td>
<td>163±18†</td>
<td>165±19†</td>
</tr>
<tr>
<td>RVR, mmHg·mL$^{-1}$·min$^{-1}$</td>
<td>22.6±2.2</td>
<td>34.9±2.8*</td>
<td>38.7±2.9</td>
<td>36.3±3.2</td>
<td>29.0±3.9</td>
<td>34.5±3.4</td>
</tr>
<tr>
<td>RBF, mL·min$^{-1}$·g$^{-1}$</td>
<td>5.3±0.45</td>
<td>3.9±0.31*</td>
<td>3.6±0.22</td>
<td>3.9±0.29</td>
<td>4.2±0.32</td>
<td>4.3±0.32</td>
</tr>
<tr>
<td>GFR, mL·min$^{-1}$·g$^{-1}$</td>
<td>0.89±0.06</td>
<td>0.85±0.05</td>
<td>0.91±0.05</td>
<td>0.95±0.06</td>
<td>0.96±0.06</td>
<td>0.93±0.06</td>
</tr>
<tr>
<td>Urine flow, µL·min$^{-1}$·g$^{-1}$</td>
<td>35.4±9.6</td>
<td>14.6±2.7*</td>
<td>14.2±3.1</td>
<td>20.4±5.9</td>
<td>20.4±5.8</td>
<td>20.6±5.9</td>
</tr>
<tr>
<td>U$_{Na}$V, µmol·min$^{-1}$·g$^{-1}$</td>
<td>3.3±1.0</td>
<td>1.5±0.52*</td>
<td>1.77±0.65</td>
<td>2.4±0.91</td>
<td>2.7±0.98</td>
<td>2.7±0.89</td>
</tr>
<tr>
<td>FE$_{Na}$, %</td>
<td>2.4±0.60</td>
<td>1.2±0.32*</td>
<td>1.3±0.4</td>
<td>1.6±0.52</td>
<td>1.8±0.54</td>
<td>1.8±0.52</td>
</tr>
<tr>
<td>U$_{K}$V, µmol·min$^{-1}$·g$^{-1}$</td>
<td>0.60±0.07</td>
<td>0.55±0.07</td>
<td>0.62±0.06</td>
<td>0.68±0.09</td>
<td>0.65±0.06</td>
<td>0.71±0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8. Nitro-L-arginine (NLA) was infused continuously at 50 µg·kg$^{-1}$·min$^{-1}$. *P < 0.05 vs. control period and †P < 0.05 vs. NLA period. Renal values are expressed per gram of kidney mass. SAP, systemic arterial pressure; RVR, renal vascular resistance; RBF, renal blood flow; GFR, glomerular filtration rate; U$_{Na}$V and U$_{K}$V, urinary sodium and potassium excretion rates, respectively; and FE$_{Na}$, fractional excretion of sodium.

Responses to NaNO$_3$ infusions on renal function. These results are summarized in Table 1 and illustrated in Figs. 2, 3, and 4. As reported earlier (13–15), NLA administration prior to NaNO$_3$ infusions resulted in significant decreases in urine flow, urinary sodium excretion, and fractional excretion of sodium with no changes in urinary potassium excretion (Table 1). All the renal values are expressed per gram of kidney mass. There were also significant decreases in urinary NO$_3$/NO$_2$ excretion rates (Fig. 2), and fractional excretion of NO$_3$/NO$_2$ (from 3.1 ± 0.62, 6.2 ± 2.3, 9.7 ± 3.5, 13.1 ± 3.2, and 15.4 ± 3.5% Fig. 4). Fractional tubular reabsorption of NO$_3$/NO$_2$ showed progressive decreases (from 97.0 ± 1.8 to 93.8 ± 6.4, 90.3 ± 9.9, 88.6 ± 8.9, and 84.6 ± 9.8% Fig. 4) in response to infusions of incremental doses of NaNO$_3$. There were no significant changes in urine flow, urinary sodium excretion, fractional excretion of sodium, and urinary potassium excretion during administration of NaNO$_3$ doses (Table 1).

Plasma levels of NO$_3$/NO$_2$ during infusions of NaNO$_3$ solutions were linearly related with net tubular reabsorption rates (r = 0.96, P < 0.001; Fig. 5A) and the urinary excretion rates of NO$_3$/NO$_2$ (r = 0.57, P < 0.001; Fig. 5B). Filtered loads of NO$_3$/NO$_2$ also showed strong positive correlation with net tubular reabsorption rates (r = 0.95, P < 0.001) and with plasma NO$_3$/NO$_2$ levels (r = 0.69, P < 0.001) observed during infusions of doses of NaNO$_3$ in these NLA-treated dogs.

**Fig. 2.** Responses to administration of NaNO$_3$ doses on urinary excretion rates of NO$_3$/NO$_2$ in NLA-treated dogs (n = 8). *P < 0.05 vs. control period. †P < 0.05 vs. period of NLA administration alone. Renal values are expressed as per gram of kidney mass.

**Fig. 3.** Responses to administration of NaNO$_3$ doses on filtered loads (● and net tubular reabsorption rates (○) of NO$_3$/NO$_2$ in NLA-treated dogs (n = 8). *P < 0.05 vs. period of NLA administration alone.
Discussion

Although NO₃⁻ has been detected in the urine of humans and other species since the later part of the last century, the source of this urinary NO₃⁻ had long remained unknown (8, 17, 18). Initial attempts to explain the origin of this urinary NO₃⁻ by Mitchell and co-workers (17) at the start of this century showed that NO₃⁻ excreted in urine was much higher than the amount ingested in the food. From these earlier experiments, it was suggested that the excess urinary excretion of NO₃⁻ was the result of endogenous biosynthesis in the body tissues. The source was not clear until recently, when it was revealed that the major precursor for endogenous NO₃⁻ is NO generated in biological tissues (12, 16, 19, 22). Studies have shown that urinary NO₃⁻ excretion is the net result of its dietary intake and its endogenous synthesis (8, 10, 17, 22, 26).

The results of the present study indicated that the urinary excretion rate of NO₃⁻ is linearly related to its plasma concentration. Filtered loads of NO₃⁻/NO₂⁻ increased in response to increases in circulating levels without changes in glomerular filtration rates. The urinary excretion rate of NO₃⁻/NO₂⁻ also increases in association with the increases in filtered loads. There were also parallel changes in the tubular reabsorption, with slight decreases in fractional reabsorption rates as the circulating levels progressively increase. Basal fractional excretion rate of NO₃⁻/NO₂⁻ in these dogs was 6 ± 1%, demonstrating that NO₃⁻/NO₂⁻ was extensively reabsorbed under normal condition. Although there were slight decreases in fractional tubular reabsorption of NO₃⁻/NO₂⁻ during progressive increases in filtered loads, there was no clear transport maximum at least within the range of filtered loads examined in this study. The filtered load was increased more than fourfold above the basal levels during infusions of incremental doses of NaNO₃ solutions. The experimental design in this study does not allow us to delineate the tubular segment responsible for the bulk of the reabsorption of the filtered NO₃⁻, it seems likely that the major portion of filtered NO₃⁻ was reabsorbed mainly in the proximal tubule (23, 25). It should be noted here that the linear relationship between plasma NO₃⁻/NO₂⁻ level and its urinary excretion rate was observed in these dogs, in which glomerular filtration rate remained unchanged or minimally affected during infusions of NaNO₃. Thus it is conceivable that, at least in the condition of minimal or no changes in glomerular filtration rate, the urinary excretion rate of NO₃⁻/NO₂⁻ would reflect the changes in in vivo generation of NO. However, it should be emphasized here that, in cases of various pathophysiological conditions in which glomerular function is severely affected, the in vivo production rate of NO may not be reflected in the excretion rate of its metabolites in the urine.

Although various NO₃⁻ salts are known to cause diuretic effects when administered orally (10), we did not observe significant diuretic effects during administration of NaNO₃. The diuretic effects of NO₃⁻ salts have usually been observed with very high doses (10–18 g/day), which occasionally led to toxic effects that contributed to the disuse of these agents as diuretics (10). The continuous infusion of the doses of NaNO₃ used in this study did not cause any changes in renal
vascular resistance, blood flow, or glomerular filtration rates. The significant increases in systemic arterial pressure noted during infusion of higher doses of NaNO₃ were most likely due to the effect of continuous infusion of NLA. It was noted that administration of increasing doses of NaNO₃ did not cause any significant increases in absolute or fractional excretion rates of sodium. There were also no significant changes in urinary potassium excretion rates during sodium nitrate administration. These findings indicate that there may be very minimal, if any, interdependence of the nitrates with these electrolytes (Na⁺, K⁺) in the renal tubular reabsorptive mechanism, at least at the nitrate concentrations studied. In a previous study (13), we have also observed that the natriuretic effects of distal tubular sodium channels blockade were not associated with any change in urinary NO₃/NO₂ excretion.

Analysis of plasma and urine for the presence of NO₃/NO₂ is generally regarded as a useful noninvasive method to quantify systemic NO production (2, 8, 24). It has been reported that 24 h of fasting would reduce the plasma nitrate levels to nearly 80%, indicating that a maximal reduction of nitrate from dietary source could be achieved within that period (8). In other studies (5, 21), it was also observed that, following fasting of at least 12 h, nitrates from the dietary source were disappearing in the plasma and that the plasma NO₂/NO₃ level was then reflective of endogenous NO production. If the dietary contribution of NO₃/NO₂ levels in plasma and urine is eliminated or minimized, the changes in total body NO production rate would generally be reflected in urinary excretion rates of NO₃/NO₂ (2, 8). In the present study, the dogs were fasting for 16–20 h prior to the start of the induction of anesthesia. Moreover, the experimental protocols were carried out following at least another 4–5 h of surgery and stabilization periods, meaning that the collections of plasma and urine samples were made after at least 24 h of fasting. Thus it is conceivable that there is minimal, if any, contribution of dietary nitrate in the observed NO₃/NO₂ levels in plasma and urine in these dogs.

As the kidney also synthesizes and releases NO (1, 11), urinary NO₃/NO₂ levels may reflect both renal and extrarenal production of NO. In this study, the relationship between plasma NO₃/NO₂ levels and its urinary excretion rate in anesthetized dogs has been examined during blockade of renal generation of NO. The dose of NLA (50 μg·kg⁻¹·min⁻¹) used in this study to inhibit renal production of NO was found to be the lowest dose capable of eliciting maximal effects on RBF and was sufficient to achieve an effective blockade of intrarenal NO activity as evident from the complete reversal of the renal vasodilator effect of ATP infused intrarenally (14), as well as marked decreases in urinary excretion rate of NO₃/NO₂ (13). In the present study, a clear predictable linear relationship between filtered load and net tubular reabsorption rates of NO₃/NO₂ is observed in dogs treated with NLA intrarenally. Therefore, any change in urinary NO₃/NO₂ levels in the absence of changes in filtered loads would be predicted to occur due to changes in renal NO production rates. However, consideration of the acute changes in urinary excretion rates of NO₃/NO₂ as a measure of changes in renal NO production has been questioned in a recent study by Suto et al. (23). In that study, the investigators failed to demonstrate a clear relationship between the urinary NO₃/NO₂ excretion rate and the predicted changes in renal NO production in chronically instrumented, conscious rats, as assessed by renal vascular responses to systemic administration of pharmacological agents (L-arginine, acetylcholine) activating the NO system. However, it should be noted that, in those experiments, plasma NO₃/NO₂ levels and filtered loads were not measured to clarify the tubular mechanism of NO₃/NO₂ excretion in urine during alterations in NO production rate. Moreover, systemic administration of NO activating agents, which would affect both renal and extrarenal NO production rate, may further complicate the interpretations of the results in those study (23). In the present study, the relationship between changes in plasma NO₃/NO₂ levels and its urinary excretion rate has been examined in a more comprehensive manner to characterize physiological processing of NO₃/NO₂ by the kidney.

In conclusion, the results of these experiments show that, within the concentration range studied, absolute tubular reabsorption of NO₃ has a linear relationship with that of filtered loads. These data demonstrate that, in the absence of renal generation, the urinary excretion rate of nitrate is linearly related to its circulating level, without exhibiting transport maximum limitation even at fourfold enhancement of its normal tubular filtered load.

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