Original Article

Nitric Oxide Buffers Renal Medullary Vasoconstriction Induced by Prostaglandins Synthesis Blockade

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The aim of this study was to examine whether nitric oxide (NO) buffers the renal medullary vasoconstriction induced by a prostaglandins (PG) synthesis inhibitor. Daily blood pressure measurements were made with implanted catheters and changes in cortical blood flow (CBF) and medullary blood flow (MBF) were determined by implanted optical fibers and laser-Doppler flow measurement techniques in conscious rats. Sodium and water balance were also determined. Infusion of meclofenamate, a nonisozyme-specific cyclooxygenase (COX) inhibitor, at $5\mu\text{g/kg/min}$ over 4 consecutive days ($n=12$ rats) elicited a transitory increase ($p<0.05$) in mean arterial pressure (MAP) and a transitory decrease ($p<0.05$) in MBF and sodium excretion without altering CBF. In contrast, the simultaneous infusion of meclofenamate and N^G -nitro-L-arginine methyl ester (L-NAME, $0.8\mu\text{g/kg/min}$), a NO synthesis inhibitor, over 4 consecutive days ($n=12$) produced a continuous increase ($p<0.01$) in MAP and a continuous decrease ($p<0.05$) in MBF and sodium excretion without altering CBF. The results of this study suggest that the renal medullary vasoconstrictor effects and sodium retention induced by meclofenamate are enhanced by a subpressor dose of L-NAME, and that NO may buffer the renal medullary vasoconstriction induced by the blockade of PG synthesis in conscious rats. (*Hypertens Res* 2001; 24: 699–704)

Key Words: nitric oxide, prostaglandin, renal medullary blood flow, hypertension

Introduction

Prostaglandins (PG) generated by cyclooxygenase (COX) are important factors in regulating renal hemodynamics. Many studies have examined the roles of PG in the long-term regulation of arterial pressure and renal function (1–3). Recently, several studies have also shown important interactions between PG and nitric oxide (NO) (4–8). To date, however, only a few attempts have been made to examine the renal regional hemodynamic interactions between PG and NO in conscious rats.

We previously reported that chronic systemic NO blockade causes a sustained decrease in medullary blood flow (MBF) without altering cortical blood flow (CBF), thereby leading to retention of sodium and development of hyperten-

sion in conscious rats (9). These findings indicate that the endogenous renal medullary NO system plays an important role in renal medullary circulation, long-term control of sodium homeostasis, and systemic arterial blood pressure. The laser-Doppler technique employed in this previous study has been adapted and developed for chronic measurements of blood flow in the medulla through puncturing of the kidney. Cowley and associates have been interested in the roles of renal medullary circulation in sodium balance and the development of hypertension. The medullary circulation is normally protected from potent vasoconstrictor actions of circulating hormones by the existence of multiple counter-regulatory paracrine systems that are much more strongly expressed in the renal medulla than in the renal cortex. These systems include NO-production mechanisms. In fact, NO is functionally the best characterized component of these sys-

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tem and has been shown to play an important role in the maintenance of the medullary circulation (10–13). We therefore formulated the hypothesis that NO can buffer the vasoconstriction induced by blockade of PG in the renal medulla.

The aim of the present study was to determine whether or not the PG blockade causes the chronic sodium retention leading to sustained hypertension in conscious rats; if not, NO may buffer the vasoconstriction induced by blockade of PG in the renal medulla.

Methods

Experiments were performed on 24 male Sprague-Dawley rats. The animals were housed in the Animal Resource Center at Toho University and given free access to normal rat chow and water *ad libitum* before the experimental protocol. All animals were closely monitored to ensure that none experienced any undue stress or discomfort.

Each rat was fitted with a chronic indwelling arterial and venous catheter, as described previously (14). The catheters were placed in the abdominal aorta *via* the femoral artery and vein, tunneled subcutaneously, and exteriorized to the top of the cage *via* a stainless steel spring that was anchored into the muscles at the back of the neck with a stitch and attached to a swivel at the top of the cage. This arrangement allowed the animal to move freely about his cage while being continuously infused. To prevent infection, the animals received a postsurgical injection of penicillin (40,000 U intra-muscle (IM)).

For measurement of changes in the renal cortical and medullary blood flows in rats of Groups 2 and 4 (see group definitions below), two 500 μ m-diameter optical fibers were implanted in the left kidney and exteriorized for blood flow measurement by laser-Doppler flowmetry, as previously described (15). Two pieces of single mode optical fiber were cut into 25 cm-lengths, and one end of each fiber was gently heated and shaped into a 1 cm-radius bend to allow for stabilization of the fibers when implanted into the dorsal pole of the left kidney. A small 1 cm-diameter piece of latex was anchored to the fiber with epoxy adhesive and was used to anchor the implanted fiber to the surface of the kidney using surgical adhesive, and the fibers were sheathed with polyethylene tubing for protection. The fibers were implanted in the renal cortex and medulla by inserting them directly into the kidney tissue through a small hole made in the renal capsule with a 26-gauge needle. The fiber tips were inserted 2 mm beneath the surface of the renal cortex to measure the net flux of red blood cells in the renal cortex or at a depth of 5 mm to monitor changes in the outer medulla. After completion of the study, the kidneys were removed for morphological examination and to precisely determine the tip placement of the optical fibers. As in the previous study, animals with incorrectly placed fibers or extensive renal damage were excluded (15).

Laser-Doppler flowmeters produce a voltage signal pro-

portional to the net flux of red blood cells in the tissue of interest. Laser light of a single frequency is directed down an optical fiber to illuminate the renal tissue. When the light strikes moving blood cells, its frequency is shifted in proportion to the velocity of the cells. The light reflected back is analyzed by a signal processor, which produces a voltage proportional to the flux of red blood cells in the regional tissue of interest.

Protocol 1: Effects of Chronic Intravenous Infusion of Meclofenamate in Normal Rats ($n=12$)

In this protocol, sodium was provided through a continuous i.v. infusion of isotonic saline. The animals were allowed free access to tap water and sodium-free rat chow *ad libitum*. A continuous intravenous infusion of isotonic saline was administered at 18 ml/day (~ 2.7 mEq/day of NaCl) on all experimental days. A nonisozyme-specific COX inhibitor, meclofenamate (5 μ g/kg/min) (Sigma, St. Louis, USA), in saline was infused intravenously from the 5th to the 8th experimental day. This infused concentration of meclofenamate was determined in the acute preliminary study to reduce MBF by more than 5–10% from the control level.

Group 1: Mean Arterial Pressure (MAP), and Sodium and Water Balance ($n=6$)

Rats fitted with chronic indwelling arterial and venous catheters were maintained in metabolic stainless steel cages for determination of daily sodium and water balance. Beginning 1 week after surgery, daily blood pressure determinations were made during a 2-hour period with solid-state pressure transducers (RM6100; Nihon Kohden, Tokyo, Japan) and the averaged mean arterial blood pressure was calculated for a 2-hour period as described previously (16). Twenty-four hour urine collections were made for each rat using metabolic cages with silicone-lubricated stainless steel funnels. The volume was measured, and a urine sample was taken for measurement of the sodium concentration. In addition, the cage was rinsed daily with distilled water, and the volume and sodium concentration of the wash were also determined.

Group 2: MAP, CBF, and MBF ($n=6$)

Chronic catheters were placed in the femoral artery and vein, and optical fibers were implanted in the renal cortex and medulla of each rat. To minimize movement artifacts from the laser-Doppler flowmetry system, the rats were trained to sit quietly in Plexiglas restrainers placed in their home cages. Starting on the 7th postsurgical day, the rats were placed in restrainers and the blood pressure and cortical and medullary blood flow were measured each day for 90 min. Blood flow in the renal cortex and medulla was measured by a laser-Doppler flowmeter (ALF20; Advance, Tokyo, Japan) as described previously (16).

Fig. 1. Changes in mean arterial pressure (MAP, top) and daily sodium balance (bottom) in the chronic intravenous infusion of meclofenamate ($5 \mu\text{g/kg/min}$) for 4 days to conscious Sprague-Dawley rats ($n=6$, Group 1). *Significant difference ($p<0.05$) from the 4th experimental day.

Protocol 2: Effects of Chronic Intravenous Infusion of Meclofenamate in L-NAME Infused Rats

A continuous intravenous infusion of isotonic saline was administered at 18 ml/day on all experimental days. After 2 days of control measurements, the NOS inhibitor L-NAME ($0.8 \mu\text{g/kg/min}$) (Sigma) was delivered into the intravenous infusate from the 3rd to the 11th experimental day. This infused concentration of L-NAME was chosen, based on the results of the acute preliminary study, as the concentration that would not reduce MBF or CBF, and that would not increase MAP. Simultaneously, meclofenamate ($5 \mu\text{g/kg/min}$) in saline with L-NAME ($0.8 \mu\text{g/kg/min}$) was infused intravenously from the 5th to the 8th experimental day.

Group 3: MAP, and Sodium and Water Balance ($n=6$)

The methods used for this group were identical to those described above for Group 1.

Group 4: MAP, CBF, and MBF ($n=6$)

The methods used for this group were identical to those described above for Group 2.

Fig. 2. Changes in mean arterial pressure (MAP, top), renal cortical blood flow (middle), and renal medullary blood flow (bottom) in the chronic intravenous infusion of meclofenamate ($5 \mu\text{g/kg/min}$) for 4 days to conscious Sprague-Dawley rats ($n=6$, Group 2). *Significant difference ($p<0.05$) from the 4th experimental day.

Statistical Analysis

Values are given as the means $\pm$ SE. One-way repeated-measures of ANOVA were performed for each group followed by Duncan's multiple-range test for significance. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Protocol 1: Effects of Chronic Intravenous Infusion of Meclofenamate in Normal Rats ($n=12$)

Group 1: MAP, and Sodium and Water Balance ($n=6$)

The daily averages of MAP and sodium balance data during chronic intravenous infusion of meclofenamate are summarized in Fig. 1. In controls, MAP averaged 96.7 ± 5.2 mmHg

Fig. 3. Changes in mean arterial pressure (MAP, top) and daily sodium balance (bottom) during intravenous infusion of L-NAME (0.8 $\mu\text{g/kg/min}$) for 8 days and chronic intravenous infusion of meclofenamate (5 $\mu\text{g/kg/min}$) for 4 days to conscious Sprague-Dawley rats ($n=6$, Group 3). *Significant difference ($p<0.05$) from the 4th experimental day.

on the 4th control day. The average MAP value in group 1 was significantly greater than this control value only on the 1st day of meclofenamate infusion; beginning on the second day of infusion, the average MAP in group 1 was not significantly different from the control value. The daily sodium balance averaged 0.27 ± 0.29 mEq/day on the 4th control day. During the 1st day of intravenous infusion of meclofenamate, the rats retained 0.75 ± 0.34 mEq of sodium, but after the 2nd day of meclofenamate infusion the daily sodium balance was not significantly different from the control value.

Group 2: MAP, CBF, and MBF ($n=6$)

As summarized in Fig. 2, MAP averaged 95.3 ± 6.9 mmHg, and the laser-Doppler signals from the implanted cortical and medullary optical fibers averaged 1.02 ± 0.23 V and 0.87 ± 0.21 V, respectively, on the 4th day of control measurements. After 24 h of meclofenamate infusion, MAP was significantly increased and medullary signals were significantly decreased to 0.58 ± 0.19 V, but the cortical signals were not different from those in controls. On the 2nd day of

Fig. 4. Changes in mean arterial pressure (MAP, top), renal cortical blood flow (middle), and renal medullary blood flow (bottom) during intravenous infusion of L-NAME (0.8 $\mu\text{g/kg/min}$) for 8 days and chronic intravenous infusion of meclofenamate (5 $\mu\text{g/kg/min}$) for 4 days to conscious Sprague-Dawley rats ($n=6$, Group 4). *Significant difference ($p<0.05$) from the 4th experimental day.

meclofenamate infusion, MAP and medullary signals had returned to levels not significantly different from those on the 4th control day.

Protocol 2: Effects of Chronic Intravenous Infusion of Meclofenamate in L-NAME Infused Rats ($n=12$)

Group 3: MAP, and Sodium and Water Balance ($n=6$)

Figure 3 summarizes the daily averages of MAP and sodium balance during chronic infusion of meclofenamate in L-NAME infused rats. MAP was insignificantly elevated from the control level of 95.2 ± 5.5 mmHg to 104 ± 12.1 and 103.5 ± 11.2 mmHg on the 1st and 2nd days of L-NAME infusion, respectively, but was significantly increased to 131.7 ± 13.2 mmHg after 24 h of meclofenamate infusion

and, over the full 4-day period of meclofenamate infusion, remained above the level in L-NAME infused rats. MAP returned to the control values on the 1st day after meclofenamate infusion.

The daily sodium balance averaged 0.28 ± 0.26 mEq/day on the 2nd control day, and was not changed during the 2 days of L-NAME infusion. However, on the 1st day of meclofenamate infusion, a net positive balance of sodium occurred (1.05 ± 0.25 mEq/day). Sodium continued to be retained during the 4 days of meclofenamate infusion as shown by the significantly elevated state of balance. Control levels were again achieved by the 1st day after meclofenamate infusion.

Group 4: MAP, CBF, and MBF (n=6)

As summarized in Fig. 4, MAP averaged 97.3 ± 5.9 mmHg, and the laser-Doppler signals from the implanted cortical and medullary optical fibers averaged 1.03 ± 0.20 V and 0.85 ± 0.18 V, respectively, on the 2nd control day, but were not changed significantly during the 2 days of L-NAME infusion. MAP was significantly increased to 127.5 ± 7.3 mmHg after 24 h of meclofenamate infusion, and remained above the level in L-NAME infused rats throughout the 4-day period of meclofenamate infusion. MAP returned to the level in controls by the 1st day after meclofenamate infusion.

Medullary signals were not changed significantly during the 2 days of L-NAME infusion, but decreased significantly to 0.60 ± 0.23 V on the 1st day of meclofenamate infusion. These medullary flow signals remained significantly reduced throughout the 4 days of meclofenamate infusion and returned to the control level by the 1st day after meclofenamate infusion. The level of cortical signals did not change significantly remained roughly constant throughout the protocol period.

Discussion

In the present study, renal vasoconstrictor effects induced by meclofenamate were significantly increased by a subpressor dose of L-NAME. In our experimental kidneys, meclofenamate decreased MBF significantly only on the first day of administration, without altering CBF, which effect would not be sufficient to cause sustained hypertension. The administration of meclofenamate with a subpressor dose of L-NAME caused sustained hypertension with decreased MBF. The present findings raise two important questions in terms of the effects of meclofenamate. There is general agreement that the renal medulla contains more NO synthase (NOS) than the cortex, and that MBF is more sensitive than CBF to the effects of NOS inhibitors (9, 14, 17–19). Therefore, the first question is why meclofenamate decreased only MBF without altering CBF in the manner of L-NAME. Originally renal medullary PG were considered to play an important role in protecting against ischemic damage. It appears that MBF is more sensitive than CBF to the dilator PG. This suggestion is supported by studies showing that dilatory PG

must be much more strongly expressed in the renal medulla than in the renal cortex to antagonize the effects of certain medullary vasoconstrictors, such as Angiotensin II and norepinephrine (10, 11).

The second subject is why meclofenamate did not cause sustained hypertension. The main aim of this study was to determine whether NO buffers the antagonized effects of dilator PG in the renal medulla. The subpressor dose of L-NAME, which did not change the MAP or renal hemodynamics, appears to reduce the buffer effects of NO in the renal hemodynamics. As for the mechanisms of long-term blood pressure control, the renal handling of sodium and water plays a very important role (12). In order to support our hypothesis, it would be better if arterial blood pressure showed only a transitory increment without sodium and water retention. Judging from the above, meclofenamate infusion could not have caused the sustained hypertension without sodium retention seen in the present study.

The renal medulla plays an important role in the regulation of sodium excretion and in the long-term control of arterial blood pressure. Small changes of arterial pressure result in an increase of MBF and vasa recta capillary pressure, and are importantly involved in the mechanism of pressure-natriuresis. Reductions of MBF are associated with a resetting of the pressure-natriuresis response to a higher set point, thereby requiring a higher renal arterial pressure perfusion pressure to achieve sodium and water homeostasis (12, 13, 20). The novel finding of the present study is that the infusion of meclofenamate caused sustained hypertension with decreased MBF in the rats treated with a subpressor dose of L-NAME. In this experiment whole kidney renal blood flow (RBF) was not measured, but could not have been decreased significantly, since Mattson *et al.* reported a strong correlation between CBF as measured using an implanted optical fiber and RBF as measured using an electromagnetic flowmeter (21). The prolonged antinatriuretic effect induced by simultaneous meclofenamate and L-NAME infusion may be secondary to an effect on decreased MBF. We confirmed that NO can buffer the vasoconstriction in the renal medulla induced by PG blockade in conscious rats. The mechanism underlying the buffer effect of NO in the renal hemodynamics is still unclear. COX inhibitors may modulate the effects of kinins on renal circulation. NO could then contribute to the action of PG in part through its effects of kinins (22–24).

It has been reported that the involvement of PG in regulating the renal hemodynamic and excretory effects when NO synthesis was reduced was mainly dependent on COX-1 activity (8). However, COX-2 may regulate MBF, based on recent studies in which COX-2 was expressed mainly in the renal medulla (25–27). Further studies using the laser-Doppler technique will be needed to evaluate which COX isoform is responsible for regulation of MBF. Such information could be useful in the treatment of hypertensive patients using nonsteroidal anti-inflammatory medicines.

In summary, the results of this study indicate that NO can

buffer the renal medullary vasoconstriction induced by PG synthesis blockade in conscious rats.

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