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Blood flow-dependent changes in intrarenal nitric oxide levels during anesthesia with halothane or sevoflurane

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Abstract

We previously demonstrated that intrarenal nitric oxide (NO) levels and renal blood flow are reduced during halothane anesthesia. Studies were performed to determine if volatile anesthetics-induced reductions in renal NO levels are associated with blood flow changes. Halothane and sevoflurane at 0.8 and 2.4 Mac were administered by inhalation to dogs, and cGMP and NOx concentrations in the renal interstitial fluid were measured by a microdialysis method. Neither halothane nor sevoflurane at 0.8 Mac altered renal blood flow and renal interstitial cyclic guanosine monophosphate (cGMP) and NOx levels, but both anesthetics significantly decreased these values at 2.4 Mac. Using an adjustable aortic clamp, renal perfusion pressure was reduced in 2 steps without halothane and sevoflurane anesthesia. Renal blood flow as well as cGMP and NOx concentrations in the renal interstitial fluid were unchanged within the autoregulatory range, but significantly decreased below the autoregulatory range. Changes in cGMP and NOx concentrations in the renal interstitial fluid were highly correlated with renal blood flow changes during halothane or sevoflurane anesthesia, and during stepwise reductions in renal perfusion pressure. The results suggested that halothane- and sevoflurane-induced decreases in intrarenal NO levels result from reductions in blood flow.

Keywords: Halothane; Sevoflurane; Nitric oxide (NO); Renal blood flow; Microdialysis

1. Introduction

Locally produced nitric oxide (NO) plays critical roles in the regulation of renal hemodynamics and tubular functions (Majid and Navar, 2001; McKee et al., 1994; Millatt and Siragy, 2000; Navar et al., 1996). NO production is regulated by various chemical stimuli, and by physical forces such as shear stress, transmural pressure and stretching of the vascular wall, which are continuously controlled by blood flow in vivo (Ballermann et al., 1998; Nakahara et al., 1997). However, there has been very little investigation into the specific role of hemodynamic forces in the regulation of NO production in the kidney. Juncos et al. (1995) showed that pressure-induced afferent arteriolar constriction is weaker in free-flow than no-flow afferent arterioles, and that this difference is abolished by endothelial disruption or NO synthase (NOS) inhibition. These data suggest the presence of intraluminal flow-dependent endothelial NO production in afferent arterioles. Studies with

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isolated rat perfused kidneys showed that augmentation of shear stress by increasing perfusate viscosity dilates renal vasculature, and that this is attenuated by NOS blockade (Endlich et al., 1999). Similarly, nitrite production in cultured inner medullary collecting duct cells is increased by pulsatile shear stress; suggesting that fluid flow within the renal tubular system also regulates NO production in the kidney (Cai et al., 2000). We recently utilized in vivo microdialysis method to demonstrate that acute reductions in renal blood flow result in significant decreases in renal interstitial cGMP and NOx levels in rabbits (Nishiyama et al., 2002). Interestingly, we found that the changes in cGMP and NOx concentrations in the renal interstitial fluid were highly correlated with the changes in renal blood flow, indicating a blood flow-dependent alterations in intrarenal NO levels.

Halothane and other volatile anesthetics exert a variety of effects on renal hemodynamics (Durieux et al., 1992; Groves et al., 1990; Holstein-Rathlou et al., 1982; Nishiyama et al., 1999). For example, a marked renal vasoconstriction was observed during halothane anesthesia, whereas cerebral blood flow was significantly increased (Durieux et al., 1992; Nishiyama et al., 1999). Furthermore, it was shown that halothane decreases renal blood flow and glomerular filtration rate, which are not caused simply by cardiovascular depression (Groves et al., 1990; Holstein-Rathlou et al., 1982). Many studies also indicate that volatile anesthetics interfere with the NO-soluble guanylate cyclase (sGC) signaling pathway; in vitro experiments using isolated vascular rings showed that halothane (Blaise et al., 1994; Hart et al., 1993; Muldoon et al., 1988; Nakamura et al., 1994; Stone and Johns, 1989; Uggeri et al., 1992), sevoflurane (Nakamura et al., 1994), enflurane (Uggeri et al., 1992) or isoflurane (Nakamura et al., 1994; Uggeri et al., 1992) interferes with endothelium-dependent vascular relaxation by NO. Previously, we demonstrated that halothane causes a renal vasoconstriction along with reductions in NO levels in the kidney (Nishiyama et al., 1999). However, the mechanisms by which halothane reduces NO levels in the kidney remained unclear.

The present study was conducted to determine if volatile anesthetics-induced reductions in renal NO levels are associated with blood flow changes. Therefore, we examined the effects of volatile anesthetics (halothane and sevoflurane) and mechanical alterations in renal blood flow (induced by using aortic clamp without halothane and sevoflurane anesthesia) on NO levels in the kidney. Intrarenal NO levels were evaluated by measuring cGMP and NOx concentrations in the renal interstitial fluid using an in vivo microdialysis method (Millatt and Siragy, 2000; Nishiyama et al., 1999, 2002; Siragy et al., 1992; Siragy and Carey, 1996; Tian et al., 2002). The results from the present study demonstrate positive associations between changes in intrarenal NO levels and renal blood flow during halothane or sevoflurane anesthesia and during mechanical reductions in renal blood flow. These data suggest that halothane- and sevoflurane-induced reductions in intrarenal NO levels result from blood flow changes.

2. Materials and methods

2.1. General procedure

Experiments were carried out on mongrel dogs, weighing 12–19 kg, that had been maintained on standard laboratory chow. All surgical and experimental procedures were performed according to the guidelines for the care and use of animals as established by the Kagawa Medical University.

The surgical preparation of the animals and basic experimental techniques are identical to those previously described (Rahman et al., 2001; Tian et al., 2002). Briefly, the animals were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and given additional doses as required. After tracheotomy, the dogs were artificially ventilated with room air (4 l/min). The right brachial artery and vein were cannulated for collecting blood and administration of an isotonic saline solution (0.2 ml/kg/min), respectively. Another catheter was placed in the aorta at the origin of the left renal artery via the right femoral artery, and renal perfusion pressure was continuously monitored with a pressure transducer (Model No. 361, NEC-San-ei, Tokyo, Japan).

The left kidney was exposed through a retroperitoneal flank incision and denervated by cutting all visible renal nerves. Renal blood flow was continuously measured by an electromagnetic flow probe (Nihon Kohden, Tokyo, Japan). The left ureter was catheterized with a polyethylene tube for collections of urine samples. Two microdialysis probes were implanted into the renal superficial cortex, and perfused with saline solution with heparin (30 unit/ml) at a rate of 5 μl/min. An adjustable clamp was placed on the aorta to be used later as an occlusion devise. To measure glomerular filtration rate, a priming dose of creatinine (100 mg/kg) was administered intravenously, at least 60 min before the initiation of the experimental protocol followed by a continuous infusion of creatinine (50 mg/kg/h, i.v.) for the entire protocol period (Rahman et al., 2001; Tian et al., 2002).

2.2. Renal microdialysis technique

For the determination of cGMP and NOx concentrations in the renal interstitial fluid, we used a microdialysis method as previously reported (Millatt and Siragy, 2000; Nishiyama et al., 1999, 2002; Siragy et al., 1992; Siragy and Carey, 1996; Tian et al., 2002). The dialysis membrane was made from cuprophan fiber, measured 15 mm in length and had a 5500-Da transmembrane diffusion cut-off (Toyobo, Otsu, Japan). Preliminary results from in vitro experiments had demonstrated that negligible amounts of cGMP and NOx

stuck to the microdialysis probes. The probes were connected to a CMA/100 microinfusion pump (Carnergie Medicine, Stockholm, Sweden) and perfused with a saline solution with heparin (30 unit/ml) at a rate of 5 μl/min. Samples were collected for 20-min sample periods and stored at −40 °C prior to analysis. At this perfusion rate, the relative equilibrium rates of cGMP and NOx were 27±2% and 30±3%, respectively (Nishiyama et al., 2002; Tian et al., 2002). Reported values were corrected by these equilibrium rates. At the end of each experiment, the kidney was removed and the location of the microdialysis membrane confirmed by surgical exposure of the probe.

2.3. Experimental protocols

2.3.1. Effects of halothane and sevoflurane on renal hemodynamics and renal interstitial cGMP and NOx

After a stabilization period of 90 min following the completion of surgery, the experimental protocol was started with renal interstitial fluid and urine collections for two consecutive 20-min periods. Then, a lower dose of halothane (0.8 Mac) was administered with room air (4 l/min) for 30 min (n=6). After 10 min of halothane (0.8 Mac) inhalation, a 20-min sample was collected. Then, a higher dose of halothane (2.4 Mac) was started. After 10 min of halothane (2.4 Mac) inhalation, a 20-min sample was collected. Then, two additional 20-min sampling periods were performed at 30 and 60 min after the cessation of the inhalation of halothane. In a separate group of animals (n=5), the effects of sevoflurane (0.8 and 2.4 Mac) were examined in a manner similar to that carried out for halothane. At the midpoint of each collection period, an arterial blood sample (2 ml) was collected into chilled tubes containing diammonium EDTA (10 mg/ml blood) to measure the plasma creatinine and cGMP concentrations.

2.3.2. Renal interstitial cGMP and NOx during stepwise reduction in renal perfusion pressure

Effects of changes in renal perfusion pressure within and below the renal blood flow autoregulatory range on cGMP and NOx concentrations in the renal interstitial fluid were examined in seven dogs. After a stabilization period of 90-min following the completion of surgery, the experimental protocol was started with dialysate and urine collections over two consecutive 20-min periods at spontaneous renal perfusion pressure. Using an adjustable aortic clamp, renal perfusion pressure was reduced within the renal blood flow autoregulatory range (around 85 mmHg: step 1). Then, renal perfusion pressure was further reduced below the renal blood flow autoregulatory range (around 50 mmHg: step 2). The pressure at each step was held for 30 min, with an extra 10 min being allowed for stabilization at each level of renal perfusion pressure, before samples were collected over a 20-min period. An additional 20-min collection was performed at 30 min after releasing the aortic clamp (recovery period). At the midpoint of each collection period, an arterial blood sample (2 ml) was collected into chilled tubes containing diammonium EDTA (10 mg/ml blood) to measure the plasma creatinine and cGMP concentrations.

2.4. Analytical procedures

Urine and plasma creatinine concentrations were measured using the method of Bonsnes and Taussky (1945). cGMP was measured by a radioimmunoassay kit (Amersham, U.S.A.). NOx was analyzed by an automated procedure based on the Griess reaction after reduction of nitrate to nitrite on a cadmium column (Kosaka et al., 1989; Nishiyama et al., 2002).

2.5. Statistical analysis

Values are presented as means±S.E.M. Statistical comparisons of differences were performed using a one-way or two-way analysis of variance for repeated measures combined with the Newman–Keuls post hoc test. Correlation of the responses were made by the Spearman test. P<0.05 was considered statistically significant.

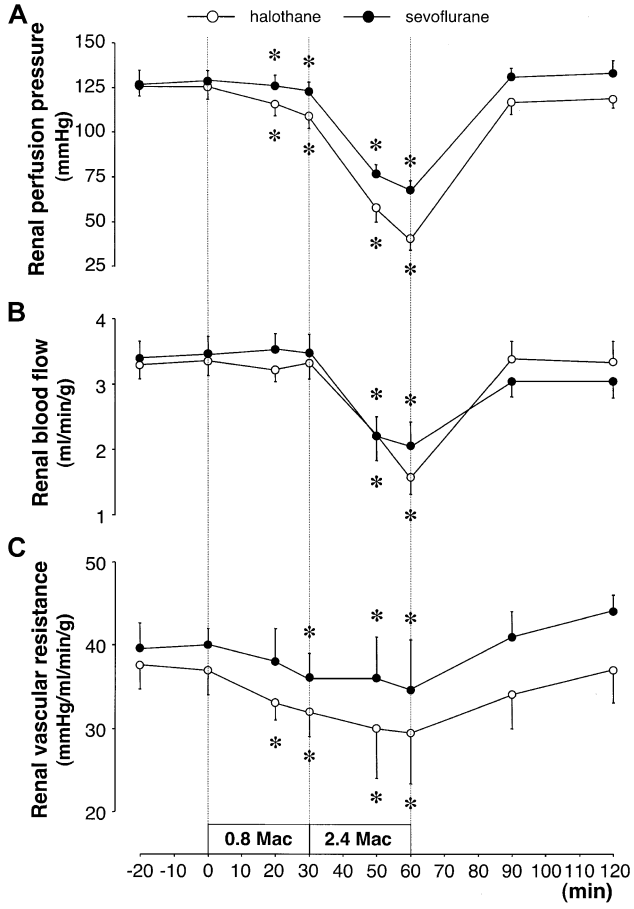

Fig. 1. Effects of halothane (n=6) and sevoflurane (n=5) on renal perfusion pressure (A), renal blood flow (B) and renal vascular resistance (C). *P<0.05 vs. control values at 0 min.

3. Results

3.1. Effects of halothane and sevoflurane on renal hemodynamics and renal interstitial cGMP and NOx

A lower dose of halothane or sevoflurane (0.8 Mac) for 30 min significantly decreased renal perfusion pressure (Fig. 1A), but did not alter renal blood flow (Fig. 1B). On the other hand, a higher dose of halothane or sevoflurane (2.4 Mac) resulted in marked decreases in renal perfusion pressure (by −66±6% and −50±5%, respectively) and renal blood flow (by −55±5% and −39±6%, respectively). During 0.8 Mac of halothane and sevoflurane, calculated renal vascular resistance was significantly decreased from 37±3 to 32±3 mmHg/ml/min/g and from 40±2 to 36±3 mmHg/ml/min/g, respectively (Fig. 1C). At 2.4 Mac, halothane and sevoflurane tended to reduce further renal vascular resistance; however, these changes were not statistically significant (from 32±3 to 29±6 mmHg/ml/min/g and from 36±3 to 34±6 mmHg/ml/min/g, respectively). All parameters returned to the respective control levels after the cessation of halothane or sevoflurane inhalation (Fig. 1). Glomerular filtration rate was not much altered by a low dose of halothane or sevoflurane (from 0.67±0.07 to 0.65±0.05 ml/min/g and from 0.71±0.07 to 0.73±0.08 ml/min/g, respectively), but was significantly decreased by both anesthetics at a high dose (0.25±0.08 and 0.35±0.04 ml/min/g, respectively); then after the cessation of either anesthetic, glomerular filtration rate returned to the respective control levels (0.70±0.05 and 0.69±0.06 ml/min/g, respectively).

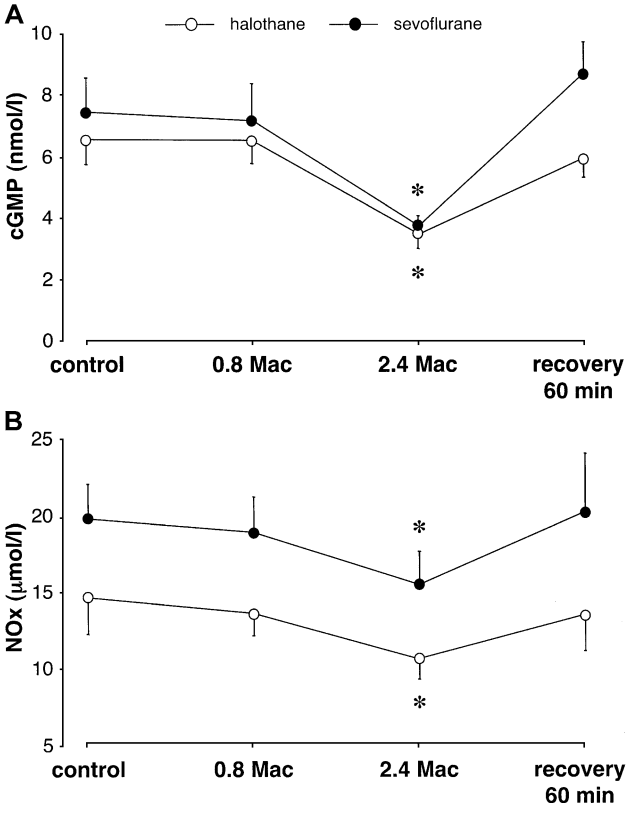

Fig. 2. Effects of halothane (n=6) and sevoflurane (n=5) on renal interstitial fluid concentrations of cGMP (A) and NOx (B). *P<0.05 vs. control values.

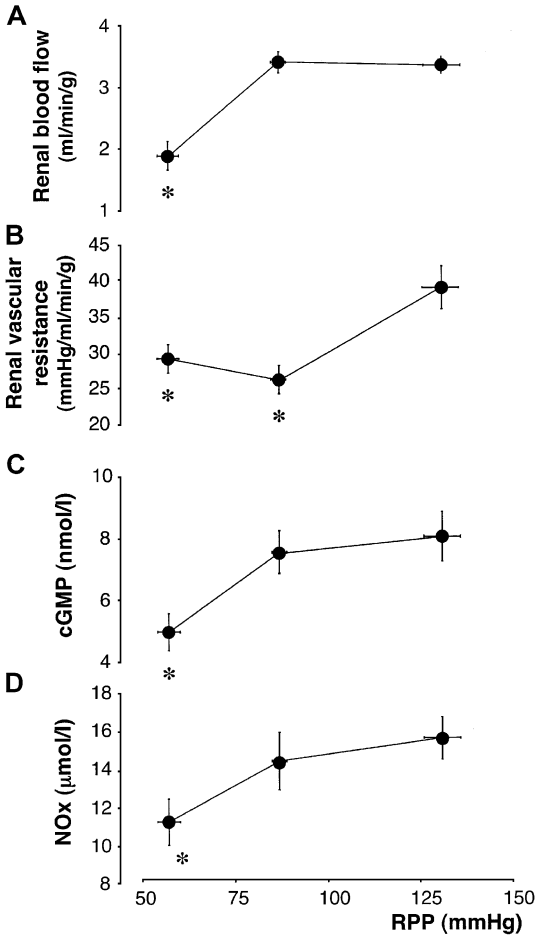

Fig. 3. Effects of stepwise reductions in renal perfusion pressure on renal blood flow (A), renal vascular resistance (B) and renal interstitial fluid concentrations of cGMP (C) and NOx (D). n=7. *P<0.05 vs. control values at spontaneous renal perfusion pressure.

Fig. 2 shows the effects of halothane and sevoflurane on cGMP and NOx concentrations in the renal interstitial fluid. cGMP and NOx concentrations in the renal interstitial fluid were not significantly altered by the lower dosage (0.8 Mac) of halothane or sevoflurane. Similarly, halothane or sevoflurane at 0.8 Mac did not alter cGMP and NOx concentrations in the renal interstitial fluid. However, halothane or sevoflurane at a high dose (2.4 Mac) resulted in significant reductions in renal interstitial cGMP (by −39±7% and −44±8%, respectively) and NOx levels (by −30±4% and −22±3%, respectively). Reduced cGMP and NOx levels returned to their respective control levels after cessation of halothane or sevoflurane inhalation (Fig. 2). As shown in Fig. 4, the percent changes in cGMP and NOx concentrations in the renal interstitial fluid were positively correlated with the percent changes in renal blood flow during treatment with halothane (r^2=0.817, P<0.0001 for

cGMP; r^2=0.754, P<0.0001 for NOx) or sevoflurane (r^2=0.677, P<0.001 for cGMP; r^2=0.620, P<0.001 for NOx). On the other hand, control plasma concentrations of cGMP were not altered by a high dose of halothane (from 8.06±1.11 to 8.94±1.27 nmol/l) or of sevoflurane (from 6.85±0.97 to 8.30±1.34 nmol/l).

3.2. Renal interstitial cGMP and NOx during stepwise reduction in renal perfusion pressure

Renal perfusion pressure was reduced in two steps from the ambient pressure (131±5 mmHg) to 83±2 mmHg (within autoregulatory range, step 1) and then to 57±3 mmHg (below the autoregulatory range, step 2). Control renal blood flow averaged 3.34±0.15 ml/min/g and remained autoregulated in step 1; however, renal blood flow was significantly decreased in step 2 (Fig. 3A). Renal vascular resistance was also decreased at step 1 from 39±3 to 26±2 mmHg/ml/min/g, but was not further changed at step 2 (29±2 mmHg/ml/min/g) (Fig. 3B). Basal cGMP and NOx concentrations in the renal interstitial fluid were 7.93±0.75 nmol/l and 15.4±1.1 μmol/l, respectively, and these concentration were not changed in step 1. However, cGMP and NOx concentrations in the renal interstitial fluid were significantly decreased in step 2 (Fig. 3C and D). When renal perfusion pressure was allowed to return to ambient conditions (133±3 mmHg), renal blood flow and vascular resistance returned to their control levels, with an average of 3.27±0.13 ml/min/g and 41±2 mmHg/ml/min/g. Similarly, cGMP and NOx concentrations in the renal interstitial fluid also returned to their control levels (7.65±0.63 nmol/l and 14.5±0.9 μmol/l, respectively). Over this pressure range, the percent changes in concentrations of cGMP and NOx were positively correlated with the percent changes in renal blood flow (r^2=0.761, P<0.0001 for cGMP; r^2=0.674, P<0.001 for NOx; Fig. 4). On the other hand, control plasma concentrations of cGMP (7.68±0.72 nmol/l) did not changed in step 1 (7.43±0.68 nmol/l) or step 2 (7.38±0.71 nmol/l).

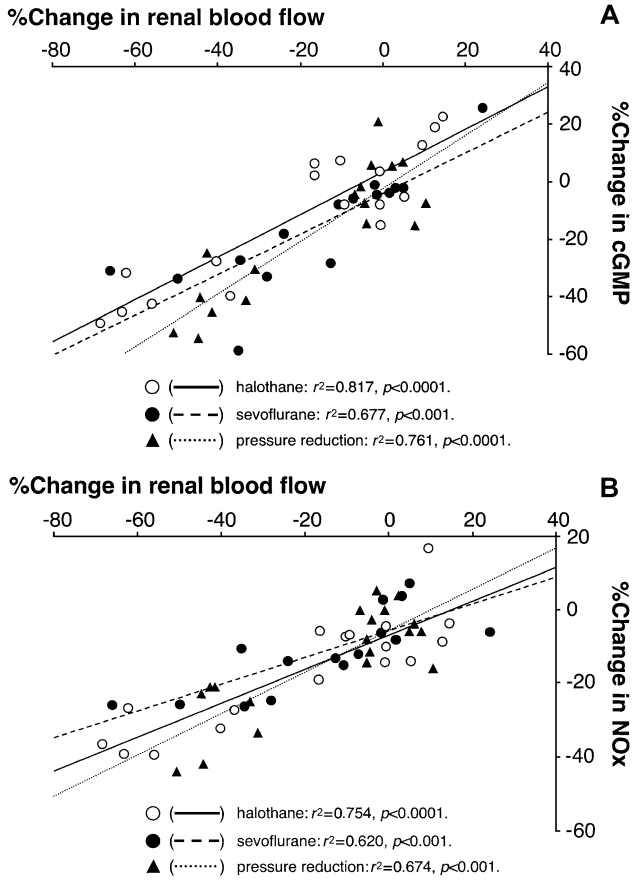

Fig. 4. (A) Relationship between percent changes in renal blood flow and percent changes in renal interstitial fluid concentrations of cGMP. (B) Relationship between percent changes in renal blood flow and percent changes in renal interstitial fluid concentrations of NOx. Data are expressed as percent change of the control values at 0 min or spontaneous renal perfusion pressure.

4. Discussion

Although many in vitro studies have indicated that volatile anesthetics interfere with endothelium-dependent vascular relaxation by NO (Blaise et al., 1994; Hart et al., 1993; Johns et al., 1995; Muldoon et al., 1988; Nakamura et al., 1994; Stone and Johns, 1989; Uggeri et al., 1992), in vivo data on the interaction between volatile anesthetics and the NO-sGC signaling pathway are limited. Wang et al. (1991) showed that NOS inhibition had no effect on blood pressure in rats anesthetized with halothane, indicating that the systemic effects of NO are blocked by halothane. Similarly, halothane anesthesia diminished regional hemodynamic responses to NOS inhibition (Sigmon et al., 1995). Previously, we utilized renal microdialysis method in rabbits to monitor cGMP and NOx concentrations in the renal interstitial fluid, and showed that halothane anesthesia caused marked renal vasoconstriction in association with reductions in renal interstitial cGMP and NOx levels (Nishiyama et al., 1999). Siragy et al. (1992) demonstrated that intra-arterial infusion of a non-specific NOS inhibitor, N^G-monomethyl-L-arginine, decreased renal interstitial cGMP levels. Similarly, it was shown that N^G-nitro-L-arginine methyl ester (L-NAME) significantly decreased cGMP and NOx concentrations in the renal interstitial fluid (Nishiyama et al., 1999). Furthermore, these responses to L-NAME could be reversed by a superimposition of L-arginine on L-NAME. We also observed that an NO donor, sodium nitroprusside, increased renal interstitial cGMP levels. Collectively, the renal microdialysis method appears to be a useful tool for monitoring the dynamics of intrarenal NO. The data obtained from the previous in vivo studies raise two possibilities; first, halothane causes renal vasoconstriction by reducing intrarenal NO levels; second, reductions in renal interstitial NO levels are associated with halothane-induced decreases in renal blood flow. The present study showed that a lower dose of halothane or

sevoflurane (0.8 Mac) slightly decreased renal perfusion pressure, but altered neither renal blood flow nor cGMP and NOx concentrations in the renal interstitial fluid. On the other hand, a higher dose (2.4 Mac) of these volatile anesthetics decreased renal perfusion pressure below the autoregulatory range and reduced renal blood flow. Interestingly, renal interstitial cGMP and NOx levels were also significantly decreased by a higher dose of these volatile anesthetics. In addition, halothane or sevoflurane-induced changes in renal interstitial cGMP and NOx levels were highly correlated with changes in renal blood flow. Thus, these data are consistent with the hypothesis that volatile anesthetic-induced reductions in renal NO levels are associated with blood flow changes.

To test this hypothesis further, we also examined the effects of the manipulations of renal blood flow on cGMP and NOx concentrations in the renal interstitial fluid. Using an aortic clamp, renal perfusion pressure was reduced in two steps to within and below autoregulatory range. Consistent with previous studies in rabbits (Nishiyama et al., 2002), cGMP and NOx concentrations in the renal interstitial fluid and renal blood flow were not altered in response to reductions in renal perfusion pressure within the autoregulatory range in dogs. However, greater reductions in renal perfusion pressure (below the autoregulatory range) consistently decreased renal interstitial cGMP or NOx levels along with reductions in renal blood flow. We also found that the changes in renal interstitial cGMP or NOx levels were highly correlated with the percent changes in renal blood flow over this pressure range. These data indicate that acute changes in renal blood flow result in alterations in NO levels in the kidney; and support the hypothesis that volatile anesthetics-induced reductions in renal NO levels are associated with blood flow changes. During a lower dose of halothane and sevoflurane (0.8 Mac) or mechanically decreasing renal perfusion pressure at step 1, renal vascular resistance was significantly decreased. On the other hand, renal vascular resistance was not altered by a higher dose of these volatile anesthetics (2.4 Mac) or further decreasing renal perfusion pressure at step 2, indicating a partial loss of renal autoregulation. These data suggest that renal blood flow-dependent changes in renal interstitial NO levels may not be directly linked to alterations in renal vascular resistance or renal perfusion pressure.

Within an renal blood flow autoregulatory range, the diameters of preglomerular vessels are increased in responses to reductions in renal perfusion pressure (Ichihara and Navar, 1999; Navar et al., 1996). Since shear stress is influenced by changes in vessel diameters (Ballermann et al., 1998), it is possible that cortical NO levels are decreased by reducing renal perfusion pressure. In vitro studies have shown that NO release from macula densa cells is also decreased in responses to reductions in renal perfusion pressure (Ichihara and Navar, 1999; Navar et al., 1996). Majid et al. (1999) utilized a NO electrode in dogs and showed that reductions in renal perfusion pressure within the autoregulatory range decreased renal cortical tissue NO levels. In the present study, however, renal interstitial cGMP and NOx levels in renal cortex were not significantly altered in response to reductions in renal perfusion pressure within the autoregulatory range (step 1). Although we have no satisfactory explanation for the differences between our results and those of Majid et al. (1999), they might be due to differential experimental conditions or regional variations in the responses of NO to renal perfusion pressure. Majid et al. (1999) elevated the basal level of renal perfusion pressure to 150 mmHg by partial occlusion of both common carotid arteries to allow examination of the pressure–flow relationship over a much wider range of renal perfusion pressure. In addition, a NO electrode was inserted into the mid-deep cortex (5-mm depth in dog kidney) (Majid et al., 1999), whereas we located the dialysis membrane at the superficial cortex.

The present experiments did not allow us to determine the molecular mechanisms by which volatile anesthetics interfere with the NO-sGC signaling pathway. We previously demonstrated that administration of a NO donor, sodium nitroprusside, causes significant increases in renal interstitial cGMP levels during treatment with halothane; suggesting that halothane does not interfere with the process of sGC activation by NO (Nishiyama et al., 1999). Several studies using in vitro approaches have shown that volatile anesthetics do not change NOS activity (Rengasamy et al., 1995; Tagliente et al., 1997). In addition, studies performed in isolated vascular rings showed that volatile anesthetics interfere with the relaxation of vascular smooth muscles by inhibiting the synthesis, release or transport of NO; whereas there are not any effects on sGC activation (Stone and Johns, 1989; Uggeri et al., 1992). However, other studies have shown that volatile anesthetics inhibit the process of sGC activation by NO (Hart et al., 1993; Nakamura et al., 1994) or reduce NO half life (Blaise et al., 1994; Nakamura et al., 1994). Furthermore, Johns et al. (1995) showed that the action site of halothane is distal to the receptor activation process and proximal to the NO-induced activation process of sGC. Collectively, although several possibilities for mechanisms responsible for the volatile anesthetic-induced interference with the NO-sGC signaling pathway have been suggested by in vitro studies, the issue has still not been clarified and further studies are needed.

In summary, the present study showed that renal blood flow and renal interstitial NO levels were significantly decreased by halothane or sevoflurane anesthesia. Furthermore, reductions in renal perfusion pressure (below the autoregulatory range) consistently decreased renal interstitial NO levels along with reductions in renal blood flow. Positive associations were observed between changes in intrarenal NO levels and renal blood flow during halothane or sevoflurane anesthesia, as well as during stepwise reductions in renal perfusion pressure. These data suggest that halothane- and sevoflurane-induced decreases in intrarenal NO levels result from reductions in renal blood flow.

Since NO plays an important role in the regulation of renal function (Majid and Navar, 2001; McKee et al., 1994; Millatt and Siragy, 2000; Navar et al., 1996), the present results might suggest caution in clinical use of high doses of volatile anesthetics in patients with renal disease.

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