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Effects of halothane on renal hemodynamics and interstitial nitric oxide in rabbits

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Abstract

The effects of halothane on renal hemodynamics and the nitric oxide (NO)–guanylate cyclase signaling pathway were examined in anesthetized rabbits using a renal microdialysis method. Halothane (0.5 and 2 vol%) caused dose-dependent decreases in blood pressure, renal blood flow and the renal interstitial concentrations of guanosine 3′,5′-cyclic monophosphate (cGMP) or nitrate (NO_2)/nitrite (NO_3). Sodium nitroprusside (20 μg kg^{-1} min^{-1}, i.v.) under the inhalation of halothane (2 vol%) increased the renal interstitial concentration of cGMP. L-Arginine (priming dose, 300 mg kg^{-1} 10 min^{-1}; sustaining dose, 50 mg kg^{-1} min^{-1}, i.v.) did not reverse halothane-induced reductions of cGMP and NO_2/NO_3. These findings demonstrate that halothane caused a renal vasoconstriction and inhibited the NO–guanylate cyclase signaling pathway in the kidney. Moreover, it is possible that the renal hemodynamic responses to halothane might have been induced, in part, through this inhibition. Finally, it can be assumed that halothane did not interfere with the activation process of guanylate cyclase by NO.

Keywords: Halothane; Nitric oxide (NO); Renal hemodynamics; cGMP; Nitrate/nitrite (NO_2/NO_3); Microdialysis

1. Introduction

Nitric oxide (NO) is synthesized in the kidney and plays an important role in regulating renal hemodynamics and functions (Tamaki and Abe, 1994). Recently, a great deal of evidence has suggested that NO is generated not only in the renal vascular endothelium but also in other renal cells such as, peripheral nerve (Tamaki et al., 1996), mesangium (Shultz et al., 1991), macula densa (Mundel et al., 1992) and tubular cell (Terada et al., 1992). Thus, NO participates in the control of renal blood flow and renal perfusion pressure, water and sodium excretion, glomerular filtration rate and renin–angiotensin system (Kiyomoto et al., 1992; Majid and Navar, 1992; Raji and Shultz, 1993; Manning and Hu, 1994; Aki et al., 1997; Majid et al., 1997).

Halothane, a volatile anesthetic, has been reported to inhibit the NO–guanylate cyclase signaling pathway in in vitro experiments using isolated vascular rings (Muldoon et al., 1988; Stone and Johns, 1989; Uggeri et al., 1992; Hart et al., 1993), cultured endothelial cells (Blaise et al., 1994) and endothelial cell-vascular smooth muscle co-culture models (Johns et al., 1995). However, the inhibitory mechanisms and the action site of halothane in the NO–guanylate cyclase signaling pathway have not been elucidated yet. Moreover, in vivo data on halothane and the NO–guanylate cyclase signaling pathway are very limited to date, due to technical difficulties of measuring NO-products in in vivo experimental systems.

Halothane also exerts a variety of renal hemodynamic and functional effects (Everett et al., 1973; Crawford et al., 1992). It decreases renal blood flow and/or glomerular filtration rate, which may not be caused by cardiovascular depression (Holstein-Rathlou et al., 1982; Groves et al., 1990). These mechanisms, however, remain unclear. In the present study, we hypothesized that the renal hemodynamic responses to halothane might be induced via depression of the NO–guanylate cyclase signaling pathway. To evaluate this hypothesis with an in vivo model, we established a microdialysis method (Nishiyama et al., 1997), and investigated the renal hemodynamic effects of halothane and the effects of halothane on the renal intersti-

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tial concentrations of guanosine 3′,5′-monophosphate (cGMP) and nitrate (NO_2)/nitrite (NO_3). This method is well suited for measuring the dynamics of intrarenal NO in various conditions, because the effects of the NO–guanylate cyclase signaling pathway on renal hemodynamics and functions depend on the renal interstitial concentrations of cGMP and NO_2/NO_3 (Siragy et al., 1992; Zou and Cowley, 1997). Using this method, we have also defined the action site of halothane in the NO–guanylate cyclase signaling pathway.

2. Materials and methods

2.1. General procedure

Experiments were carried out using adult male New Zealand white rabbits weighing from 2.8 to 3.2 kg. All surgical and experimental procedures were performed under the guidelines for the care and use of animals as established by the Kagawa Medical University. The animals were anesthetized with sodium pentobarbital (25 mg kg^{-1}, bolus and 5 mg kg^{-1} h^{-1}, infusion), and were ventilated with room air (4 l min^{-1}) using a mechanical ventilator after tracheotomy. Arterial blood gas and *pH* were measured periodically and ventilation was maintained within normal levels. Pancuronium bromide (0.25 mg kg^{-1}; bolus and 0.125 mg kg^{-1} h^{-1}; infusion) which had a minor effect on hemodynamics was given intravenously for muscle relaxation. A catheter was inserted into the right femoral vein for the infusion of isotonic saline or drug solution which were infused at a rate of 4 ml kg^{-1} h^{-1} throughout the experiment. Another catheter was also placed in the abdominal aorta via the right femoral artery, and mean blood pressure and heart rate were continuously measured and recorded with a pressure transducer (NEC-San-ei Model No. 361, Japan) and a polygraph (NEC-San-ei Model No. 361). The left kidney was exposed through a retroperitoneal flank incision. An electromagnetic flowmeter (MFV-1200, Nihon Kohden, Tokyo, Japan) was positioned around the renal artery and renal blood flow was continuously monitored.

The microdialysis probes were implanted into the renal cortex as shown in Fig. 1. The probes were connected to a microinfusion pump (Carnergie Medicine, Stockholm, Sweden) and were perfused with isotonic saline solution with heparin (30 units ml^{-1}) at a rate of 5 μl min^{-1}. The dialysates were collected into chilled tubes for 10-min sample periods and analyzed for cGMP and NO_2/NO_3. Samples were stored at −70°C until analysis. After the surgery, animals were allowed to stabilize for 90 min.

2.2. Experimental protocols

The experiments were carried out according to the following protocols.

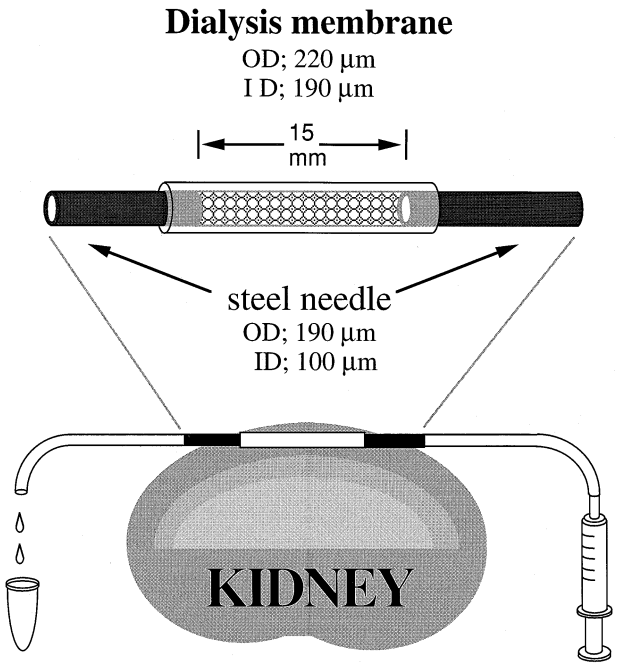

Fig. 1. Schematic illustration of a newly developed microdialysis probe. The dialysis membrane is made from cuprophan fiber, measuring 15 mm in length and 0.22 mm in outer diameter and with a 5500 Da transmembrane diffusion cut-off. The steel needles were inserted into the both sides of cuprophan fiber. The probe was gently implanted into the renal cortex. The probe was connected to a microinfusion pump and perfused with saline solution at a rate of 5 μl min^{-1}.

2.2.1. The effects of N^G-nitro-L-arginine methyl ester (L-NAME) and L-arginine on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3

After three 10-min sampling periods, L-NAME (30 mg kg^{-1}; bolus and 50 mg kg^{-1} h^{-1}; infusion) was infused intravenously in six rabbits. The dialysates were collected at 15, 30 and 60 min after starting the L-NAME infusion. Then, L-arginine (priming dose, 300 mg kg^{-1} 10 min^{-1}; sustaining dose, 50 mg kg^{-1} min^{-1}) was superimposed to L-NAME for an additional 60 min. The dialysates were collected again at 30 and 60 min after starting the L-arginine infusion.

2.2.2. The effects of halothane on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3

We studied the effects of two doses of halothane, 0.5 and 2 vol%. Following three 10-min sampling periods, 0.5% halothane ($n = 5$) or 2% halothane ($n = 8$) was administered with room air (4 l min^{-1}). The dialysates were collected at 30 and 60 min after starting the inhalation of halothane. Mean blood pressure, heart rate and renal blood flow were measured at the midpoint of each 10-min sampling period. Three additional 10-min periods were performed at 30, 60 and 120 min after the cessation of the inhalation of halothane.

2.2.3. The effects of sodium nitroprusside under halothane on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3

After two 10-min sampling periods, halothane (2 vol%) was administered with room air (4 l min^{-1}) and dialysates were collected in the same manner as described above. At 60 min after starting the inhalation of halothane, sodium nitroprusside was infused intravenously at a rate of 20 μg kg^{-1} min^{-1} in nine rabbits while continuing the inhalation of halothane. The dialysate was collected at 30 min after starting the sodium nitroprusside infusion. The inhalation of halothane (2 vol%) was continued for an additional 40 min after the cessation of sodium nitroprusside infusion, and more dialysate was obtained at 30 min after the cessation of sodium nitroprusside infusion. After the cessation of the halothane inhalation, three additional 10-min sample periods were performed at 30, 60 and 120 min.

2.2.4. The effects of L-arginine under halothane on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3

In 12 rabbits, L-arginine (priming dose, 300 mg kg^{-1} 10 min^{-1}; sustaining dose, 50 mg kg^{-1} min^{-1}) was also infused intravenously under the inhalation of halothane (2 vol%). The experimental procedures were identical to the sodium nitroprusside experiments described above.

2.3. Microdialysis probe

In this study, a newly developed microdialysis probe, constructed in our laboratory, was used (He et al., 1995). The dialysis membrane is made from cuprophan fiber, measuring 15 mm in length and 0.22 mm in outer diameter and with a 5500 Da transmembrane diffusion cut-off. The steel needles were inserted into both sides of cuprophan fiber (Fig. 1). The efficiency of this microdialysis probe was determined as follows: probes were placed into beakers containing an isotonic saline solution with different quantities of cGMP or NO_2/NO_3. Each probe was perfused at different perfusion rates with saline solution containing heparin (30 units ml^{-1}). The dialysates were collected, and the recovery rates of cGMP or NO_2/NO_3 were calculated by dividing the concentrations in the dialysate by the concentration in the medium. At a perfusion rate of 5 μl min^{-1}, the recovery rates of cGMP and NO_2/NO_3 were 26.9 ± 1.9% and 29.7 ± 3.2%, respectively. These recovery rates were stable for 6–8 h and were higher than those obtained by regular probes.

2.4. Drugs

Drugs used include: N^G-nitro-L-arginine methyl ester, L-arginine, sodium nitroprusside (all from Sigma, St. Louis,

Table 1
Effects of N^G-nitro-L-arginine methyl ester (L-NAME) and L-arginine on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3 in rabbits

	Mean blood pressure (mm Hg)	Renal blood flow (ml min^{-1} g^{-1})	Renal vascular resistance (mm Hg ml^{-1} g^{-1} min^{-1})	cGMP (nM)	NO_2/NO_3 (μM)
Control					
10 min	96±3	2.97±0.53	32.4±2.7	15.8±4.3	79.0±4.7
20 min	96±3	2.93±0.50	32.7±2.9	16.2±4.5	79.8±7.7
30 min	96±3	2.93±0.50	32.7±2.9	16.1±4.3	79.0±5.4
L-NAME (30 mg kg^{-1}; bolus and 50 mg kg^{-1} h^{-1}; infusion)					
15 min	118±1[a]	1.51±0.37[a]	78.2±5.2[a]	13.1±3.6	76.2±5.7
30 min	119±1[a]	1.29±0.34[a]	92.1±6.4[a]	12.6±3.3[a]	64.9±7.7[a]
60 min	119±1[a]	1.17±0.26[a]	101.5±6.9[a]	11.1±3.5[a]	59.5±6.7[a]
L-NAME (30 mg kg^{-1}; bolus and 50 mg kg^{-1} h^{-1}; infusion) plus L-arginine (priming dose, 300 mg kg^{-1} for 10 min; sustaining dose, 50 mg kg^{-1} min^{-1})					
30 min	92±7	2.54±0.40	36.1±4.3	14.6±4.4	62.6±7.4[a]
60 min	94±5	2.86±0.49	32.8±3.1	16.2±4.5	77.5±7.1

Values are means ± S.E. $n = 6$. Kidney weight: 7.76 ± 0.57 g, cGMP: guanosine 3′,5′-cyclic monophosphate, NO_2/NO_3: nitrate/nitrite, L-NAME: N^G-nitro-L-arginine methyl ester, [a]indicates significant difference from the third control value ($P < 0.05$).

MO, USA), and halothane (from Hoechxt Japan, Tokyo, Japan). Pancuronium bromide was a gift from Sankyo-Pharmaceutical (Tokyo, Japan). N^G-nitro-L-arginine methyl ester, sodium nitroprusside, L-arginine and pancuronium bromide were dissolved in isotonic saline solution.

2.5. Analytical procedures

The cGMP in dialysate was measured by radioimmunoassay kits (Amersham USA). The NO_2/NO_3 was analyzed with high performance liquid chromatography (Hitachi Model No. 638-50, Japan) on an anion exchange column (Tosoh TSK-GEL IC-ANION-PWXL) equilibrated with 2.5 mM KH_2PO_4 and 1 mM K_2HPO_4 at a flow rate of 0.8 ml min^{-1}. Ten microliters of the sample were injected and the elution was monitored by a UV detector (Hitachi Model No. L-4000, Japan) at a wave length of 210 nm. The chromatogram was recorded by a Hitachi recorder (Model No. 056, Japan).

2.6. Statistical analysis

The values are presented as means ± S.E. Statistical differences of data means were determined by Student's *t* and paired *t*-tests. Differences with a *P*-value below 0.05 were considered statistically significant.

3. Results

3.1. The effects of L-NAME and L-arginine on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3

L-NAME increased mean blood pressure, and decreased renal blood flow and heart rate. The calculated renal vascular resistance was increased significantly at 60 min after starting the infusion. These results showed a tendency to return to the respective control level after a superimposition of L-arginine on L-NAME. L-NAME significantly decreased the renal interstitial concentrations of cGMP and NO_2/NO_3. These parameters tended to return to the respective control level after a superimposition of L-arginine on L-NAME (Table 1).

3.2. The effects of halothane on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3

The lower dose of halothane (0.5 vol%) decreased mean blood pressure by 9 ± 1 mm Hg and renal blood flow by 0.75 ± 0.10 ml g^{-1} min^{-1}, but did not affect heart rate. The renal vascular resistance significantly increased at 60 min after starting the inhalation. The renal vascular resistance in this group was higher than that in other groups and this difference might be due to the differences in kidney weight (Tables 1–3). However, the renal hemodynamic responses to halothane were not qualitatively different from those induced by the higher dose of halothane. The renal interstitial concentrations of cGMP and NO_2/NO_3 decreased by 29% and 28%, respectively (Table 2).

Halothane (2 vol%) also decreased mean blood pressure by 39 ± 5 mm Hg, renal blood flow by 1.79 ± 0.14 ml g^{-1} min^{-1} and heart rate by 15 ± 3 beats min^{-1}. These data showed a tendency of returning to the respective control levels after the cessation of halothane inhalation. The renal vascular resistance which increased significantly at 60 min after starting the inhalation of halothane, indicated a renal vasoconstriction (Table 3). The basal concentrations of cGMP and NO_2/NO_3 in the renal interstitial space, which were measured at 90 min after the implantation of the

Table 2
Effects of halothane (0.5 vol%) on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3 in rabbits

	Mean blood pressure (mm Hg)	Renal blood flow (ml min^{-1} g^{-1})	Renal vascular resistance (mm Hg ml^{-1} g^{-1} min^{-1})	cGMP (nM)	NO_2/NO_3 (μM)
Control					
10 min	96 ± 4	2.26 ± 0.44	42.8 ± 2.8	14.8 ± 3.6	74.8 ± 7.1
20 min	96 ± 4	2.37 ± 0.50	40.5 ± 2.4	15.1 ± 3.5	75.4 ± 7.7
30 min	96 ± 4	2.49 ± 0.60	38.6 ± 2.2	15.1 ± 3.7	75.1 ± 6.4
Halothane (0.5 vol%)					
30 min	87 ± 4*	1.74 ± 0.74[a]	50.0 ± 2.3[a]	11.0 ± 1.5[a]	58.9 ± 3.0[a]
60 min	87 ± 6*	1.77 ± 0.54[a]	49.4 ± 2.4[a]	10.1 ± 2.9[a]	47.8 ± 3.7[a]
Recovery					
30 min	94 ± 5	2.54 ± 0.69	37.0 ± 2.8	13.8 ± 3.0	66.0 ± 5.4
60 min	95 ± 5	2.54 ± 0.69	37.2 ± 2.9	15.2 ± 3.3	75.4 ± 6.7

Values are means ± S.E. $n = 5$. Kidney weight: 9.87 ± 0.81 g, cGMP: guanosine 3′,5′-cyclic monophosphate, NO_2/NO_3: nitrate/nitrite, [a]indicates significant difference from the third control value ($P < 0.05$).

Table 3
Effects of halothane (2 vol%) on renal hemodynamics in rabbits

	Mean blood pressure (mm Hg)	Renal blood flow (ml min^{-1} g^{-1})	Renal vascular resistance (mm Hg ml^{-1} g^{-1} min^{-1})	Heart rate (beats min^{-1})
Control				
10 min	95 ± 3	3.42 ± 0.16	29.5 ± 2.2	306 ± 15
20 min	95 ± 3	3.42 ± 0.17	29.9 ± 2.4	307 ± 14
30 min	95 ± 3	3.42 ± 0.20	30.1 ± 2.8	303 ± 14
Halothane (2 vol%)				
30 min	58 ± 6*	1.70 ± 0.14[a]	36.2 ± 5.5[a]	290 ± 13[a]
60 min	56 ± 4*	1.63 ± 0.07[a]	41.1 ± 2.0[a]	288 ± 14[a]
Recovery				
30 min	91 ± 2	2.18 ± 0.06[a]	36.1 ± 2.3[a]	310 ± 16
60 min	94 ± 2	2.59 ± 0.12[a]	34.4 ± 4.0	311 ± 17
120 min	95 ± 2	3.26 ± 0.14	29.6 ± 1.6	311 ± 17

Values are means ± S.E. $n = 8$. Kidney weight: 6.26 ± 0.55 g, [a] indicates significant difference from the third control value ($P < 0.05$).

microdialysis probes, were 18.3 ± 2.1 nM and 72.6 ± 7.9 μM, respectively. At 60 min after the inhalation of halothane, cGMP and NO_2/NO_3 concentrations decreased from 19.2 ± 2.2 and 71.5 ± 9.0 to 9.6 ± 1.8 nM and 43.6 ± 5.5 μM, respectively. These values returned to the respective control levels soon after the cessation of halothane inhalation (Fig. 2).

3.3. The effects of sodium nitroprusside under halothane on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3

Halothane (2 vol%) decreased mean blood pressure by 36 ± 4 mm Hg and renal blood flow by 1.27 ± 0.17 ml g^{-1} min^{-1}. An intravenous infusion of sodium nitroprusside at a rate of 20 μg kg^{-1} min^{-1} under the inhalation of halothane (2 vol%) caused an additional decrease of mean blood pressure by 16 ± 2 mm Hg and renal blood flow by 0.49 ± 0.04 ml g^{-1} min^{-1}. After the cessation of sodium nitroprusside infusion, mean blood pressure and renal blood flow tended to return to the respective values of halothane alone. At 30 min after the infusion of sodium nitroprusside under the inhalation of halothane, the renal interstitial concentration of cGMP increased significantly from 11.2 ± 0.7 to 17.8 ± 1.1 nM, but the NO_2/NO_3 concentration was not affected (Fig. 3). Soon after the cessation of sodium nitroprusside infusion, the cGMP concentration decreased from 17.8 ± 1.1 to 10.4 ± 1.5 nM. The concentrations of cGMP and NO_2/NO_3 returned to their respective basal levels 60 min after the cessation of halothane inhalation (Fig. 3).

3.4. The effects of L-arginine under halothane on renal hemodynamics and the renal interstitial concentrations of cGMP and NO_2/NO_3

The superimposition of L-arginine to halothane (2 vol%) did not affect mean blood pressure, renal blood flow or heart rate. L-Arginine also did not affect the renal intersti-

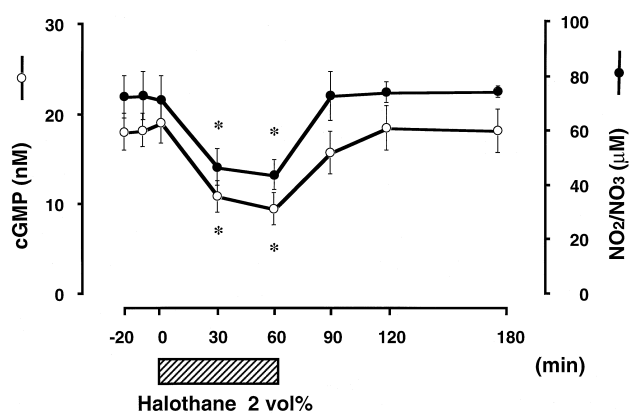

Fig. 2. Effects of halothane (2 vol%) on the renal interstitial concentrations of cGMP and NO_2/NO_3. * indicates significant difference from the third control value ($P < 0.05$). $n = 8$.

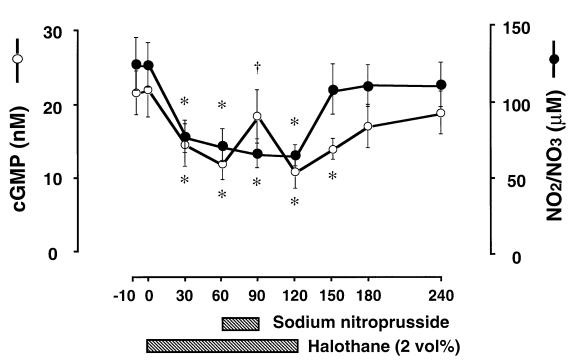

Fig. 3. Effects of intravenous infusion of sodium nitroprusside (20 μg kg^{-1} min^{-1}) under the inhalation of halothane (2 vol%) on the renal interstitial concentrations of cGMP and NO_2/NO_3. * and † indicate significant difference from the second control value and halothane alone, respectively ($P < 0.05$). $n = 9$.

tial concentrations of NO_2/NO_3 and cGMP under the inhalation of halothane (data not shown).

4. Discussion

It is generally accepted that halothane exerts a variety of renal hemodynamic and functional effects and affects the NO–guanylate cyclase signaling pathway. In the present study, we hypothesized that the renal responses to halothane might be induced via modulation of the NO–guanylate cyclase signaling pathway. To test this hypothesis, we decided to investigate the renal hemodynamic effects of halothane and the effects of halothane on the NO–guanylate cyclase signaling pathway using a renal microdialysis method. Halothane (2 vol%) caused significant decreases in mean blood pressure, renal blood flow and heart rate, but significantly increased the calculated renal vascular resistance, indicating a renal vasoconstriction. Halothane (2 vol%) decreased the renal interstitial concentrations of cGMP and NO_2/NO_3. Similar changes except the change in heart rate were observed during the inhalation of a lower dose of halothane (0.5 vol%). Thus, the present data showed that halothane caused dose-dependent renal vasoconstriction and inhibition of the NO–guanylate cyclase signaling pathway in the kidney. These results, therefore, suggest that halothane inhibited the NO–guanylate cyclase signaling pathway in the kidney and the renal responses to halothane might have been induced, in part, through this inhibition.

Microdialysis techniques have been applied for several tissues, but have not been widely used in the kidney because of the tissue injury induced by the insertion of the microdialysis probe. We could minimize tissue injury by making a fiber type probe with a thinner diameter. As shown in Fig. 1, the outer diameter of the newly developed probe is only 0.22 mm, which is one-third the diameter of commercially available probes. In addition, the length of dialysis membrane is 1.5 cm which is 3–4 times longer than that of a regular probe. As a result, the dialysis efficiency of the new probe was greater than that of a regular probe and we could shorten the sampling time (He et al., 1995). Using these newly developed probes, we measured for the first time the interstitial concentrations of cGMP and NO_2/NO_3 levels when the NO synthase was inhibited. Intravenous administration of L-arginine analogue antagonist, L-NAME, significantly decreased the renal interstitial concentrations of cGMP and NO_2/NO_3. These responses to L-NAME could be reversed by a superimposition of L-arginine on L-NAME (Table 1). Moreover, the changes in the interstitial concentrations of cGMP and NO_2/NO_3 were closely related to those in renal blood flow. Thus, this microdialysis method appears to be a useful tool for monitoring the dynamics of intrarenal NO.

The mechanism by which halothane interferes with the NO–guanylate cyclase signaling pathway is not yet defined. The initial studies by Stone and Johns (1989) and Uggeri et al. (1992) showed that halothane interferes with the NO-dependent relaxation of vascular smooth muscle by inhibiting the synthesis, release or transport of NO, but it does not have any effects on guanylate cyclase activation. In contrast to these reports, Hart et al. (1993) found that halothane inhibits the process of guanylate cyclase activation by NO. Using an endothelial cell-vascular smooth muscle co-culture model, Blaise et al. (1994) reported that halothane does not affect NO release from endothelial cells or the activation of guanylate cyclase, but halothane rather impairs either the NO half-life or its activated redox form. Johns et al. (1995) recently showed that halothane inhibits the NO–guanylate cyclase signaling pathway, and that the action site of halothane may be distal to the receptor activation process and proximal to the NO-induced activation process of guanylate cyclase in endothelial cells. Thus, several possibilities of this mechanism have been suggested in recent studies which have employed in vitro settings, however, the action site of halothane in the NO–guanylate cyclase signaling pathway has been highly controversial.

In vivo data on halothane and the NO–guanylate cyclase signaling pathway are very limited to date. Especially, few direct measurements of NO-products are available because of technical difficulties. Therefore, most of the in vivo work to date has been limited to the responses to NO synthase inhibitors, such as L-NAME or N^G-monomethyl-L-arginine (Wang et al., 1991; Koenig et al., 1993; Sigmon et al., 1995). In this study, we established an in vivo microdialysis method and showed that halothane decreased the renal interstitial concentrations of cGMP and NO_2/NO_3, indicating that halothane inhibited the NO–guanylate cyclase signaling pathway in the kidney. Thus, these data provide the first evidence to assess this pathway in an in vivo setting where direct measurement of the cGMP and NO_2/NO_3 were made.

The present in vivo study clearly demonstrates that halothane decreased the renal interstitial concentrations of cGMP and NO_2/NO_3, and the administration of sodium nitroprusside, which is a NO synthase-independent NO donor, caused a significant increase of cGMP concentration under the inhalation of halothane. In a separate series of experiments, we have examined the effects of sodium nitroprusside on renal interstitial concentration of cGMP in non-treated rabbits ($n = 8$). An intravenous infusion of sodium nitroprusside at a rate of 20 $\mu g\ kg^{-1}\ min^{-1}$ increase cGMP from 17.0 ± 2.1 to 31.1 ± 4.0 nM, 30 min after starting the sodium nitroprusside infusion (data were not shown). There were no differences between the rates of increase in cGMP concentration induced by sodium nitroprusside with and without halothane. Thus, these results suggest that halothane inhibited the NO–guanylate cyclase signaling pathway, but did not interfere with the process of guanylate cyclase activation by NO. Our results also indicate that the action site of halothane was proximal to the

NO–guanylate cyclase signaling pathway. These findings are inconsistent with those reported by Hart et al. (1993). The reason for this discrepancy is not clear. However, since their studies were performed in vitro using rat aorta, it might be due to differences in experimental conditions, species and tissue.

L-Arginine did not reverse halothane-induced reductions in cGMP and NO_2/NO_3 (data were not shown). However, the significant reduction in the cGMP and NO_2/NO_3 levels were observed during the administration of L-NAME, and these changes were reversed by a superimposition of L-arginine on L-NAME (Table 1). Thus, L-arginine administration did not affect the cGMP and NO_2/NO_3 levels during the inhalation of halothane, but did increase these parameters under the inhibition of NO synthase. These findings suggest that halothane did not competitively inhibit NO synthase as L-NAME did.

The intravenous administration of sodium nitroprusside under the inhalation of halothane caused a significant increase of cGMP concentration, but did not affect the renal interstitial concentrations of NO_2/NO_3. The result for NO_2/NO_3 was not anticipated. However, the plasma concentration of sodium nitroprusside at 30 min after starting the sodium nitroprusside infusion was estimated as about 10 μM. If the biotransformation of sodium nitroprusside had been neglected, the actual concentration of sodium nitroprusside in the kidney would have been lower than the above estimated value. Since the basal concentration of NO_2/NO_3 in the renal interstitial space was around 100 μM, we might not have been able to detect changes in NO_2/NO_3 concentration induced by sodium nitroprusside.

It is known that NO production and release are regulated by various factors such as vascular shear stress, some chemical stimuli and the nitroxidergic nervous system (Furchgott and Vanhoutte, 1986; Rubanyi et al., 1986; Toda et al., 1994), which were affected by halothane. Based on the present experiments, we could not define whether halothane directly or indirectly affected the NO–guanylate cyclase signaling pathway. Considering both the present in vivo results and previous in vitro findings, it can be speculated that the inhibition of NO–guanylate cyclase signaling pathway by halothane in the kidney played a role in halothane-induced renal hemodynamic changes, at least in part.

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References

Aki, Y., Tomohiro, A., Nishiyama, A., Kiyomoto, K., Kimura, K., Abe, Y., 1997. The role of basally synthesized nitric oxide in modulating the renal vasoconstrictor action of angiotensin II. Hypertens. Res. 20, 251–256.

Blaise, G., To, Q., Parent, M., Lagarde, B., Asenjo, F., Saure, R., 1994. Does halothane interfere with the release, action, or stability of endothelium-derived relaxing factor/nitric oxide?. Anesthesiology 80, 417–426.

Crawford, M.W., Lerman, J., Saldivia, V., Carmichael, F.J., 1992. Hemodynamic and organ blood flow responses to halothane and sevoflurane anesthesia during spontaneous ventilation. Anesth. Analg. 75, 1000–1006.

Everett, G.B., Allen, G.D., Kennedy, W.F. Jr., Gerbershagen, H.U., Cutler, R.E., Sawyer, T.K., 1973. Renal hemodynamic effects of general anesthesia in outpatients. Anesth. Analg. 52, 470–479.

Furchgott, R.F., Vanhoutte, P.M., 1986. Endothelium-derived relaxing and contracting factors. FASEB J. 3, 2007–2018.

Groves, N.D., Leach, K.G., Rosen, M., 1990. Effects of halothane, enflurane and isoflurane anesthesia on renal plasma flow. Br. J. Anesth. 65, 796–800.

Hart, J.L., Jing, M., Bina, S., Freas, W., Van Dyke, R.A., Muldoon, S.M., 1993. Effects of halothane on EDRF/cGMP-mediated vascular smooth muscle relaxations. Anesthesiology 79, 323–331.

He, H., Miura, K., Aki, Y., Kimura, S., Tamaki, T., Iwao, H., Abe, Y., 1995. Adenosine metabolism in the renal interstitial space during ischemia and hypertonic saline infusion. Am. J. Hypertens. 8, 156A.

Holstein-Rathlou, N.-H., Christensen, P., Leyssac, P.P., 1982. Effects of halothane–nitrous oxide inhalation anesthesia and inactin on overall renal and tubular function in Sprague–Dawley and Wistar rats. Acta Physiol. Scand. 114, 193–201.

Johns, R.A., Tichotsky, A., Muro, M., Spaeth, J.P., Cras, T.D.L., Rengasamy, A., 1995. Halothane and isoflurane inhibit endothelium-derived relaxing factor-dependent cyclic guanosine monophosphate accumulation in endothelial cell–vascular smooth muscle co-cultures independent of an effect on guanylyl cyclase activation. Anesthesiology 93, 823–834.

Kiyomoto, H., Matsuo, H., Tamaki, T., Aki, Y., He, H., Iwao, H., Abe, Y., 1992. Effect of L-N^G-nitro-arginine, inhibitor of nitric oxide synthesis, on autoregulation of renal blood flow in dogs. Jpn. J. Pharmacol. 58, 147–155.

Koenig, H.M., Pelligrino, D.A., Albrecht, R.F., 1993. Halothane vasodilation and nitric oxide in rat pial vessels. J. Neurosurg. Anesth. 5, 264–271.

Majid, D.S.A., Navar, L.G., 1992. Suppression of blood flow autoregulation plateau during nitric oxide blockade in canine kidney. Am. J. Physiol. 262, F40–F46.

Majid, D.S.A., Godfreym, M., Navar, L.G., 1997. Pressure natriuresis and renal medullary blood flow in dogs. Hypertension 29, 1051–1057.

Manning, R.D., Hu, L., 1994. Nitric oxide regulates renal hemodynamics and urinary sodium excretion in dogs. Hypertension 23, 619–625.

Muldoon, S.M., Hart, J.L., Bowen, K.A., Freas, W., 1988. Attenuation of endthelium-mediated vasodilation by halothane. Anesthesiology 68, 31–37.

Mundel, P., Bachmann, S., Bader, M., Fischer, A., Kummer, W., Mayer, B., Kriz, W., 1992. Expression of nitric oxide synthase in kidney macula densa cells. Kidney Int. 42, 1017–1019.

Nishiyama, A., Miyatake, A., Aki, Y., Kimura, S., Abe, Y., 1997. Measurements of nitric oxide and cyclic GMP in the renal interstitial space—using microdialysis method. Nephrology 3, P1576, (suppl. 1).

Raji, L., Shultz, P.J., 1993. Endothelium-derived relaxing factor, nitric oxide: effects on and production by mesangial cells and the glomerulus. J. Am. Soc. Nephrol. 3, 1435–1441.

Rubanyi, G.M., Romero, J.C., Vanhoutte, P.M., 1986. Flow-induced release of endothelium-derived relaxing factor. Am. J. Physiol. 250, H1145–H1149.

Shultz, P.J., Tayeh, M.A., Marletta, M.A., Raji, M.A., 1991. Synthesis and action of nitric oxide in rat glomerular mesangial cells. Am. J. Physiol. 261, F600–F606.

Sigmon, D.H., Florentino-Pineda, I., Dyke, R.A.V., Beierwaltes, W.H., 1995. Halothane impairs the hemodynamic influence of endthelium-derived nitric oxide. Anesthesiology 82, 135–143.

Siragy, H.M., Johns, R.A., Peach, M.J., Carey, R.M., 1992. Nitric oxide alters renal function and guanosine 3′,5′-cyclic monophosphate. Hypertension 19, 775–779.

Stone, D.J., Johns, R.A., 1989. Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. Anesthesiology 71, 126–132.

Tamaki, T., Abe, Y., 1994. Control of renal circulation—with special reference on nitric oxide. Asia Pac. J. Pharmacol. 9, 97–112.

Tamaki, T., Kiyomoto, K., Nishiyama, A., Tomohiro, A., He, H., Kimura, S., Aki, Y., Abe, Y., 1996. Nitrodergic nerves in isolated microperfused rabbit afferent arterioles. J. Hypertens. 14, S170, (suppl. 1).

Terada, Y., Tomita, K., Nonoguchi, H., Marumo, F., 1992. Polymerase chain reaction localization of constitutive nitric oxide synthase and soluble guanylate cyclase messenger RNAs in microdissected rat nephron segments. J. Clin. Invest. 90, 659–665.

Toda, N., Kitamura, Y., Okamura, T., 1994. Role of nitroxidergic nerve in dog retinal arterioles in vivo and arteries in vitro. Am. J. Physiol. 266, H1985–H1992.

Uggeri, M.J., Proctor, G.J., Johns, R.A., 1992. Halothane, enflurane, and isoflurane attenuate both receptor- and non-receptor-mediated EDRF production in rat thoracic aorta. Anesthesiology 76, 1012–1017.

Wang, Y.-X., Zhou, T., Chou, T.C., Pang, C.C.Y., 1991. Effects of inhalation and intravenous anaesthetic agents on pressor response to N^G-nitro-L-arginine. Eur. J. Pharmacol. 198, 183–188.

Zou, A.-P., Cowley, A.W., 1997. Nitric oxide in renal cortex and medulla. An in vivo microdialysis study. Hypertension 29, 194–198.