Isoflurane mediates protection from renal ischemia-reperfusion injury via sphingosine kinase and sphingosine-1-phosphate-dependent pathways

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Isoflurane mediates protection from renal ischemia-reperfusion injury via sphingosine kinase and sphingosine-1-phosphate-dependent pathways. Am J Physiol Renal Physiol 293: F1827–F1835, 2007. First published September 26, 2007; doi:10.1152/ajprenal.00290.2007.—The inhalational anesthetic isoflurane has been shown to protect against renal ischemia-reperfusion (IR) injury. Previous studies demonstrated that isoflurane modulates sphingolipid metabolism in renal proximal tubule cells. We sought to determine whether isoflurane stimulates sphingosine kinase (SK) activity and synthesis of sphingosine-1-phosphate (S1P) in renal proximal tubule cells to mediate renal protection via the S1P signaling pathway. Isoflurane anesthesia reduced the degree of renal failure and necrosis in a murine model of renal IR injury. This protection with isoflurane was reversed by SK inhibitors (DMS and SKI-II) as well as an S1P receptor antagonist (VPC23019). In addition, mice deficient in SK1 enzyme were not protected from IR injury with isoflurane. SK activity as well as SK1 mRNA expression increased in both cultured human proximal tubule cells (HK-2) and mouse kidneys after exposure to isoflurane. Finally, isoflurane increased the generation of S1P in HK-2 cells. Taken together, our findings indicate that isoflurane activates SK in renal proximal tubule (HK-2) cells (29).

The lysophospholipid sphingosine-1-phosphate (S1P) has been shown to function as both an extracellular ligand for specific G protein-coupled receptors (GPCRs) as well as an intracellular second messenger in promoting cell growth and survival and the inhibition of apoptosis (40, 50). Activation of sphingosine kinase (SK), the enzyme catalyzing the formation of S1P from its precursor sphingosine, with a resultant increase in levels of S1P, and activation of S1P receptors with specific agonists have been shown to be involved in protection from IR injury in the heart (24, 53, 55), liver (35), and kidney (4, 32).

Most volatile anesthetics are lipophilic molecules (2) and have been shown to increase membrane fluidity and activate sphingomyelin hydrolysis in the renal cortex (33). Based on this knowledge, we hypothesized that volatile anesthetics increase SK activity and S1P formation to mediate renal protection and lead to new therapeutic applications of inhalational anesthetics during the perioperative period.

Acute renal failure; inflammation; necrosis; reverse transcriptase-polymerase chain reaction; volatile anesthetics

ACUTE RENAL FAILURE (ARF) continues to be a major contributor to morbidity and mortality in the perioperative period. A recent review found that the reported incidence of ARF after major vascular surgery ranged from 1.7 to 25% (52) and in a study of 2,672 patients undergoing coronary artery bypass grafting, development of ARF was associated with a 14-fold increase in mortality (10). Despite continued advances in the understanding of the pathophysiology of ARF, a recent meta-analysis of randomized clinical trials found no conclusive evidence that any of the currently available treatments, including pharmacological and fluid hydration regimens, offered renal protection during surgery (58).

We previously reported that certain volatile anesthetics, including sevoflurane [2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane], protected rats from renal ischemia-reperfusion (IR) injury by attenuating the inflammatory response as well as necrosis (31). We also demonstrated that sevoflurane, another volatile anesthetic, had direct anti-inflammatory and anti-necrotic effects in cultured human kidney proximal tubule (HK-2) cells (29).

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Manassas, VA) were grown and passaged in 75-cm² cell culture flasks to assess the degree of renal tubular necrosis in the outer medullary grading scale of 0 to 4, as outlined by Jablonski et al. (23), was performed by an experienced renal pathologist who was unaware of the method based on the Jaffe reaction (22).

Effect of SK antagonists and S1P receptor antagonist on renal IR injury. To test the effect of SK inhibition on renal IR injury, the SK inhibitors DMS and SKI-II were administered to mice undergoing renal IR injury. DMS (5 mg/kg) was given intraperitoneally 30 min pre- and 4 h postischemia. SKI-II (50 mg/kg) was administered subcutaneously 15 min pre- and 4 h postischemia. In addition, VPC23019, a specific S1P1/S1P3 receptor antagonist (10-fold more selective at the S1P1 vs. the S1P3 receptor), was administered to test the effects of S1P1 receptor blockade. VPC23019 (1 mg/kg) was given intraperitoneally 10 min preischemia and 10 min prererperfusion.

Measurement of plasma creatinine. Renal function was assessed by measuring plasma creatinine 24 h after ischemia using a colorimetric method based on the Jaffe reaction (22).

Histological detection of necrosis. Morphologic assessment was performed by an experienced renal pathologist who was unaware of the treatment that each animal had received. A renal injury score grading scale of 0 to 4, as outlined by Jablonski et al. (23), was used to assess the degree of renal tubular necrosis in the outer medullary area after renal IR injury as described previously (27, 28).

HK-2 cell culture. HK-2 cells (American Type Culture Collection, Manassas, VA) were grown and passaged in 75-cm² cell culture flasks containing culture medium (1:1 mixture of DMEM/F12 with 10% fetal bovine serum; Invitrogen, Carlsbad, CA) and antibiotics (100 \( \mu \)g/ml penicillin G, 100 \( \mu \)g/ml streptomycin, and 0.25 \( \mu \)g/ml amphotericin B; Invitrogen) at 37°C in a 100% humidified atmosphere of 5% CO₂-95% air. This cell line has been characterized extensively and retains the phenotypic and functional characteristics of proximal tubule cells in culture (47). They were plated in six-well plates when 80% confluent and used in the experiments described below when confluent.

Exposure of HK-2 cells to isoflurane. For isoflurane treatment, HK-2 cells were placed in an air-tight, 37°C, humidified modular incubator chamber connected to an in-line agent-specific calibrated vaporizer (Datex-Ohmeda, Madison, WI) to deliver isoflurane (2.5% or 2 MAC) mixed with 95% air-5% CO₂ (carrier gas) at 10 l/min, as described previously (29). Exposure to isoflurane lasted 3–16 h. Control cells were exposed to carrier gas in a modular incubator chamber.

In vivo kidney enzyme preparation from mice. To measure the effects of isoflurane anesthesia on SK activity in vivo, we anesthetized mice with either 1.2% isoflurane or pentobarbital sodium for 3 h. Twenty-one hours later, the kidneys were collected and homogenized manually with a pestle in buffer \( E \) (100 mM sucrose, 1 mM EGTA, 20 mM MOPS, pH 7.4, 5% Percoll, 0.01% digitonin, protease \( \{ \text{4-2-aminoethyl}-\text{benzenesulfonyl fluoride, aprotinin, bestatin, E-64, leupeptin, pepstatin A (Calbiochem, San Diego, CA)}\} \), and phosphatase \( \{ \text{Na₂VO₄, Na₃MoO₄, sodium tartrate, imidazole (Sigma, St. Louis, MO)}\} \) inhibitors on ice. After a 1,000-g spin for 5 min, the supernatant was collected and spun at 100,000 g for 30 min to separate the membrane and cytosolic fractions. The pellet was resuspended in lysis buffer (50 mM Tris, pH 7.5, 1% Triton X-100, 10 mM EDTA, 150 mM NaCl, 100 mM NaF, 1 mM Na₂VO₄, protease and phosphatase inhibitors) as the membrane fraction. Protein concentrations were determined for both fractions.

In vitro enzyme preparations from HK-2 cells. For whole cell assays, HK-2 cells (treated with either 2.5% isoflurane or carrier gas) were scraped in lysis buffer for the SK activity assay. For separation of membrane and cytosolic fractions, cells were scraped in buffer \( F \) and the lysate was spun at 1,000 g for 5 min. The supernatant was then spun at 100,000 g for 30 min to separate the fractions. The pellet was resuspended in lysis buffer. The protein concentrations of both fractions were determined.

SK activity assay. SK activity was measured as described by Vessey et al. (54). Briefly, enzyme preparations from kidneys (20 \( \mu \)g total protein) or HK-2 cells (120–240 \( \mu \)g total protein) were incubated in assay buffer \( \{ \text{0.5% Triton X-100, 250 mM KCl, 0.05 \( \mu \)M \[\text{3H}\]sphingosine (20 Ci/mmol; American Radiolabeled Chemicals), 5 mM ATP, 10 mM MgCl₂, 100 mM Tris pH 8.0} \) in a total volume of 100 \( \mu \)l at 20°C for 15 (renal cortices) or 60 min (HK-2 cells). These incubation times yielded a linear increase in SK activity (data not shown). \[\text{[3H]}\]S1P was extracted using 1.2 ml of methanol:chloroform:isooctane (20:20:1). After vortexing and centrifugation for 5 min at 2,700 g, the upper aqueous phase was collected.

For whole cell assays, HK-2 cells (treated with either 2.5% isoflurane or carrier gas) were then exposed to either 2.5% isoflurane or the carrier gas for 6 h. The media were aspirated and the cells were scraped in 100-\( \mu \)l lysis buffer. The lysate was spun at 100,000 g for 30 min to separate the fractions. The pellet was resuspended in lysis buffer. The protein concentrations of both fractions were determined.

Measurement of \[\text{[3H]}\]S1P synthesis in HK-2 cells. Cellular \[\text{[3H]}\]S1P formation in HK-2 cells was measured using a method similar to that described by Lavieu et al. (26). Confluent cells in six-well plates were incubated overnight in serum-free media containing 0.02 \( \mu \)M \[\text{[3H]}\]sphingosine (20 Ci/mmol) to allow for equilibration. The cells were then exposed to either 2.5% isoflurane or the carrier gas for 6 h. The media were aspirated and the cells were scraped in 100-\( \mu \)l lysis buffer. The lysate was collected and \[\text{[3H]}\]S1P was extracted as described above and counted.

RT-PCR for SK1 mRNA. Samples from mouse renal cortices (21 h following 3 h of exposure to either 1.2% isoflurane or pentobarbital sodium) or HK-2 cells (following exposure to 3, 6, or 16 h of 2.5% isoflurane) were collected and total RNA was extracted for semiquantitative RT-PCR analysis as described previously (31). The expression of SK1 mRNA was measured using primers designed based on published GenBank sequences for mice and humans (Table 1). Primers were purchased from Sigma Genosys (The Woodlands, TX).

Table 1. Primer sequences

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<th>Primers</th>
<th>Species</th>
<th>Size, bp</th>
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<th>Sequence (Sense/Antisense)</th>
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SK, sphingosine kinase.
To verify the results of our semiquantitative RT-PCR analysis, we also performed quantitative real-time RT-PCR analysis on the mouse kidneys and one time point (6-h isoflurane treatment) of the HK-2 cell samples. The results were consistent with the semiquantitative analysis (data not shown). Q-RTPCR was performed with the MyIQ Real Time Detection System (Bio-Rad, Hercules, CA) using SYBR Green I Brilliant Mastermix (Stratagene, La Jolla, CA). The cDNA template was synthesized using Omniscript Reverse Transcriptase and oligo-dT primer (Qiagen, Valencia, CA). Specificity of the amplification was checked by melting curve analysis and by agarose gel electrophoresis. All reactions were performed in duplicate with appropriate negative controls. The Ct values were determined by using Mx3000P software. Values were normalized for GAPDH mRNA.

Protein determination. Protein content was determined with the Pierce Chemical bicinchoninic acid protein assay reagent with bovine serum albumin as a standard.

Statistical analysis. The data were analyzed with Student’s t-test when comparing means between two groups. One-way ANOVA plus Dunnett’s post hoc multiple comparison test was used when comparing multiple groups. The ordinal values of the Jablonski scale renal injury score were analyzed by the Mann-Whitney nonparametric test. In all cases, a probability statistic <0.05 was taken to indicate significance. All data are expressed throughout the text as means ± SE.

RESULTS

Isoflurane protects against renal IR injury in mice. We determined in our preliminary studies that systemic arterial blood pressure did not differ significantly between 1.2% isoflurane- and pentobarbital sodium-anesthetized mice. The average systolic arterial pressures for the pentobarbital sodium-anesthetized group at 1, 2, and 3 h after induction of anesthesia were 132 ± 6, 136 ± 8, and 139 ± 6 mmHg (n = 3), respectively. The average systolic arterial pressures for the isoflurane-anesthetized group at 1, 2, and 3 h after induction of anesthesia were 132 ± 6, 136 ± 8, and 139 ± 6 mmHg (n = 3), respectively. Finally, we maintained the temperature (measured with an infrared temperature sensor during laparotomy and reperfusion under anesthesia) at 37°C at all times. The average temperatures for the isoflurane-anesthetized group at 1, 2, and 3 h after induction of anesthesia were 37 ± 1, 36 ± 1, and 36 ± 1°C (n = 4), respectively. The average temperatures for the pentobarbital sodium-anesthetized group at 1, 2, and 3 h after induction of anesthesia were 37 ± 1, 37 ± 1, and 36 ± 1°C (n = 4), respectively.

Twenty-four hours after renal IR injury, C57BL/6 mice anesthetized with pentobarbital sodium developed significant renal dysfunction indicated by a rise in plasma creatinine (Cr = 2.4 ± 0.2 mg/dl, n = 11, vs. sham 0.5 ± 0.0 mg/dl, n = 3, P < 0.01; Fig. 1A). Mice anesthetized with ketamine-xylazine also developed significant renal dysfunction (Cr = 3.1 ± 0.3 mg/dl, n = 3) indicating that the worsening of renal function was not a function of barbiturate anesthesia. In contrast, mice treated with 1.2% isoflurane (1 MAC) during renal ischemia and the first 3 h of reperfusion (Iso IR) had significantly lower plasma creatinine values compared with pentobarbital sodium-anesthetized mice (Cr = 1.3 ± 0.1, n = 6, P < 0.01; Fig. 1A).

The renal protection mediated by isoflurane was abrogated upon treatment with the SK inhibitors DMS (14, 16) (Cr = 2.7 ± 0.1 mg/dl, n = 6, P < 0.01 vs. Iso IR; Fig. 1B) and SKI-II (18) (Cr = 3.5 ± 0.1 mg/dl, n = 5, P < 0.01 vs. Iso IR; Fig. 1B), as well as the S1P1 receptor antagonist VPC23019 (13) (Cr = 2.6 ± 0.3 mg/dl, n = 7, P < 0.01 vs. Iso IR; Fig. 1B).

Fig. 1. Plasma creatinine values after ischemia-reperfusion (IR) injury. A: plasma creatinine (Cr; mg/dl; 24 h after ischemia) from sham-operated mice (Sham) and mice subjected to IR under pentobarbital sodium anesthesia (PB IR), ketamine-xylazine anesthesia (Ket-Xyl IR), or isoflurane anesthesia (Iso IR). B: plasma creatinine from mice subjected to IR under isoflurane anesthesia with the S1P1 receptor antagonist VPC23019 (Iso+VPC23019 IR) or the sphingosine kinase (SK) inhibitor N,N-dimethylsphingosine (Iso+DMS IR) and SKI-II (Iso+SKI-II IR), or under pentobarbital sodium anesthesia with VPC23019 (PB+VPC23019 IR), DMS (PB+DMS IR), or SKI-II (PB+SKI-II IR). C: plasma creatinine from SK1 knockout mice subjected to IR under isoflurane (SK1KO Iso IR) or pentobarbital sodium (SK1KO PB IR) anesthesia. #P < 0.01 vs. PB IR group. *P < 0.01 vs. Iso IR group. †P < 0.05 vs. Iso IR group. Error bars represent SE. Statistical analysis performed using 1-way ANOVA with Dunnett’s post hoc multiple comparison test.
from renal IR injury with isoflurane (SK1KO Iso IR; Cr background. Mice lacking SK1 enzyme were not protected previously (1) and they are congenically derived on a C57BL/6 strain of mice that were deficient in SK1 enzyme. The generation and characterization of these mice have been described (Fig. 2). The protective effects of isoflurane were not seen.

Isoflurane anesthesia reduces renal tubular necrosis. In Fig. 2, the renal-protective effects of isoflurane anesthesia are further supported by representative histological slides. Thirty minutes of renal ischemia followed by 24 h of reperfusion under pentobarbital sodium anesthesia resulted in significant renal injury as evidenced by severe tubular necrosis, medullary congestion and hemorrhage, and the development of proteinaceous casts (Fig. 2B). Isoflurane anesthesia significantly attenuated this drastic necrosis after IR injury (Fig. 2C). When the mice were treated with inhibitors of SK (DMS and SKI-II; Fig. 2, D and E) or an S1P1 receptor antagonist (VPC23019; Fig. 2F), the protective effects of isoflurane were not seen.

The Jablonski scale renal injury histology grading scores are shown in Fig. 3. Thirty minutes of renal ischemia under pentobarbital sodium anesthesia and 24 h of reperfusion resulted in severe acute tubular necrosis as demonstrated by the renal injury score (2.8 ± 0.4, n = 8). In contrast, renal injury was less severe with isoflurane anesthesia (0.8 ± 0.3, n = 4, P < 0.01 vs. pentobarbital sodium). Mice anesthetized with pentobarbital sodium without subsequent IR injury had normal renal histology as demonstrated by a renal injury score of 0.0 ± 0.0 (n = 5).

Isoflurane increases SK activity in vivo. Mice were anesthetized with 1.2% isoflurane or pentobarbital sodium for 3 h (without IR injury) and allowed to recover for 21 h and their renal cortices were extracted for SK activity measurement. Mice anesthetized with isoflurane demonstrated higher renal SK activity compared with those mice anesthetized with pentobarbital sodium (Fig. 4). Kidneys of isoflurane-anesthetized mice exhibited an increase in renal SK activity in the membrane fractions (1.17 ± 0.06-fold, n = 6, P < 0.05) compared with pentobarbital sodium-anesthetized mice (1.00 ± 0.05-fold, n = 6), while the cytosolic fractions did not exhibit such an increase (Fig. 4). The kidneys of mice were also collected immediately after exposure to isoflurane or pentobarbital sodium for 3 h (no recovery) but there were no differences in renal SK activity between the two treatment groups (data not shown).

Isoflurane increases SK activity in vitro. Treatment of HK-2 cells with 3–16 h of 2.5% isoflurane demonstrated a time-
dependent increase in SK activity (Fig. 5). When measured in whole cell lysate fractions, SK activity did not increase after 3 h of isoflurane exposure but increased 1.28 ± 0.06-fold \((n = 6, P < 0.01\) vs. control) and 1.37 ± 0.10-fold \((n = 6, P < 0.01\) vs. control) after 9 and 16 h of isoflurane exposure, respectively (Fig. 5A).

When SK activity was measured in HK-2 cells separated into membrane and cytosolic fractions, there was a time-dependent increase in SK activity in the membrane fraction (Fig. 5B). In the membrane fraction, there was no increase in SK activity after 3 h of isoflurane exposure but SK activity increased 1.19 ± 0.06-fold \((n = 6, P < 0.05\) vs. control) and 1.22 ± 0.07-fold \((n = 6, P < 0.01\) vs. control) after 9 and 16 h of isoflurane exposure, respectively (Fig. 5B). In the cytosolic fractions, there were no changes in SK activity after 3 and 9 h of isoflurane exposure but after 16 h of isoflurane exposure, there was a decrease to 0.90 ± 0.02-fold \((n = 6, P < 0.01\) vs. control; Fig. 5B).

**Isoflurane increases \([3H]S1P\) formation in HK-2 cells.** Consistent with our findings of increased SK activity with isoflurane exposure, HK-2 cells treated with 2.5% isoflurane for 6 h had higher cellular \([3H]S1P\) levels (1.45 ± 0.01-fold, \(n = 6, P < 0.01\) compared with control) (Fig. 6).

**Isoflurane increases synthesis of SK1 mRNA in mice.** We measured renal SK1 mRNA expression in kidneys of mice 21 h after being exposed to 2.5% isoflurane or 1.2% isoflurane to determine whether the increased SK activity levels were correlated with an increase in SK1 mRNA synthesis (Fig. 7A). We found that in mice exposed to isoflurane, renal SK1 mRNA expression increased 1.43 ± 0.10-fold \((n = 6, P < 0.01\) over mice exposed to pentobarbital sodium (1.00 ± 0.05-fold, \(n = 6\); Fig. 7B).

**Isoflurane increases SK1 mRNA synthesis in HK-2 cells.** In HK-2 cells, there was an increase in SK1 mRNA expression with increasing exposure to 2.5% isoflurane that peaked with...
mechanism dependent on activation of the SK as renal necrosis in a murine model of renal IR injury by a after renal ischemia, reduces the degree of renal failure as well concentration (1 MAC) of isoflurane, given both during and

as an in vitro model of cultured human proximal tubule cells mRNA transcription in both an in vivo murine model as well as an in vitro model of cultured human proximal tubule cells (HK-2).

DISCUSSION

The major finding of this study is that a clinically relevant concentration (1 MAC) of isoflurane, given both during and after renal ischemia, reduces the degree of renal failure as well as renal necrosis in a murine model of renal IR injury by a mechanism dependent on activation of the SK→S1P signaling pathway. In addition, isoflurane increases SK activity and SK1 mRNA transcription in both an in vivo murine model as well as an in vitro model of cultured human proximal tubule cells (HK-2).

We demonstrated that in rats, volatile anesthetics, including isoflurane, provided significant protection against renal IR injury with improved renal function, reduction in renal tubular necrosis, improved preservation of renal proximal tubular architecture, and inhibition of neutrophil influx (31). Subsequently, we showed that the volatile anesthetic sevoflurane reduced necrosis and inflammation in HK-2 cells (29). This in vitro protection was associated with activation of the prosurvival kinases ERK and Akt. Therefore, it is likely that volatile anesthetics have direct effects on renal tubules to reduce injury (necrosis and inflammation) after IR. However, the signaling mechanisms of volatile anesthetics before ERK and Akt activation are not known. Therefore, in this study, we aimed to determine the proximal signaling pathways involved in this volatile anesthetic-mediated renal protection.

In our current study, we showed that, consistent with our previous results in rats (31), the volatile anesthetic isoflurane protected mice against renal IR injury compared with pentobarbital sodium anesthesia, as demonstrated by reduction in plasma creatinine values as well as reduced necrosis. Pentobarbital sodium, ketamine, and isoflurane anesthesia at doses utilized in our study did not affect systemic blood pressure or renal blood flow in mice or rats (30, 31) making it unlikely that altered hemodynamics explain the differences between groups. In addition, pentobarbital sodium anesthesia did not worsen the degree of renal failure compared with ketamine-xylazine anesthesia, indicating that there was no specific effect of barbiturate anesthesia causing worsening of renal failure. The volatile anesthetic methoxyflurane has been shown to be nephrotoxic due to its metabolism to inorganic fluoride. However, isoflurane is minimally metabolized and has not been linked to fluoride nephrotoxicity in rats or humans (11).

We aimed to determine whether there was a role for SK/S1P signaling in mediating this protection. We found that administration of the SK inhibitors, DMS and SKI-II, reversed the isoflurane-mediated protection from renal IR injury. DMS is a well-known inhibitor of SK enzyme (14, 16), whereas SKI-II is a newly developed SK inhibitor with a higher potency and selectivity (18). We were able to demonstrate that SK-Ii inhibited SK activity in a concentration-dependent fashion in mouse kidney and HK-2 cell extracts (M. Kim and H. T. Lee et al., unpublished data). We also examined the role of S1P receptor blockade using the S1P receptor antagonist, VPC23019, which behaves as a competitive antagonist at both the S1P1 and S1P3 receptors, although it is 10-fold selective for S1P1 vs. S1P3 (13). Administration of VPC23019 was also associated with a reversal of isoflurane-mediated renal protection. In addition, we demonstrated that mice lacking SK1 enzyme were not protected against renal IR injury with isoflurane. Due to the inherent concerns regarding the use of genetic knockout mice (e.g., alterations in the expression of unrelated proteins), we used both pharmacological inhibitors as well as genetic knockout mice to study the role of SK in renal IR injury. Taken together, these data indicate that both increased S1P formation, via upregulation of SK1, as well as S1P1 receptor activation are involved in mediating the protective effects of isoflurane in renal IR injury.

Renal IR injury is an inflammatory process involving multiple cellular and systemic responses, including complement activation, activation of proinflammatory cytokines and chemokines, and infiltration by leukocytes such as neutrophils,
S1P, an important regulator of the sphingolipid rheostat. Many agents are known to stimulate SK activity, including agonists of growth factor receptors (e.g., PDGF, VEGF, NGF, and EGF), TGF-β, and TNF-α (21). Our study is the first to show that isoflurane activates SK. SK activation can occur via several different posttranslational mechanisms, such as phosphorylation, ubiquitination, and palmitoylation, and these effects are typically only seen for a few minutes following stimulation (21). SK activation lasting for longer periods of time has also been demonstrated, likely from upregulation of transcription, following PMA treatment in HEL cells (7) and estrogen treatment in MCF-7 breast cancer cells (51). An important aspect of SK activation is its translocation from the cytosol to the plasma membrane, as it is mainly a cytosolic protein while its substrate, sphingosine, is present in cell membranes (21, 44, 45).

In our study, HK-2 cells exposed to isoflurane exhibited increased SK activity at 9 and 16 h of volatile anesthetic exposure, but not at 3 h, suggesting that there is a time-dependent upregulation of SK transcription that is responsible for the increased SK activity seen after isoflurane exposure. In addition, the increase in SK activity was found in the plasma membrane fraction but not in the cytosolic fraction, suggesting that there is preferential localization of SK to the membrane where it can be in direct contact with its substrate. Our in vitro data are supported by our findings in mice showing that after 3 h of isoflurane exposure and 21 h of recovery, renal SK activity also increased in the membrane fraction relative to the cytosolic fraction. We also demonstrated that there was an increase in SK1 mRNA levels after isoflurane exposure both in vivo and in vitro.

One limitation of our study is that volatile anesthetics may have both local and systemic effects in renal IR injury, so it is difficult to determine in an in vivo study whether volatile anesthetics affect the release of S1P into the circulation.

Two recent studies demonstrated the role of the S1P₁ receptor in mediating protection from renal IR injury and this was associated with lymphopenia as well as the inhibition of the proinflammatory cytokines TNF-α, P-selectin, and ICAM-1 (32) along with reduced vascular permeability (4). These results are consistent with our findings that S1P₁ receptor antagonism abrogates the renal protection mediated by isoflurane, although the exact mechanisms have yet to be elucidated.

SK, the enzyme catalyzing the conversion of sphingosine to S1P, is an important regulator of the sphingolipid rheostat.
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GRANTS

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