J Anesth (2004) 18:203–209
DOI 10.1007/s00540-004-0237-5

Journal of Anesthesia



# The volatile anesthetics halothane and isoflurane differentially modulate proinflammatory cytokine-induced p38 mitogen-activated protein kinase activation

Tatsuya Itoh[1], Kiichi Hirota[1,2], Taizo Hisano[1], Tsunehisa Namba[1], and Kazuhiko Fukuda[1]

[1] Department of Anesthesia, Kyoto University Hospital, Kyoto University, Kyoto, Japan
[2] Department of Anesthesia, The Tazuke Kofukai Medical Research Institute Kitano Hospital, 2-4-20 Ohgimachi, Kita-ku, Osaka 530-8480, Japan

**Abstract**
*Purpose.* Volatile anesthetics affect the cardiovascular and immune systems. Toward a better understanding of the molecular mechanisms behind the modulation exerted by these agents, we focused on the effects of halothane and isoflurane on the activation of p38 mitogen-activated protein kinase (MAPK), which plays a critical role in the cellular responses to extracellular stimuli such as lipopolysaccharide (LPS) and proinflammatory cytokines, including tumor necrosis factor (TNF) and interleukin 1 (IL-1).
*Methods.* Human umbilical vein endothelial cells and HeLa cells, an established cell line, were examined by molecular biological methods. Cells were treated with proinflammatory compounds with or without the volatile anesthetics. p38 MAPK activation was investigated by Western blotting analysis with phosphospecific anti-p38 MAPK antibodies.
*Results.* Isoflurane activated p38 MAPK by itself, but halothane did not. Halothane or isoflurane augmented the LPS- and TNF-induced activation of p38 MAPK. In contrast, neither halothane nor isoflurane enhanced the p38 MAPK activation induced by IL-1. Neither of the anesthetics affected $H_2O_2$- or MAPK kinase 3 (MKK3)-induced p38 MAPK activation.
*Conclusion.* Our in vitro results indicate that the volatile anesthetics used in the clinical field and in animal experiments modify the p38 MAPK signaling cascade and suggest that the target molecules of the anesthetics are not unique and the anesthetics regulate them differentially at clinically relevant doses.

**Key words** Halothane · Isoflurane · Inflammatory cytokines · LPS · P38 MAPK

## Introduction

Volatile anesthetics are among the most commonly used drugs for clinical anesthesia. There has been an explosion of studies over the past several decades about how and where volatile anesthetics act [1]. A large number of studies have demonstrated that volatile anesthetics directly affect ligand-gated ion channels, such as glutamate receptors, nicotinic acetylcholine receptors, and $GABA_A$ receptors; and voltage-gated ion channels, such as $Na^+$ channels, $K^+$ channels, and $Ca^{2+}$ channels [2,3]. In addition to their hypnotic effects on the central nervous system, volatile anesthetics have been shown to affect the cardiovascular and immune systems; modulating vascular tone, cardiac contraction, and the production of cytokines, and all of these effects cannot be explained by modulation of the channels by anesthetics. Not only ion channels in the cell membrane but also intracellular proteins, such as protein kinase C [4], have been shown to be target molecules of anesthetics. However, so far, few studies have investigated the effects of the volatile anesthetics on intracellular signaling pathways using cultured cells and molecular biological methods.

Many of the signal transduction pathways from the cell surface to the nucleus have been elucidated. Among them, mitogen-activated protein kinase (MAPK) cascades are pivotal in mediating cellular reactions to extracellular stimuli [5–7]. Three distinct subtypes of MAPK have been identified. The "classical" MAPK, extracellular signal-related kinases, are usually activated by stimuli that induce cell growth and proliferation [5]. On the other hand, c-Jun N-terminal kinase/stress-activated protein kinase and p38 MAPK are activated by extracellular stresses such as the inflammatory cytokines tumor necrosis factor (TNF) and interleukin 1 (IL-1), and toxins such as lipopolysaccharide (LPS), resulting in the modulation of the cardiovascular and immune systems through regulation of the downstream

*Address correspondence to*: K. Hirota
Received: September 8, 2003 / Accepted: February 13, 2004

gene expressions of *IL-6*, *IL-8*, vascular endothelial growth factor, and inducible nitric oxide synthase [6–8]. We and others have shown that μ opioid agonists, including morphine and fentanyl, activate MAPKs [9,10]. In addition, there is a report which provides evidence that certain local anesthetics modulate G-protein-coupled receptor-mediated MAPK activation, although the details of the mechanism have not been elucidated yet [11]. However, there have been no studies of the effects of volatile anesthetics on MAPK activity elicited by proinflammatory cytokines and reagents. In this study, we focused on the signaling pathways from proinflammatory substances to p38 MAPK activation, using human umbilical vein endothelial cells (HUVECs) and HeLa cells, an established cell line. We demonstrated that two widely used volatile anesthetics, halothane and isoflurane, differentially modulated the activation of p38 MAPK induced by LPS, TNFα, or IL-1β.

## Materials and methods

### *Reagents*

Anti-p38 MAPK antibody (C-20) (rabbit IgG) and anti-$Thr^{180}/Tyr^{182}$ phosphorylated-p38 MAPK antibody (rabbit IgG) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and New England Biolabs (Beverly, MA, USA), respectively. Horseradish peroxidase (HRP)-conjugated anti-rabbit IgG antibody and an Enhanced Chemiluminescence Plus (ECL + Plus) kit was obtained from Amersham Biosciences (Piscataway, NJ, USA). CS-C complete medium was obtained from Cell Systems (Kirkland, WA, USA). Recombinant human TNFα and IL-1β were obtained from Roche Diagnostics (Basel, Switzerland). LPS (serotype 055:B5) was obtained from Sigma (St. Louis, MO, USA). Halothane and isoflurane were obtained from Takeda Chemical Industries (Osaka, Japan) and Dainabott (Tokyo, Japan), respectively. Other chemicals were obtained from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical (Osaka, Japan). An expression plasmid of MKK3 (MAPK kinase 3), pSRαMKK3 [12], was kindly provided by Dr. Eisuke Nishida (Kyoto University).

### *Cell culture*

Human umbilical vein endothelial cells (HUVECs) were obtained from Kurabo (Osaka, Japan) and cultured in CS-C complete medium on plastic dishes coated with 0.5% gelatin at 37°C under a humidified atmosphere of 5% $CO_2$. Cells between passages 1 and 5 were seeded in CS-C complete medium onto six-well plates, at a density of $3 \times 10^5$ per well, and used for the experiments. HeLa cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics (50 $U \cdot ml^{-1}$ penicillin and 100 $\mu g \cdot ml^{-1}$ streptomycin) at 37°C under a humidified atmosphere of 5% $CO_2$.

### *Application of volatile anesthetics*

For experiments, cells were maintained in a multigas incubator (APMW-36; Astec Tokyo, Japan). Each volatile anesthetic was delivered through an agent-specific vaporizer with a carrier gas; 5% carbon dioxide and 95% air mixture. Gas concentration in the incubator was monitored with an anesthetic gas analyzer (Type 1304; Blüel & Kjær, Nærum, Denmark) that was calibrated before each study with a commercial standard gas ($O_2$, 47%; $CO_2$, 5.6%; $N_2O$, 47%; sulfur hexafluoride, 2.05%). The anesthetic concentration in CS-C complete medium was measured by gas chromatography (5890A; Hewlett Packard, Palo Alto, CA, USA), as previously described [13]. Preliminary experiments using gas chromatography showed that 30 min was sufficient to ensure equilibrium of the CS-C complete medium by the anesthetics (data not shown).

### *Western blot analysis*

After treatments, cells were lysed with a buffer (5 mM Hepes; 250 mM NaCl, 10% ([v/v]) glycerol, 0.5% Nonidet P-40 [v/v], 100 μM $Va_3VO_4$ supplemented with Complete protease inhibitor cocktail (Roche Diagnostics). Aliquots 50 μM of whole cell lysate were separated on 12% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. Western blotting analysis was performed following a protocol previously described [14,15]. Briefly, the membrane was incubated with a primary antibody, rabbit anti-phospho-p38 MAPK antibody (dilution, 1:1000). The membrane was then washed three times in Tris-buffered saline Tween 20 (20 mM, Tris-HCl, pH 7.6; 137 mM NaCl; 0.1% Tween 20), and incubated with HRP-conjugated anti-rabbit IgG antibody (dilution, 1:2000). The blots were developed using the ECL + Plus kit (Amersham Biosciences) according to the manufacturer's instructions. In order to normalize any variation in the amount of p38 MAPK loaded in each lane, the same membranes were reblotted with anti-p38 MAPK antibody (C-20, 1:2000).

### *Transfection*

HeLa cells were transfected with pSRα-MKK3, using Fugene 6 reagent (Roche Diagnostics) following a protocol provided by the company. Twelve hours after transfection, HeLa cells were serum-starved for 12 h in the incubator with or without volatile anesthetics. Then

they were harvested and subjected to Western blot analysis.

*Densitometry analysis*

The amount of phospho-p38 MAPK or p38 MAPK in each sample was estimated by analysis of the density of each band, using a computerized densitometer [16]. The intensities of phospho-p38 MAPK-bands were normalized to the corresponding intensities of p38 MAPK-bands. Values for results were expressed as means ± SD in bar graphs. Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by Bonferroni's test; $P < 0.05$ was accepted as statistically significant.

## Results

*Activation of p38 MAPK by LPS, TNFα, and IL-1β in HUVECs*

p38 MAPK is a serine/threonine kinase which is activated in response to phosphorylation by MAPK kinases such as MKK3 and MKK6. The activity of p38 MAPK is determined by the phosphorylation status of specific amino-acid residues, and the phosphorylation status can be determined by using specific antibodies raised against phosphorylated peptides derived from p38 MAPK. In HUVECs, treatment with LPS (100 ng·ml$^{-1}$), TNFα (10 ng·ml$^{-1}$), or IL-1β (200 pg·ml$^{-1}$) for 20 min induced phosphorylation of p38 MAPK (Fig. 1A). Densitometry study showed that LPS, TNF, and IL-1 enhanced p38 phosphorylation by 2.7-, 5.8-, and 3.1-fold compared to the control, respectively (Fig. 1B).

*Differential activation of p38 MAPK by halothane and isoflurane in HUVECs*

We next examined the effects of the volatile anesthetics on p38 MAPK activation in HUVECs. HUVECs were treated for 1 h with the anesthetics in the incubator in which the concentration of anesthetics was monitored. As shown in Fig. 2, any concentration of halothane tested caused only marginal activation of p38 MAPK. In contrast, isoflurane showed statistically significant activation of p38 MAPK in a concentration-dependent manner. Thus, halothane and isoflurane show different properties in the context of the regulation of p38 MAPK phosphorylation.

*p38 MAPK activation by proinflammatory reagents in HUVEC is influenced by halothane and isoflurane*

We examined the effects of volatile anesthetics on p38 MAPK activation induced by proinflammatory substances; LPS (100 ng·ml$^{-1}$), TNFα (10 ng·ml$^{-1}$), or IL-1β (200 pg·ml$^{-1}$). HUVECs were pretreated with the anesthetics for 30 min and were then treated with the proinflammatory substances for 20 min with the anesthetics. Both halothane and isoflurane augmented LPS-induced p38 MAPK phosphorylation in a dose-dependent manner (Fig. 3A). Studies with densitometry demonstrated that the effect of isoflurane was stronger than that of halothane at concentrations of 1% (1.2- vs 2.7-fold), 2% (1.3- vs 4.3-fold), and 4% (1.9- vs

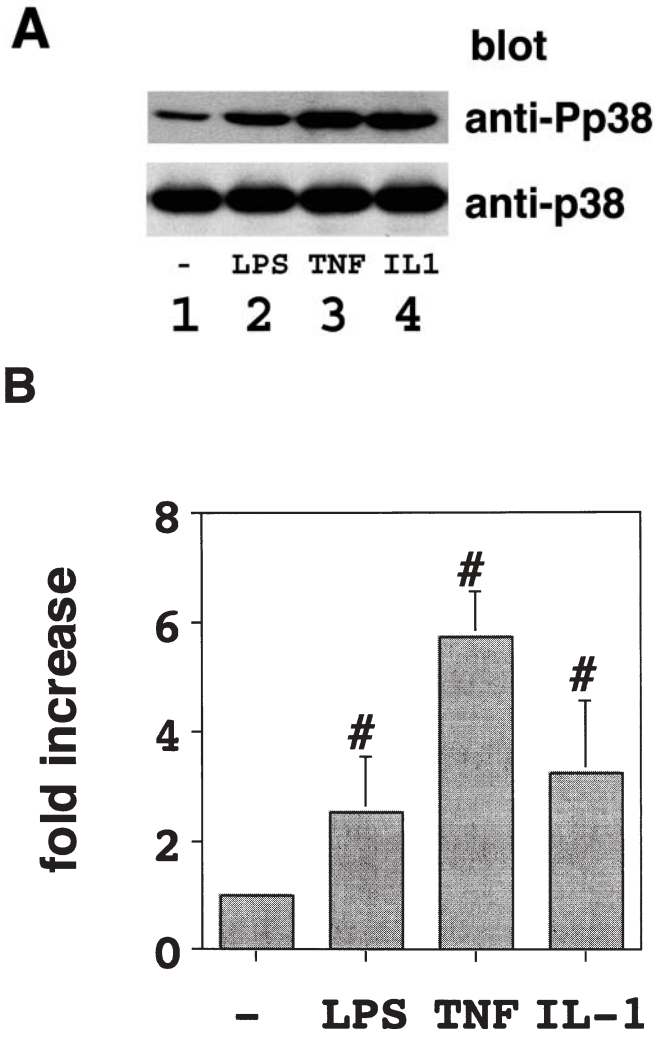


**Fig. 1A,B.** Activation of p38 mitogen-activated protein kinase (MAPK) by lipopolysaccharide (*LPS*), tumor necrosis factor (*TNF*)α, and interleukin 1 (*IL-1β*) in human umbilical vein endothelial cells (HUVECs). HUVECs were incubated with LPS (100 ng·ml$^{-1}$), TNFα (10 ng·ml$^{-1}$), or IL-1β (200 pg·ml$^{-1}$) for 20 min at 37°C. After the incubation, the cells were lysed and the lysates were subjected to Western blot analysis. A representative Western blot (**A**) and normalized phospho-p38 MAPK protein levels from three independent experiments (**B**) are presented, as described in "Materials and methods". Values are means ± SD of the three independent experiments. $^{\#}P < 0.05$ vs control

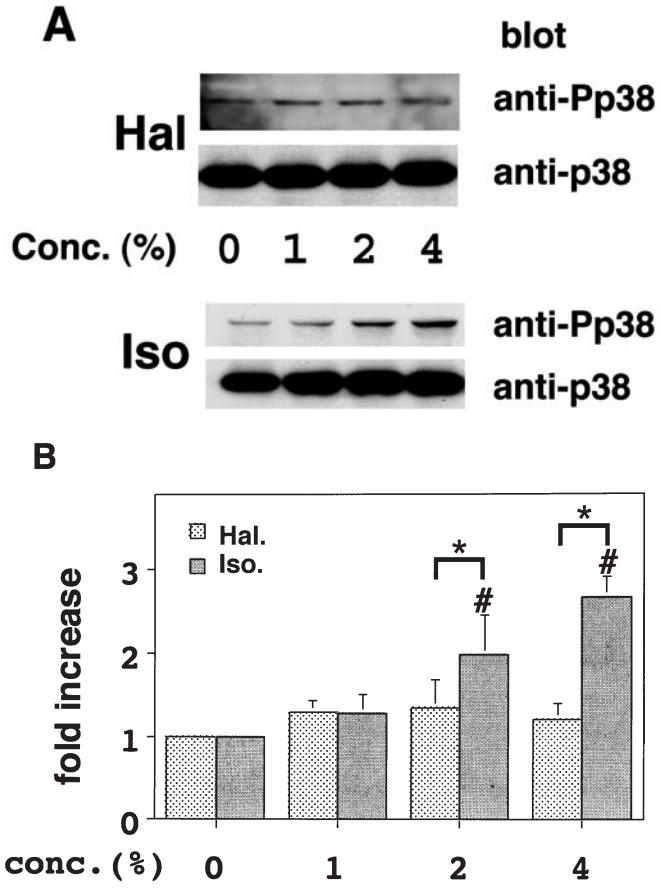


**Fig. 2A,B.** Activation of p38 MAPK by halothane (*Hal*) and isoflurane (*Iso*) in HUVECs. HUVECs were incubated with halothane or isoflurane for 1 h at 37°C. After the incubation, the cells were lysed and the lysates were subjected to Western blot analysis. A representative Western blot (**A**) and normalized phospho-p38 MAPK protein levels (**B**) are presented, as described in "Materials and methods". Values in *bar graphs* are expressed as fold increases from control. Values are means ± SD of three independent experiments. $^{\#}P < 0.05$ vs control: $^{*}P < 0.05$ between anesthetics

5.0-fold). TNF-induced p38 MAPK phosphorylation was also enhanced by treatment with the anesthetics at the concentrations tested (Fig. 3B). Notably, in contrast to the case of LPS, we failed to detect any significant difference between the anesthetics (1%, 1.3 vs 1.2-fold; 2%, 2.8- vs 2.3-fold; 4%, 3.5- vs 3.0-fold). We next examined the effects of the anesthetics on IL-1-induced p38 MAPK activation. As shown in Fig. 3C, these results were completely different from those for LPS and TNF. Neither halothane nor isoflurane enhanced IL-1-induced p38 MAPK phosphorylation. By contrast, 4% halothane showed a statistically significant inhibitory effect.

### *$H_2O_2$-induced or MKK3-induced p38 MAPK activation is not sensitive to halothane or isoflurane*

In order to elucidate site(s) of action of the anesthetics, we adopted molecular biological methodology. HeLa cells (derived from human uterine cervical carcinoma) were tested. p38 MAPK activation in HeLa cells was affected by the volatile anesthetics in a manner similar to that in HUVECs (data not shown). Reactive oxygen species (ROS), especially hydrogen peroxide ($H_2O_2$), act as essential signaling intermediates in the proinflammatory cytokine-induced p38 MAPK activation pathway [17,18]. $H_2O_2$ activates the MAPKK Kinase (MAPKKK) apoptosis signal-regulating kinase (ASK1), resulting in the activation of downstream signaling cascades [17–19]. As shown in Fig. 4A, $H_2O_2$ induced p38 MAPK activation (lane 2). Neither of the anesthetics affected the activation induced by $H_2O_2$ treatment (lanes 3 and 4). HeLa cells were transfected with an expression vector of the MAPK kinase MKK3 and treated with or without 2% halothane and isoflurane. Neither halothane nor isoflurane modulated

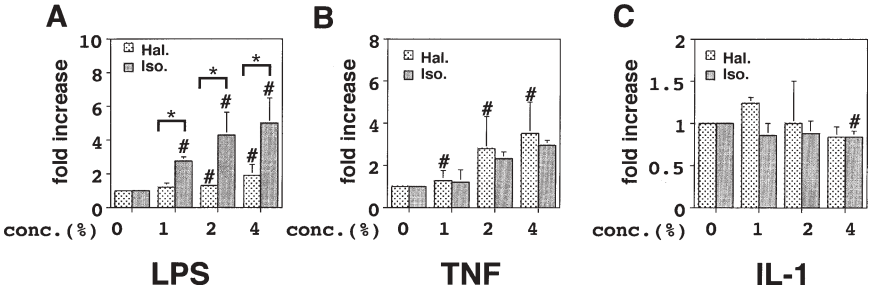


**Fig. 3A–C.** Effects of the anesthetics on cytokine-induced p38 MAPK activation in HUVECs. HUVECs were incubated with halothane or isoflurane for 1 h at 37°C. After 1-h incubation, the cells were further treated with LPS (100 ng·ml$^{-1}$); (**A**), TNFα (10 ng·ml$^{-1}$); (**B**), or IL-1β (200 pg·ml$^{-1}$); (**C**) for 20 min with the anesthetics. After the treatment, the cells were lysed and subjected to Western blot analysis. The *graphs* represent the phospho-p38 MAPK protein level normalized as described in "Materials and methods". Values in *bar graphs* are expressed as fold increases from control (0% anesthetics with cytokine/LPS treatment). Values are means ± SD of three independent experiments. $^{\#}P < 0.05$ vs control; $^{*}P < 0.05$ between anesthetics

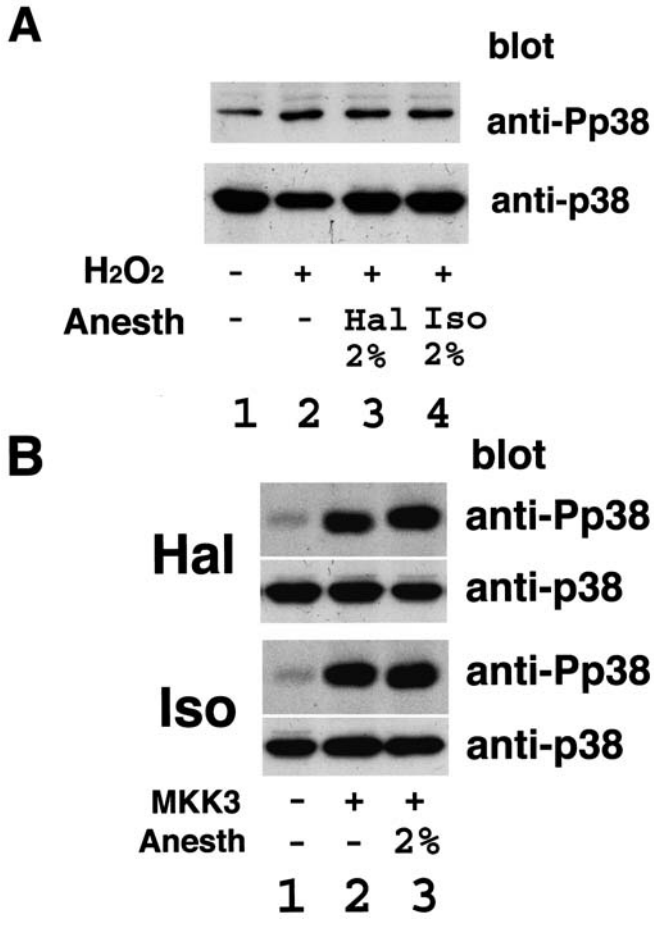


**Fig. 4A,B.** Effects of the anesthetics on $H_2O_2$- or MKK3-induced p38 MAPK activation in HeLa cells. **A** HeLa cells that had been serum-starved for 12 h were incubated without (*lanes 1 and 2*) or with halothane (*lane 3*) or isoflurane (*lane 4*) for 1 h at 37°C, and were then treated with $H_2O_2$ (200 μM) for 20 min. $H_2O_2$ 200 mM was added directly to the culture media. Cells were harvested and the lysates were subjected to Western Blot analysis. **B** HeLa cells were transfected with 1 μg of pSRα-HA-MKK3 (*lanes 2 and 3*) or pSRα-HA plasmid (*lane 1*). After 24 h, cells were serum-starved for 12 h and then treated with (*lane 3*) or without (*lanes 1 and 2*) the anesthetics for 1 h. The lysates were subjected to Western Blot analysis. *MKK3*, MAPK Kinase 3

MKK3-induced p38 MAPK phosphorylation (Fig. 4B, lane 3). Together, these lines of evidence suggest that the target(s) of the anesthetics are located upstream of ROS, as signaling intermediates.

## Discussion

Considerable progress has been made in identifying the upstream kinases and phosphatases that regulate each member of the MAPK family [5]. Elaborate balances between kinases and phosphatases determine the activation status of MAPKs. All signalling intermediates, including cytokine receptors, receptor-associated molecules, MAPKs, MAPK kinases (MAPKKs), MAPKK kinases (MAPKKKs), and corresponding phosphatases are potential targets of the anesthetics (Fig. 5). Receptors for TNFα, IL-1β, and LPS are in the same class of membrane proteins, and a variety of receptor-associated molecules which are recruited to the receptors have been identified. Among these components, members of the TNF receptor-associated factor (TRAF) family, are essential for the signalling pathway which leads to MAPK activation. The signals of TNFα and IL-1β are transduced to TRAF2 and TRAF6, respectively [20,21]. The LPS signal is transduced to TRAF6 via Toll-Like receptor and IL-1 receptor-associated kinase [22]. Downstream signalling pathways distal to TRAF2 and TRAF6 converge to MAPKKs; MKK3 and MKK6 via certain MAPKKKs, such as ASK1 in the case of TNF-α, and transforming growth factor-β-activated kinase 1 (TAK1) in the case of LPS and IL-1β [20,21,23]. As shown clearly in Fig. 2, halothane and isoflurane have different properties in the regulation of the p38 MAPK activation process. These results strongly suggest that the sensitivies of intermediate signalling molecules to the anesthetics are different from each other. Another important finding in this study is that p38 MAPK activation by LPS, TNF, and IL-1 was differentially regulated by the anesthetics (Fig. 3). Although TNFα, IL-1β, and LPS utilize a very similar intracellular signal transduction mechanism, the sensitivity of each intermediate of the cytokine signals to the individual anesthetics does not seem to be identical. Notably, halothane, which has no significant effect on p38 MAPK activation, is a more potent enhancer of LPS- and TNF-induced activation than isoflurane. Moreover, our data using an established cell line derived from human cervical carcinoma, HeLa cells, and an expression plasmid of MKK3, suggest that MKK3 is not a direct target of the anesthetics. Taken together, the evidence suggests that the target molecules of the anesthetics are not unique, and that the anesthetics regulate them differentially. In this study, we treated cells with the anesthetics for rather short periods. In the clinical setting, human bodies are usually exposed to anesthetics for longer periods than we used here, and longer exposure may elicit a different type of reaction. Further studies are needed from the point of view of time.

Based on the medium-gas partition coefficient of the anesthetics obtained in this study and the previously reported values for the blood-gas partition coeffecient, the concentration of the anesthetics we used would be as high as the clinical dose. As p38 MAPK is expressed ubiquitously in the body, including in lymphocytes and neuronal cells, the modulatory effect of anesthetics may be observed in various organs and tissues. In surgi-

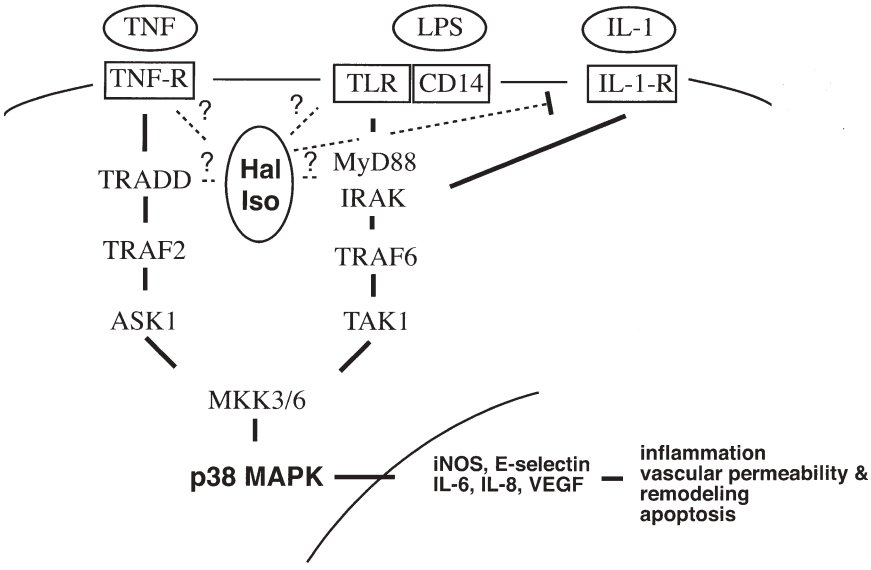


**Fig. 5.** Schematic representation of signalling pathway from LPS, TNF, and IL-1 receptors to p38 MAPK. LPS, TNF, and IL-1 induce p38 MAPK activation and subsequent gene expression via their own specific receptors. Intracellular signal transduction is executed by certain pathway-specific intermediates. Some intermediates are shared by different signalling systems. Finally, the signals converge into MKK3 or MKK6 and result in p38 MAPK activation. *TLR*, toll-like receptor; *TRADD*, TNF receptor (*TNF-R*)1-associated death domain protein; *IRAK*, IL-1 receptor(*R*)-associated kinase. *iNOS*, inducible nitric oxide synthase; *VEGF*, vascular endothelial growth factor; *TRAF*, TNF receptor-associated factor; *ASK1*, MAPKK kinase apoptosis signal-regulating kinase; *TAK1*, transforming growth factor-β-activated kinase. See text for further details

cal operations and in the septic state, inflammatory cytokines and endotoxins from bacteria insult vital organs, including the heart, liver, and lungs [24]. In particular, the endothelia of blood vessels are highly susceptible to these stimuli. Adhesion molecules, such as E-selectin and intercellular adhesion molecule (ICAM); and growth factors, such as vascular endothelial growth factor (VEGF) and inducible nitric oxide synthase (iNOS), are induced by TNF, IL-1, and/or LPS, and their gene products determine vascular tone, permeability, apoptosis, and the remodeling of vessels [25]. A series of studies have elucidated the involvement of p38 MAPK in these processes [26,27]. In this study, we presented not all but at least part of the molecular basis for these biological phenomena. In order to understand better the biological significance of the effect of volatile anesthetics on p38 MAPK activation, further studies using cells from various tissues and whole animals are required.

*Acknowledgments.* We thank Dr. Gotaro Shirakami for his critical reading of the manuscript, Dr. Patrick Connor Mahon for correcting the English, Dr. Kenjiro Mori for encouragement, Mrs. Kanako Murata for technical assistance, and Miss Etsuko Kobayashi for secretarial help. This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (to K.H. and K.F.).

## References

1. Kobin DD (1994) Mechanism of action. In: Millar RD (ed) Anesthesia, 4th edn. Churchill Livingstone, New York, pp 67–99
2. Franks NP, Lieb WR (1998) Which molecular targets are most relevant to general anesthesia? Toxicol Lett 100:1–8
3. Campagna JA, Miller KW, Forman SA (2003) Mechanisms of actions of inhaled anesthetics. New Engl J Med 348:2110–2124
4. Slater S, Cox K, Lombardi J, Ho C, Kelly M, Rubin E, Stubbs C (1993) Inhibition of protein kinase C by alcohols and anaesthetics. Nature 364:82–84
5. Widmann C, Gibson S, Jarpe MB, Johnson GL (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol Rev 79:143–180
6. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, Davis RJ (1995) Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation of tyrosine and threonine. J Biol Chem 270:7420–7426
7. Sanghera JS, Weinstein SL, Aluwalia M, Girn J, Pelech SL (1996) Activation of multiple proline-directed kinases by bacterial lipopolysaccharide in murine macrophages. J Immunol 156:4457–4465
8. Suzuki M, Tetsuka T, Yoshida S, Watanabe N, Kobayashi M, Matsui N, Okamoto T (2000) The role of p38 mitogen-activated protein kinase in IL-6 and IL-8 production from the TNF-α- or IL-1β-stimulated rheumatoid synovial fibroblasts. FEBS Lett 467:23–27
9. Fukuda K, Kato S, Morikawa H, Shoda T, Mori K (1996) Functional coupling of the delta-, mu-, and kappa-opioid receptors to mitogen-activated protein kinase and arachidonate release in Chinese hamster ovary cells. J Neurochem 67:1309–1316

10. Gutstein HB, Rubie EA, Mansour A, Akil H, Woodgett JR (1997) Opioid effects on mitogen-activated protein kinase signaling cascades. Anesthesiology 87:1118–1126
11. Tan Z, Dohi S, Ohguchi K, Nakashima S, Nozawa Y (1999) Local anesthetics inhibit muscarinic receptor-mediated activation of extracellular signal-regulated kinases in rat pheochromocytoma PC12 cells. Anesthesiology 91:1014–1024
12. Moriguchi T, Toyoshima F, Gotoh Y, Iwamatsu A, Irie K, Mori E, Kuroyanagi N, Hagiwara M, Matsumoto K, Nishida E (1996) Purification and identification of a major activator for p38 from osmotically shocked cells. Activation of mitogen-activated protein kinase kinase 6 by osmotic shock, tumor necrosis factor-α, and $H_2O_2$. J Biol Chem 271:26981–26988
13. Namba T, Ishii TM, Ikeda M, Hisano T, Itoh T, Hirota K, Adelman JP, Fukuda K (2000) Inhibition of the human intermediate conductance Ca(2+)-activated K(+) channel, hIK1, by volatile anesthetics. Eur J Pharmacol 395:95–101
14. Hirota K, Murata M, Sachi Y, Nakamura H, Takeuchi J, Mori K, Yodoi J (1999) Distinct roles of thioredoxin in the cytoplasm and in the nucleus: a two step mechanism of redox regulation of transcription factor NF-κB. J Biol Chem 274:27891–27897
15. Itoh T, Namba T, Kazuhiko F, Semenza LG, Hirota K (2001) Reversible inhibition of hypoxia-inducible factor 1 activation by exposure of hypoxic cells to the volatile anesthetic halothane. FEBS Lett 509:225–229
16. Hirota K, Nakamura H, Arai T, Ishii H, Bai J, Itoh T, Fukuda K, Yodoi J (2000) Geranylgeranylacetone enhances expression of thioredoxin and suppresses ethanol-induced cytotoxicity in cultured hepatocytes. Biochem Biophys Res Commun 275:825–830
17. Nishitoh H, Saitoh M, Mochida Y, Takeda K, Nakano H, Rothe M, Miyazono K, Ichijo H (1998) ASK1 is essential for JNK/SAPK activation by TRAF2. Mol Cell 2:389–395
18. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H (1998) Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 17:2596–2606
19. Ichijo H, Nishida E, Irie K, ten-Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, Gotoh Y (1997) Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science 275:90–94
20. Wong BR, Josien R, Lee SY, Vologodskaia M, Steinman RM, Choi Y (1998) The TRAF family of signal transducers mediates NF-κB activation by the TRANCE receptor. J Biol Chem 273:28355–28359
21. Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV (1996) TRAF6 is a signal transducer for interleukin-1. Nature 383:443–446
22. Zhang G, Ghosh S (2001) Toll-like receptor-mediated NF-κB activation: a phylogenetically conserved paradigm in innate immunity. J Clin Invest 107:13–19
23. Ichijo H (1999) From receptors to stress-activated MAP kinase. Oncogene 18:6087–6093
24. McBride WT, Armstrong MA, McBride SJ (1996) Immunomodulation: an important concept in modern anaesthesia. Anaesthesia 51:465–473
25. Baldwin AS (2001) The transcription factor NF-κB and human disease. J Clin Invest 107:3–6
26. Da-Silva J, Pierrat B, Mary JL, Lesslauer W (1997) Blockade of p38 mitogen-activated protein kinase pathway inhibits inducible nitric-oxide synthase expression in mouse astrocytes. J Biol Chem 272:28373–28380
27. Han J, Jiang Y, Li Z, Kravchenko VV, Ulevitch RJ (1997) Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. Nature 386:296–299