Original Article

Impairment of Thermal Tolerance in Heat-Shocked SH-SY5Y Cells by a High Dose of Sevoflurane

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Abstract

Background: Sevoflurane has a cytoprotective effect, while the heat shock 70-kDa proteins 1A and 1B（HSPA1A/B）play major roles in the restoration of denatured proteins. The purpose of this study was to disclose the effects of sevoflurane on the expression and cytoprotective effect of HSPA1A/B.

Methods: Human SH-SY5Y neuroblastoma cells were randomly assigned to 8 groups. Cells of the control group were incubated under a normal condition. Cells of the S1, S2, and S5 groups were exposed to 1.25%, 2.5%, and 5% sevoflurane, respectively. Cells of the H group were incubated under heat shock（HS）at 42℃. Cells of the S1H, S2H, and S5H groups were exposed to 1.25%, 2.5%, and 5% sevoflurane under HS, respectively. Each group was challenged for 1 h and then incubated for an additional 2 or 4h. Immunoblots and real-time polymerase chain reactions were conducted to determine the expression of HSPA1A/B and their mRNAs. Finally, each group was exposed to an additional lethal HS（44℃, 1 h）, and cytotoxicity was evaluated after additional incubation（16h）.

Results: HSPA1A/B and their mRNAs were significantly increased in the H group and the S1H group compared with their levels in the control group. In the cytotoxicity assay, the H group and S1H group showed significantly lower cytotoxicity than the control group.

Conclusion: Sevoflurane dose-dependently attenuated the HS-induced expression of HSPA1A/B and its mRNAs, and also impaired cytoprotection against lethal HS.

（Key words: sevoflurane, heat stress, chaperone, preconditioning, malignant hyperthermia）

Introduction

Proteins are denatured, and cellular homeostasis is critically disrupted, by physical stressors such as heat shock. Chaperones assist with the conversion of newly synthesized proteins to maturity and protect cells from stress by isolating them from the denatured proteins and normalizing them.[1,2] The heat shock protein 70（HSP70） family is highly conserved from bacteria to humans.[3] Within the HSP70 family, heat shock 70-kDa proteins 1A and 1B（HSPA1A and HSPA1B, respectively） are well-known as inducible 70-kDa heat shock proteins and also have many aliases, such as HSP70i, HSP70, HSP70-1 and 2, and so

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on.[2,4,5] Preconditioning is generally induced by previous stress, including heat shock, and gives cells the ability to survive subsequent lethal stress. Late preconditioning develops several hours after the stress and evidently correlates with the regulation of cell-protective genes such as HSP70.[6-8]

Sevoflurane, a volatile general anesthetic drug, regulates the expression of cell-protective genes, including chaperones,[8-10] whereas the influence of sevoflurane on HSPA1A and HSPA1B (HSPA1A/B) expression has not been fully elucidated. In addition to heat shock, other stressors have also been shown to induce HSP70.[2,5] Therefore, research on the influence of sevoflurane on HSPA1A/B expression is important in order to clarify the contribution made by sevoflurane to the cytoprotection against various stressors. This study was conducted to elucidate the influence of sevoflurane on the cytoprotection conferred by HSPA1A/B when cells are simultaneously exposed to heat shock and sevoflurane. In this study, the human neuron-like SH-SY5Y neuroblastoma cell line was used to assess whether neuroprotection against heat stress was related to expression of HSPA1A/B and administration of sevoflurane.

Materials and methods

I. Preparation of cultured cells

SH-SY5Y cells were cultured in high glucose Dulbecco's Modified Eagle's Medium (D-MEM; Wako Pure Chemical, Osaka, Japan) that contained 100 U/ml penicillin (GIBCO/Invitrogen, Tokyo, Japan), 100 μg/ml streptomycin (GIBCO/Invitrogen), and 15% heat-inactivated fetal bovine serum (Japan Bioserum, Tokyo, Japan). D-MEM was replaced with CO_2-Independent Medium (GIBCO/Invitrogen) before the following experiments, and the cells were separately placed inside airtight containers (AnaeroPack; Mitsubishi Gas Chemical, Tokyo, Japan) with a thermometer, cotton, and a gel pack to maintain temperature. If required, liquid sevoflurane (Sevofrane; Maruishi Pharmaceutical, Osaka, Japan) was dripped on the cotton, a 20-mL syringe was connected to the sampling port, and a plunger was pulled and pushed ten times to vaporize the liquid sevoflurane.

II. Time course of HSPA1A/B expression after administration of sevoflurane and/or heat shock

These preliminary experiments were performed to evaluate the time point of maximum HSPA1A/B expression, and whether the time point was shifted by administration of sevoflurane. SH-SY5Y cells were challenged by various concentrations of sevoflurane and/or heat shock at 42℃ for 1 h, as shown by the graph in Fig. 1. The containers were opened after the challenge, and the medium was replaced with D-MEM. The cells were additionally incubated under a normal condition for 2h, 4h, 8h, 16h, and 24h, respectively. SH-SY5Y cells under normal conditions served as a control. HSPA1A/B was detected by immunoblot analysis as described below.

III. Analysis of the effect of sevoflurane on HSPA1A/B expression and cytoprotection

These experiments were performed in order to compare the maximum amounts of HSPA1A/B proteins and mRNAs, and whether tolerance to lethal heat shock was changed by the administration of sevoflurane. Other SH-SY5Y cells were randomly assigned to 8 groups and challenged for 1 h as follows. Cells of group C were incubated under a normal condition as a control; group S1 cells were exposed to 1.25% sevoflurane; group S2 cells were exposed to 2.5% sevoflurane; group S5 cells were exposed to 5%

sevoflurane; group H cells were exposed to heat shock at 42℃ ; group S1H cells were exposed to 1.25% sevoflurane and heat shock; group S2H cells were exposed to 2.5% sevoflurane and heat shock; and group S5H cells were exposed to 5% sevoflurane and heat shock（Fig. 2）. The concentrations of sevoflurane were measured with an anesthetic gas monitor（Multigas Unit AG-920R; Nihon-Kohden, Tokyo, Japan）before and after the preconditioning state. The containers were opened after the challenge, and the medium was replaced with D-MEM for immunoblot and real-time polymerase chain reaction（RT-PCR）assays or Opti-MEM I Reduced-Serum Medium（GIBCO/Invitrogen） for cytotoxicity assays. The cells were additionally incubated under a normal condition for various periods of time. Cells for the cytotoxicity assay were subsequently exposed to lethal heat shock（44℃, 1h） and further incubated under normal conditions for 16 h. Immunoblotting, RT-PCR, and cytotoxicity assays were performed by the methods described below.

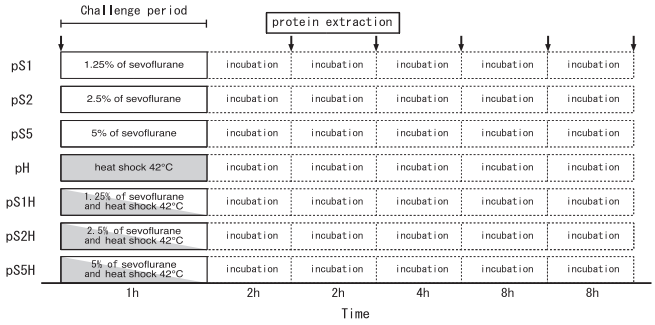

Figure 1

Time course of the preliminary examinations to evaluate the maximum HSPA1A/B expression time. All cells were exposed to heat shock（42℃） and/or various concentrations of sevoflurane for 1h（Challenge period）. The extractions of total protein and immunoblots were conducted at baseline and 2h, 4h, 8h, 16h, and 24h after the challenge period. pH = heat shock; pS1 = 1.25% sevoflurane; pS2 = 2.5% sevoflurane; pS5 = 5% sevoflurane; pS1H = 1.25% sevoflurane and heat shock; pS2H = 2.5% sevoflurane and heat shock; pS5H = 5% sevoflurane and heat shock.

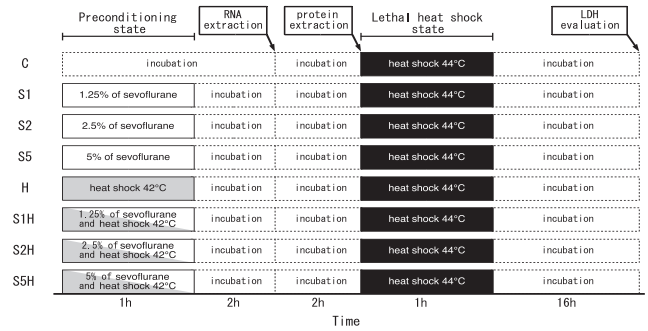

Figure 2

Time course of the examinations to compare the expressions of HSPA1A/B protein and mRNA and cytotoxicity levels. All groups except the control group were exposed to heat shock（42℃） and/or various concentrations of sevoflurane for 1 h（Preconditioning state）. Two or four hours after this procedure, total RNA or total protein was extracted. In the cytotoxicity assay, 4 h after this procedure, all groups were exposed to lethal heat shock（44℃） for 1 h（Lethal heat shock state）, all cells were returned to 37℃ for 16 h, and LDH was examined. C = control group; H = heat shock group; LDH = lactate dehydrogenase; S1 = 1.25% sevoflurane group; S2 = 2.5% sevoflurane group; S5 = 5% sevoflurane group; S1H = 1.25% sevoflurane and heat shock group; S2H = 2.5% sevoflurane and heat shock group; S5H = 5% sevoflurane and heat shock group.

IV. Immunoblotting

The cells were lysed by scraping and pipetting in ice-cold radioimmunoprecipitation assay buffer containing 25 mM Tris HCl (pH 7.6), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1/100 Protease Inhibitor Cocktail (Sigma-Aldrich, Tokyo, Japan), and 0.2 u/ml Benzonase (Merck, Tokyo, Japan). A total of 2 μg of protein was loaded for SDS-polyacrylamide gradient gel electrophoresis and transferred to nitrocellulose membranes using the NuPAGE system (Invitrogen). The amount of protein for the electrophoresis was determined by our preliminary experiments for detection of HSPA1A/B, heat shock 70-kDa protein 8 (HSPA8, also known as HSC70), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). A long duration of electrophoresis was necessary for separation of proteins in the comparative experiment. The membranes were blocked for 1h in phosphate buffered saline containing 5% skimmed milk (Morinaga Nyugyo, Tokyo, Japan), then incubated with the primary and secondary antibodies. Bands were developed with a chemiluminescence detection system (ECL Western Blotting Detection Reagents; GE Healthcare/Amersham Biosciences, Buckinghamshire, United Kingdom) and subsequently exposed to X-ray film. The intensity of each band was determined by a densitometer and expressed relative to the level of control cells after normalization to the GAPDH signal. The following antibodies were used. As primary antibodies, anti-HSP70 mouse monoclonal antibody (Becton Dickinson, San Jose, CA), which recognizes both HSPA1A/B and HSPA8,[11] and anti-GAPDH mouse monoclonal antibody (Millipore, Bedford, MA) were used. As a secondary antibody, anti-mouse IgG sheep antibody conjugated to horseradish peroxidase (GE Healthcare/Amersham Biosciences) was used.

V. RT-PCR

Complementary DNA (cDNA) was synthesized using a TaqMan Gene Expression Cells-to-CT Kit (Applied Biosystems, Tokyo, Japan). Solutions for RT-PCR were assembled with the cDNA and TaqMan Gene Expression Assays (Hs00359147_s1 for HSPA1A/B, Hs99999905_m1 for GAPDH; Applied Biosystems). RT-PCR was carried out by an ABI PRISM 7700 sequence detector (Applied Biosystems). The relative amounts of HSPA1A/B and GAPDH mRNAs were calculated by threshold cycle (C_t) values. Finally, the amounts of HSPA1A/B mRNAs were normalized by dividing them by the amount of GAPDH mRNA.

VI. Cytotoxicity by lethal heat shock

Release of lactate dehydrogenase (LDH) into the culture medium was detected according to a protocol provided with the LDH-based Cytotoxicity Detection Kit (Roche Diagnostics, Tokyo, Japan). Spontaneous LDH release was used as a low control, and whole-cell lysate was used as a high control. The percentage of cytotoxicity was determined by the following equation:

[(experimental value - low control) / (high control - low control)] × 100.

VII. Statistical analysis

Data are expressed as the mean ± SD. For data corresponding to temperature and sevoflurane concentrations, a two-factor repeated measures analysis of variance was used to evaluate differences. For all other data, statistical analysis and multiple comparisons were performed using the Kruskal-Wallis test

and the Scheffe *post hoc* test. A value of $P < 0.05$ was considered statistically significant. All statistics were evaluated using the program StatView 5.0 for the Macintosh OS (Sas Institute, Cary, NC).

Results

A preliminary experiment was conducted to analyze the timing of maximal HSPA1A/B expression after administration of sevoflurane and/or heat shock at 42℃ for 1 h to SH-SY5Y cells. This experiment was performed one time. Immunoblot analysis revealed that HSPA1A/B were highly expressed at 2 and 4 h after the heat shock and remained at high levels for 16h, but returned to the control levels at 24 h. However, sevoflurane did not alter HSPA1A/B expression. In addition, sevoflurane did not change the time point of maximum HSPA1A/B expression in the cells exposed to heat shock (Fig. 3). Based on these results, we postulated that HSPA1A/B proteins or mRNAs would be maximally expressed at 4 or 2 h after the heat shock, respectively.

Additional examinations were performed to analyze the amounts of the HSPA1A/B proteins and mRNAs, and whether these variations altered the cytoprotective effects of the HSPA1A/B proteins and mRNAs against subsequent lethal heat shock. The series of these experiments was performed three times. There were no differences in temperature among the normal temperature groups or among the high temperature groups. There were also no differences in the concentration of sevoflurane among groups.

Expression of HSPA1A/B was compared among 8 groups using immunoblot analysis. Four hours after the heat shock at 42℃ for 1 h, the HSPA1A/B levels in group H and group S1H were significantly higher than those in group C ($P < 0.0001$ and $P = 0.0043$, respectively; 160 ± 10% and 142 ± 19% compared with group C); however, the levels of expression in the other groups were not different from those in group C. The levels of expression in group S2H and group S5H were significantly lower than those in group H ($P = 0.0018$ and $P = 0.0003$, respectively; 114 ± 3% and 107 ± 2% compared with group C) (Fig. 4). Thus, it was suggested that the expression of HSPA1A/B was enhanced by the heat shock, and a high dose of sevoflurane inhibited the enhancement. Under normal conditions, sevoflurane did not change the expression of HSPA1A/B from the baseline levels.

Expression of HSPA1A/B mRNAs was determined by RT-PCR to determine whether or not the enhanced expression of HSPA1A/B was relevant to the expression levels of HSPA1A/B mRNAs. Two hours after the heat shock at 42℃ for 1 h, the levels of HSPA1A/B mRNAs in group H and group S1H were significantly higher than those in group C ($P < 0.0001$ and $P = 0.0006$, respectively; 687 ± 73% and 453 ± 27% compared with group C), but the levels of expression in other groups were not different from those in group C. The levels of expression in group S2H and in group S5H were significantly lower than those in group H ($P < 0.0001$ and $P < 0.0001$, respectively; 192 ± 44% and 202 ± 42% compared with group C) (Fig. 5). Thus, it was demonstrated that heat shock enhances the expression of HSPA1A/B mRNAs. Sevoflurane had an inhibitory effect on the enhancement in a dose-dependent fashion, and did not alter the baseline expression of HSPA1A/B mRNAs. These findings were similar to the alterations of HSPA1A/B.

A cytotoxicity assay was performed 16 h after the exposure to lethal heat shock at 44℃ for 1h to determine whether the attenuation of HSPA1A/B by sevoflurane diminished the cytoprotective effect against the lethal heat shock. The levels of cytotoxicity in group H (3.5 ± 3.2%) and group S1H (20.9

± 1.8%) were significantly lower than that in group C (41.5 ± 3.3%) ($P < 0.0001$ and $P < 0.0001$, respectively) at 16 h after the lethal heat shock, but the levels of cytotoxicity in the other groups were not different from those in group C. Those in group S2H (33.0 ± 3.9%) and group S5H (43.3 ± 8.4%) were significantly higher than those in group H ($P < 0.0001$ and $P < 0.0001$, respectively) (Fig. 6). In this way, the cytotoxic effects of lethal heat shock were decreased in heat shock-preconditioned cells, but were increased in sevoflurane-administered cells with heat shock preconditioning. Whether or not sevoflurane-induced anesthetic preconditioning decreased the cytotoxicity was not examined in these experiments.

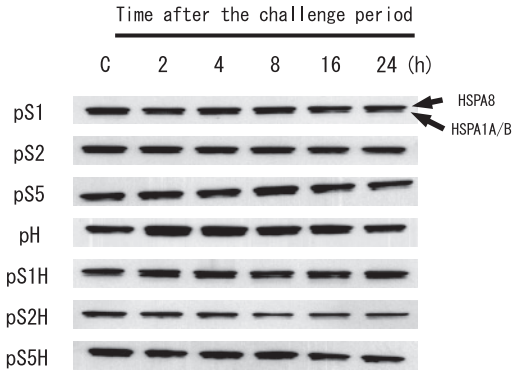

Figure 3

Immunoblots of HSPA1A/B proteins before and after the challenge period (Fig. 1). HSPA1A/B was markedly expressed at 2 and 4 h after the heat shock. Under the heat condition, 1.25% sevoflurane slightly inhibited HSPA1A/B expression at 4 h after the challenge. The blots in other cells were similar to that in the control cells. C = control; HSPA1A/B = heat shock 70-kDa protein 1A and 1B; HSPA8 = heat shock 70-kDa protein 8; pH = heat shock; pS1 = 1.25% sevoflurane; pS2 = 2.5% sevoflurane; pS5 = 5% sevoflurane; pS1H = 1.25% sevoflurane and heat shock; pS2H = 2.5% sevoflurane and heat shock; pS5H = 5% sevoflurane and heat shock.

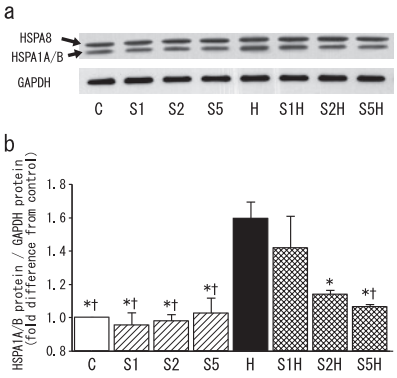

Figure 4

Immunoblot analysis of HSPA1A/B proteins at 4 h after the preconditioning state (Fig. 2). a, Representative immunoblot showing the changes in HSPA1A/B and GAPDH after exposure to heat shock at 42℃ and/or various concentrations of sevoflurane for 1 h. b, Band intensity of the immunoblots. Heat shock significantly increased heat-induced HSPA1A/B expression. In the 1.25% sevoflurane and heat shock group, the expression was significantly higher than in the control group. However, 2.5% and 5% sevoflurane limited the emergence of HSPA1A/B expression under the heat condition. C = control group; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; H = heat shock group; HSPA1A/B = heat shock 70-kDa protein 1A and 1B; HSPA8 = heat shock 70-kDa protein 8; S1 = 1.25% sevoflurane group; S2 = 2.5% sevoflurane group; S5 = 5% sevoflurane group; S1H = 1.25% sevoflurane and heat shock group; S2H = 2.5% sevoflurane and heat shock group; S5H = 5% sevoflurane and heat shock group. Values are the means ± SD and normalized to the control. n=3 in each group; *P<0.05 compared with group H; † P<0.05 compared with group S1H.

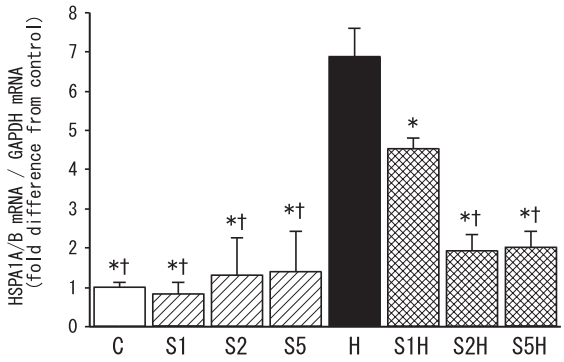

Figure 5

Real-time polymerase chain reaction analysis of HSPA1A/B mRNAs at 2 h after the preconditioning state (Fig. 2). Heat shock significantly increased the heat-induced expression of HSPA1A/B mRNAs. In the 1.25% sevoflurane and heat shock group, the expression was significantly higher than in the control group. However, 2.5% and 5% sevoflurane limited the emergence of HSPA1A/B expression under the heat condition. C = control group; H = heat shock group; HSPA1A/B = heat shock 70-kDa protein 1A and 1B; S1 = 1.25% sevoflurane group; S2 = 2.5% sevoflurane group; S5 = 5% sevoflurane group; S1H = 1.25% sevoflurane and heat shock group; S2H = 2.5% sevoflurane and heat shock group; S5H = 5% sevoflurane and heat shock group. Values are the means ± SD and normalized to the control. n=3 in each group; *P<0.05 compared with group H; † P<0.05 compared with group S1H.

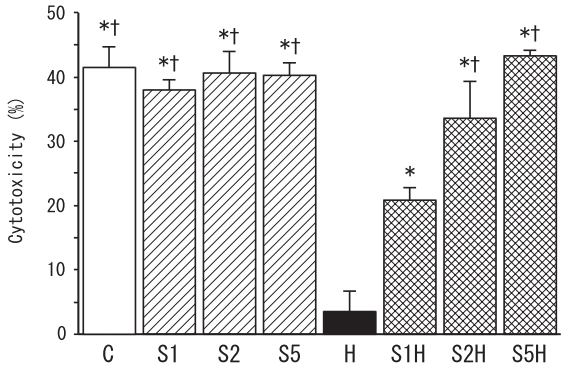

Figure 6

Cytotoxicity at 16 h after the lethal heat shock state (Fig. 2). Heat shock significantly decreased the heat-induced cytotoxicity. In the 1.25% sevoflurane and heat shock group, the cytotoxicity was significantly lower than in the control group. However, 2.5% and 5% sevoflurane obstructed the heat shock-induced cytoprotection. C = control group; H = heat shock group; S1 = 1.25% sevoflurane group; S2 = 2.5% sevoflurane group; S5 = 5% sevoflurane group; S1H = 1.25% sevoflurane and heat shock group; S2H = 2.5% sevoflurane and heat shock group; S5H = 5% sevoflurane and heat shock group. Values are the means ± SD and normalized to the control. n=3 in each group; *P<0.05 compared with group H; † P<0.05 compared with group S1H.

Discussion

Our experiments revealed that sevoflurane dose-dependently attenuated the expression of HSPA1A/B and their mRNAs, which ought to have been increased by the concurrent heat shock, and consequently these factors could not protect the heat-shocked cells against the subsequent lethal heat shock. These results indicated that sevoflurane has a counteracting effect on late preconditioning induced by heat shock.

This is the first report of the effect of heat shock and sevoflurane on HSPA1A/B expression in SH-SY5Y cells. The human HSPA1A and HSPA1B (NCBI Reference Sequence: NP_005336.3 and NP_005337.2, respectively) have identical amino acid sequences and the same functions.[5] In these experiments, the levels of HSPA1A/B proteins and mRNAs in the heat-stressed cells were approximately 1.6- and 7-fold higher than those of the control, respectively, and the up-regulated HSPA1A/B protected the cells against the subsequent lethal heat shock. The expression levels of HSPA8 protein were not significantly different among the groups (data not shown). HSPA8 is also a protein chaperone and shares many similar characteristics with HSPA1A/B, but its expression is abundant under normal circumstances.[2] Heat stress induces some HSP70 family proteins, strongly HSPA1A/B.[4] Therefore, HSPA1A/B may play a pivotal role in the survival of the heat shock-preconditioned SH-SY5Y cells during the particular time period. Additional research for protective effect of chaperones against heat shock may be required because expression of chaperones is probably influenced by various situations such as cell type, an elapsed time from heat shock, intensity of stress, and so on.

Many reports have demonstrated the effects of sevoflurane on HSP70 expression. Administration of 0.5 minimum alveolar concentration (MAC) sevoflurane for 30 min does not influence the expression of HSP70 in rabbits.[12] Another study showed that the levels of HSPA1A/B mRNAs were not affected by 1 h administration of 0.5-1.0% sevoflurane in white blood cells in volunteers.[8] In addition, HSPA1A/B expression was not affected by 3 h administration of 1.0 MAC sevoflurane in the rat brain.[10] We assumed that sevoflurane had no effect in these previous studies because it was used at such low doses. In our study, it was hoped that a high dose of sevoflurane would influence HSPA1A/B expression even if the administration time was short; however, a high dose of sevoflurane had no effect on HSPA1A/B proteins and mRNAs expression. It is also reported that long-term administration of 2.2% sevoflurane increases HSP70 and reduces oxidant-induced necrosis in human kidney tubule cells.[9] According to these facts, it is reasonable to assume that a short-term administration of sevoflurane is unlikely to enhance HSP70 levels.[13]

The mechanisms of sevoflurane-induced inhibition of HSPA1A/B expression are unclear; however, sevoflurane probably influences the transcriptional and post-transcriptional regulation of HSPA1A/B mRNA. Heat shock factor protein 1 (HSF1) is the major heat shock transcription factor. Under heat conditions, translocation of HSF1 from the cytoplasm to the nucleus is an initial step in the expression of HSPA1A/B mRNAs via the binding of HSF1 to the promoter (heat shock element: HSE) of the HSPA1A/B gene.[1] Expression of heat shock proteins is mostly regulated at the level of their mRNA transcription.[14] Sevoflurane may control the activity of regulatory factors, including HSF1. Recently, some researchers have reported that heat shock stress can reduce mRNA transport from the nucleus to the cytoplasm, except in the case of certain mRNAs, including HSP mRNA.[15] The mRNAs in the nucleus rapidly disappear along with the increase in degradation activity induced by heat shock stress. HSP mRNA in the cytoplasm tends to increase protein translation due to the reduction of the other mRNAs. Sevoflurane also might dose-dependently reduce the selective transportation of mRNAs from the nucleus to the cytoplasm by inhibition of some kinases such as PKC and intracellular Ca^{2+} regulation.

Sevoflurane mimics the cardioprotective effects of early preconditioning and enhances the ischemic preconditioning occurring subsequent to ischemic stimuli via the activation of mitochondrial adenosine triphosphate-regulated potassium (K_{ATP}) channels.[16] According to clinical research, sevoflurane plays

important roles in cardioprotection via anesthetic preconditioning or the sevoflurane-related enhancement of ischemic preconditioning.[17, 18] In an animal study, administration of sevoflurane was shown to reduce the cerebral infarction size and to inhibit the ischemia-induced apoptosis of neural cells.[19] Thus, it is clear that sevoflurane plays an important role in cytoprotection via early preconditioning. Meanwhile, the present study revealed that sevoflurane attenuated the HSPA1A/B-related cytoprotection against lethal heat shock and inhibited the heat shock-induced late preconditioning. HSP70 is highly induced by various stressors,[2, 20] but it is unclear whether sevoflurane also suppresses the expression of HSPA1A/B induced by stressors other than heat shock. In conclusion, the sevoflurane-related cytoprotection may differ according to the time elapsed since the stresses, including heat shock. Further investigation of the precise mechanism of sevoflurane-related regulation of HSPA1A/B expression will be needed.

Malignant hyperthermia, a hypermetabolic syndrome caused by abnormal calcium regulation in skeletal muscle, can be induced by inhalational anesthetics, including sevoflurane.[21] In this life-threatening disease, the core body temperature can be increased to 44℃.[21] Exposure to hyperthermic heat shock at 42℃ for 15 min elicits maximal expression of HSP70 at 24 h after stress in the dorsomedial medulla of rats.[22] There has been no report as to whether HSPA1A/B is up-regulated in human brain cells in malignant hyperthermia; however, HSPA1A/B expression ought to be elevated by high body temperature. Within 72h, 25% of patients with malignant hyperthermia will experience a recrudescence of the syndrome.[21] If intraoperative administration of a high dose of sevoflurane inhibits HSPA1A/B expression in malignant hyperthermia, HSPA1A/B-induced neuroprotection against heat stress may not be exerted in the relapsing syndrome. Therefore, high dose of sevoflurane may worsen the prognosis of hyperthermia due to the mechanism showed in this study. Certainly, sevoflurane is one of the most used volatile anesthetics, and the drug evidently has the cytoprotective effect against ischemic stress during an intraoperative and perioperative period as the above-mentioned reports suggest. It is unclear whether inhibition of HSPA1A/B expression by high dose of sevoflurane will have any effect on ischemia in many organs, and in vivo experiments may be needed to reveal the relationship among expression levels of HSPA1A/B, cytoprotection against ischemia, and various doses of sevoflurane.

Conclusion

Our study has demonstrated that co-administration of a high dose of sevoflurane and heat shock attenuated the heat shock-induced expression of HSPA1A/B proteins and their mRNAs, and impaired cytoprotection against the lethal heat stress. The mechanisms of the sevoflurane-induced attenuation of cytoprotection must be clarified in the future.

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熱ショックおよびセボフルランがHSPA1A/Bタンパク質およびmRNAの発現に及ぼす影響

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要　約

セボフルランは様々な細胞保護効果を持つ。heat shock 70-kDa proteins 1Aおよび1B（HSPA1A/B）は，細胞機能の維持に重要な分子シャペロンである。本研究では，熱ショックおよびセボフルランがHSPA1A/Bタンパク質の発現に与える影響を神経芽腫由来細胞を用いて検討した。軽度の熱ショック（HS）により，HSPA1A/Bタンパク質およびmRNAは有意に発現が増強し，致死的熱ショック（LHS）に対する耐性が発揮された。HS下において，セボフルランはHSによるHSPA1A/Bタンパク質およびmRNAの発現を濃度依存性に抑制し，LHSに対する耐性を減弱した。セボフルラン単独ではHSPA1A/Bタンパク質およびmRNAの発現に影響を及ぼさなかった。セボフルランによるHSPA1A/Bタンパク質発現の抑制メカニズムを含め，今後も細胞保護に関する更なる検討を要する。

（キーワード：セボフルラン，熱ショック，分子シャペロン，プレコンディショニング，悪性高熱症）

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