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Inhibitory Effects of the Anesthetics Propofol and Sevoflurane on Spontaneous Lymphatic Vessel Activity in Rats

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Background: **The effects of propofol and sevoflurane on lymphatic vessel activity are unknown. This study aimed to clarify the effects of these anesthetics on lymphatic vessel activity in rats by the use of a technique for mechanical removal of the endothelium.**

Methods: **The authors first examined the effects of propofol (8 mg/kg) and sevoflurane (2.0%) on *in vivo* lymphatic flow by injection of dye into the femoral regions of rats. In the *in vitro* study, the ends of the vessel segments of rat thoracic duct were connected to a syringe and stopcock, respectively. Spontaneous changes in diameter of each segment were monitored, and the extraluminal side of each segment was exposed to propofol ($1 \times 10^{-6} \sim 3 \times 10^{-5}$ M) or sevoflurane (0.5∼2.0%). Endothelial function was eliminated by perfusion of air into the lumen.**

Results: **In the dye uptake study, 80% of iliac lymphatic nodes were positively stained in a control group, whereas only 10% and 20% were positively stained in propofol and sevoflurane groups, respectively. In the *in vitro* study, both of the anesthetics significantly decreased the amplitude of spontaneous activity of lymphatic vessels with or without endothelial function. Sevoflurane inhibited the frequency of lymphatic vessel activity but propofol had no effect on it. When the endothelial function was eliminated, both anesthetics decreased the frequency of spontaneous activity of lymphatic vessels.**

Conclusions: **Propofol and sevoflurane seem to have some different effects on endothelial function, which regulates the pacemaking of spontaneous contraction of lymphatic vessels.**

MAINTENANCE of extracellular fluid volume requires control of fluid and salt transport between plasma and the interstitium across the capillary membrane through which salt and water can easily pass.[1] Lymphatic circulation plays an important role in regulation of the transport of extracellular fluids and macromolecular substances in tissues.[1,2] Thus, lymphatic vessels act to return fluid and proteins that escape from the capillary blood vessels to the circulation. The total lymphatic flow in humans is thought to be as high as 2∼4 l a day,[1] and it is well known that failure of lymphatic circulation can cause lymphedema of the extremities, especially after removal of metastatic subaxillary or inguinal lymph nodes. Lymphatic flow is maintained by passive and active driving forces,[1,2] the passive driving forces being respiration, intestinal peristalsis, limb movements, external compression and massage, and blood vessel pulsation, and the active driving force being spontaneous contractions of lymphatic vessels.

Anesthetics such as halothane and barbiturates are known to reduce lymphatic flow.[3,4] This undoubtedly results in part from the effect of these agents on the forces necessary for lymph propulsion, and the depressant effect has usually been attributed to the effect of immobilization of the animal and thus removal of the extrinsic muscle pump believed to be necessary for lymph propulsion. However, there is growing evidence that such extrinsic forces are not so important[5–7] and that the essential motor of lymph propulsion is the intrinsic spontaneous contractility of lymphatics themselves.[1,2] McHale and Thornbury[8] first reported that the conventional anesthetics pentobarbital sodium and halothane at clinically used concentrations depressed lymphatic contractility in isolated segments of bovine mesenteric lymphatic vessels. The direct inhibitory mechanisms of the actions of anesthetics, however, are still unknown.

It has recently been shown that endothelial factors are important contributors to the tone and pumping activity of lymphatics *in vivo*.[9–11] It has also been shown that the lymphatic endothelium produces and releases nitric oxide, leading to a decrease in the efficacy of spontaneous transient depolarizations to generate action potentials and resultant contractions.[12,13] These findings suggest that the endothelium seems to regulate the spontaneous activity of lymphatic vessels,[10] and it is important to know the effect of anesthetics on endothelial function.

We therefore investigated the inhibitory effects of the most widely used intravenous and volatile anesthetics, propofol and sevoflurane, on *in vitro* activity of lymphatic vessels from rats by the use of a technique for mechanical removal of the endothelium.[10,11] We also investigated in *in vivo* dye uptake study the effects of these anesthetics on lymphatic flow.

Materials and Methods

The experimental protocols used in this study were approved by the Sapporo Medical University Animal

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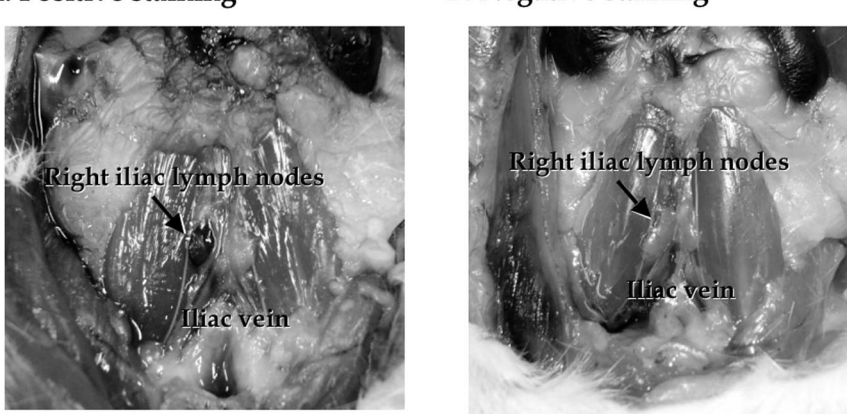

Fig. 1. Representative photographs obtained in the *in vivo* dye staining study. After 60 min of the staining, the abdomen was opened and the iliac lymph nodes were exposed and observed. The right iliac lymph nodes were stained positively in the left inset (*A*) whereas the nodes were stained negatively in the right inset (*B*).

Care and Use Committee (Sapporo, Hokkaido, Japan). Seventy-two male Wistar rats (6∼8 weeks old, weighing 200∼250 g) were used for this study. The rats were housed in an environmental-controlled vivarium and fed a normal diet and water.

In Vivo *Dye Uptake Study of the Effects of Anesthetics on Lymphatic Flow*

Thirty rats were randomly divided into three groups: a control group (n = 10), a propofol group (n = 10), and a sevoflurane group (n = 10). Hexamethylpararosaniline chloride (gentian violet; 1%, 0.2 ml), which can be absorbed selectively into lymphatic vessels,[14] was injected into the femoral region (approximately 1 cm above the knee) of each rat. Just after the injection, the rats in the propofol and sevoflurane groups were sedated with propofol and sevoflurane, respectively, whereas the rats in the control group received no anesthesia. Propofol was continuously infused *via* the tail vein (40 $mg \cdot kg^{-1} \cdot h^{-1}$ after a bolus injection of 8 mg/kg, sedative dose in rats)[15] in the propofol group, whereas the rats in the sevoflurane group was exposed to 2% (0.75 minimum alveolar concentration in rats)[16] sevoflurane within mixed gases at a high flow rate (6 l/min). All of the rats tested in this study breathed spontaneously in an atmosphere of 30% oxygen, and each rat was placed in a small cage so as to be completely immobilized.[3,4]

After 60 min of dye staining, the rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg) and then killed by exsanguination. The abdomen was opened immediately, and the iliac lymph nodes were exposed and observed by a dissecting microscope (× 50∼100). When gentian violet was transferred to the iliac lymph nodes within the 60-min staining period, the lymph nodes were dyed a violet color, and the staining was judged to be positive by an independent observer (fig. 1).

In Vitro *Study of the Effects of Anesthetics on Lymphatic Vessel Activity With or Without Endothelial Function*

Forty-two rats were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally). As soon as a surgical concentration of anesthesia had been attained, the thoracic duct was carefully isolated from each animal by using microsurgical instruments and a microscope (× 100∼200), and it was placed in a Petri dish containing ice-cold Krebs bicarbonate solution aerated with a gas mixture of 95% N_2 and 5% CO_2. The Krebs solution contained 120 mM NaCl, 5.9 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 5.5 mM glucose and 25 mM $NaHCO_3$.[10,11] The thoracic duct, dissected free from connective tissue, was cut into a single vessel segment (approximately 600 μm in maximum external diameter and 5∼7 mm in length) in the Petri dish and placed in a chamber designed by us. After immersion of the thoracic ducts in the Krebs solution, both proximal and distal ends of the thoracic ducts were cannulated with glass micropipettes and secured with fine nylon sutures. The cannulated proximal and distal ends of the vessel were connected to a 50-ml plastic syringe *via* a Tygon tube and to a stopcock, respectively.

After the thoracic duct had been cannulated, the chamber containing the thoracic duct was placed on the stage of an inverted microscope (IX-70; Olympus, Tokyo, Japan) equipped with a video camera (WAT-308A; WATEC, Yamagata, Japan), and the images of the vessel were projected onto a monitor screen (fig. 2). Changes in lymphatic vessel internal diameters were measured using a Video Dimension Analyzer system (Living Systems Instrumentation, Burlington, VT). Measurements of diameters were recorded using a personal computer (Power Macintosh G3® 266 MB; Apple, Cupertino, CA) connected to the analyzer. The Krebs bicarbonate solution, aerated with 95% N_2 and 5% CO_2 (pH 7.40 ± 0.02; n = 5), was warmed at 37°C using an automatic temperature controller (TC-324B; Warner Instrument Co., Hamden, CT) during the study period, and the replacement of the chamber solution was carried out by changing the inflow perfusate (flow rate, 35 ml/min). Partial pressure of O_2 of the solution in the chamber was within the range of 35 to 50 mm Hg (n = 5), and the O_2 tension was appropriate for isolated lymphatic vessel activity.[17] The thoracic duct was warmed slowly to 37°C and allowed to

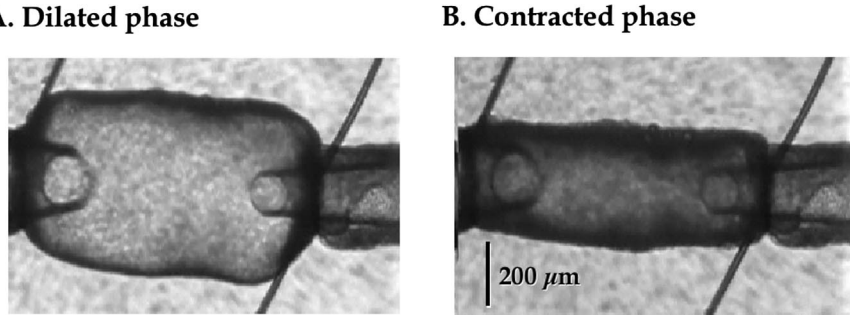

Fig. 2. Representative images of the vessel segment projected onto a monitor screen. Changes in lymphatic vessel internal diameters were determined using a video dimension analyzer system. (*A*) Dilated phase; (*B*) contracted phase.

equilibrate for 60 min. The intraluminal pressure in the thoracic ducts was kept at +5.0 cm H_2O by controlling a level of the syringe connected to the inflow tubing. The syringe was also filled with the Krebs bicarbonate solution and aerated with 95% N_2 and 5% CO_2, and the outflow tubing was closed with a stopcock. At the pressure of +5.0 cm H_2O, active constriction of the isolated rat lymphatic vessels was observed spontaneously and constantly, as assessed by changes in lymphatic diameter.

After equilibration, acetylcholine chloride (ACh, 10^{-6} M) was administered extraluminally to all of the lymph vessels before starting the following experimental protocols to evaluate functional viability of the lymphatic endothelial cells.[11,18,19] After demonstrating ACh-induced dilation of maximum diameter (D_{max}) by more than 10% and cessation of the spontaneous contraction,[19] the solution in the chamber was washed out to obtain control activity again. The vessel segment was then accumulatively exposed to propofol (1×10^{-6} M $\sim 3 \times 10^{-5}$ M, relevant clinical dose in rats)[15,20] by changing the perfusate to one that included each single concentrations of propofol. In separate experiments, other similarly prepared segments of the lymphatic vessels were accumulatively exposed to sevoflurane (0.5% [0.2 minimum alveolar concentration]$\sim$ 2.0% [0.75 minimum alveolar concentration] in rats)[16] by changing the perfusate to one that had been vigorously bubbled with the anesthetic. We estimated that the exchange of solution in the chamber was essentially completed extraluminally within 3 min.

To further demonstrate the effects of these anesthetics on lymphatic vessel activity, we investigated the effects of the anesthetics on the force and frequency of spontaneous contraction under an endothelium-denuded condition. Briefly, endothelial function was eliminated by gentle perfusion of air (approximately 300 μl) into the lumen of each thoracic duct strip for 3 min.[10,11] After confirming that 10^{-6} M ACh had no effect on lymphatic vessel activity, similar experimental protocols as those described above were performed under this condition.

Measurement of Sevoflurane Concentrations in the Gas Phase and in a Bath Solution

Sevoflurane concentrations were measured according to the previously described method.[21] Briefly, a vaporizer for sevoflurane (0.5, 1.0, 1.5, and 2.0%) was calibrated with an infrared anesthetic gas monitor (5250 RGM; Datex-Ohmeda, Madison, WI). Concentrations of the anesthetic agent in bath solution samples were analyzed using a gas chromatograph (GC-17A; Shimadzu, Kyoto, Japan). The mean concentration of sevoflurane in the solutions (0.10, 0.18, 0.28, and 0.36 mM, respectively; n = 3 each) was similar to that reported previously.[21]

Materials

The following drugs and chemicals were used: Gentian Violet, acetylcholine chloride, EDTA, and salts (Sigma Chemical, St. Louis, MO), sevoflurane (Sevofrane®; Maruishi Pharmaceutical, Osaka, Japan), and propofol (Diprivan®; AstraZeneca Japan, Osaka, Tokyo). We used commercially available propofol, which included 10% Intralipid® (Pharmacia-Upjohn, Stockholm, Sweden) as a solvent (10% vol/vol soybean oil, 2.5% glycerol, and 1.2% purified egg lecithin) and 0.005% w/v EDTA. The effects of Intralipid and EDTA *per se* were therefore tested in each experiment, and the concentration of each of these agents used alone corresponded to each concentration of propofol. Neither Intralipid nor EDTA affected any parameters tested in the *in vitro* study (n = 3 each, data not shown).

Statistical Analysis

After the force, frequency, and duration of the contraction had reached a steady state, mean values of maximum diameter (D_{max}, μm), minimum diameter (D_{min}, μm), amplitude ($D_{max} - D_{min}$, μm) and frequency (min^{-1}) of spontaneous lymphatic pump activity for a period of 2 min were determined (fig. 2). Drug-induced lymphatic constriction is expressed as a percentage change in spontaneous pump activity before the administration of the anesthetics. Data are expressed as means $\pm$ SD. Data obtained from the *in vivo* dye uptake study were compared by the χ squared test. Other data obtained from in the *in vitro* study were analyzed by the unpaired, two-tailed Student *t* test between two groups or by using one-way analysis of variance for repeated measurements, and the Tukey-Kramer test was used as a *post hoc* test. In all comparisons, a *P* value < 0.05 was considered significant.

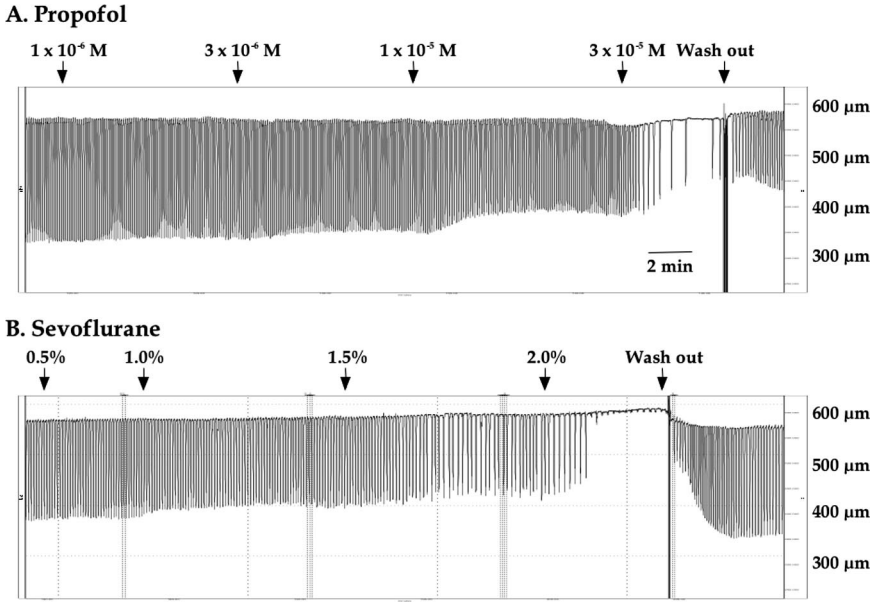

Fig. 3. Representative tracings of the effects of the anesthetics (*A*) propofol ($1 \times 10^{-6} \sim 3 \times 10^{-5}$ M) and (*B*) sevoflurane (0.5∼2.0%) on spontaneous activity in isolated lymph vessel segments with intact endothelial function. Both of the anesthetics caused dose-dependent inhibition of spontaneous lymphatic activity (amplitude). Sevoflurane decreased the frequency of vessel contraction in a dose-dependent manner, but propofol seemed to have little effect on it up to a concentration of 1×10^{-5} M.

Results

In Vivo *Effects of the Anesthetics Propofol and Sevoflurane on Lymphatic Flow*

Gentian violet is absorbed by peripheral lymphatic vessels in limbs and circulates to right iliac lymphatic nodes.[14] Figure 1 shows positive and negative staining of right iliac lymphatic nodes after subcutaneous injection of 1% gentian violet in the right femoral region. Eight (80%) of the ten rats in the control group showed positive staining, whereas only one (10%) of the ten rats in the propofol group and two (20%) of the ten rats in the sevoflurane group showed positive staining 60 min after injection of the dye ($P < 0.05$, respectively). The results of this *in vivo* study showed that both propofol and sevoflurane can inhibit lymphatic flow at clinically relevant concentrations.

In Vitro *Study of the Effects of Anesthetics on Lymphatic Vessel Activity With or Without Endothelial Function*

Isolated lymphatic vessels of the rat thoracic duct exhibited stretch-induced spontaneous contraction and dilation at an intraluminal pressure of +5 cm H_2O with endothelial function (figs. 2 and 3). D_{max} and D_{min} of the lymphatic vessels were 580 ± 80 μm and 315 ± 135 μm, respectively (n = 27). Mean amplitude ($D_{max} - D_{min}$) and frequency of spontaneous activity of the lymphatic vessels were 260 ± 50 μm and 12 ± 4 min^{-1}, respectively (n = 27). ACh (10^{-6} M) significantly increased D_{max} by 12 ± 2% (n = 27) and caused complete cessation of spontaneous contraction (raw data not shown).

Figure 3 shows representative tracings of the effects of propofol ($1 \times 10^{-6} \sim 3 \times 10^{-5}$ M) and sevoflurane (0.5% ∼ 2.0%) on spontaneous activity in isolated lymph vessels with intact endothelial function. Both of the anesthetics caused dose-dependent reduction in amplitude of spontaneous lymphatic activity. Although sevoflurane decreased the frequency of spontaneous contraction of lymphatic vessels in a dose-dependent manner, propofol had no effect on the frequency up to a concentration of 1×10^{-5} M. Both anesthetics at the highest concentrations tested completely suppressed lymphatic activity. The spontaneous contractions recovered immediately after the agents had been washed out.

To further demonstrate the effects of the anesthetics on lymphatic vessel activity, we performed the same experimental protocol using different vessel segments under an endothelium-denuded condition. After eliminating endothelial function by perfusion of air into the lumen,[10,11] we confirmed that 10^{-6} M ACh had little effect on the amplitude of lymphatic vessel activity before starting each experiment (n = 15, data not shown). Figure 4 shows representative tracings of the effects of the anesthetics on spontaneous activity in isolated lymph vessels without endothelial function. Even without endothelial function, isolated vessels exhibited stretch-induced spontaneous contraction. The D_{max} and D_{min} of the lymphatic vessels were 500 ± 50 μm and 350 ± 50 μm, respectively (n = 15). Mean amplitude and frequency of spontaneous activity of the lymphatic vessels were 160 ± 40 μm and 16 ± 4 min^{-1}, respectively (n = 15). This means, in essence, that the amplitude of the lymphatic vessels in this study decreased by 38%, whereas the frequency increased by 33% after eliminating endothelial function. Similar to the results of the experiments with intact endothelial function, the effects of the anesthetics were demonstrated in a series of experiments without endothelial function. Both anesthetics caused dose-dependent inhibition of spontaneous lymphatic frequency as well as amplitude.

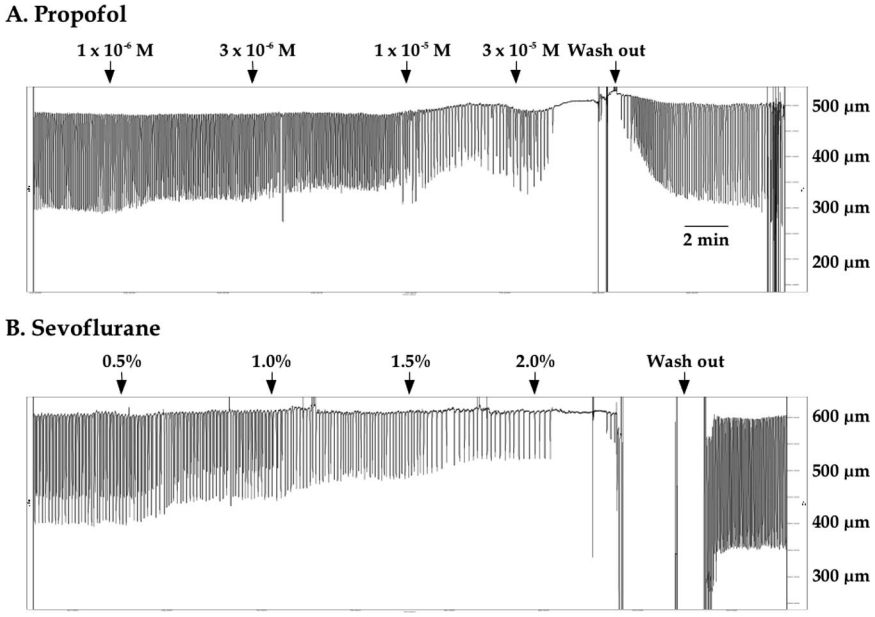

Fig. 4. Representative tracings of the effects of the anesthetics (*A*) propofol ($1 \times 10^{-6} \sim 3 \times 10^{-5}$ M) and (*B*) sevoflurane (0.5∼2.0%) on spontaneously activity in isolated lymph vessel segments without endothelial function. Even without endothelial function, isolated vessels exhibited stretch-induced spontaneous contraction. As was found in the experiment with intact endothelial function, both anesthetics caused dose-dependent inhibition of spontaneous lymphatic activity (amplitude). Both of the anesthetics also caused dose-dependent inhibition of the frequency of spontaneous lymphatic activity.

The experimental findings are summarized in figures 5 and 6. As the highest concentrations of the anesthetics tested completely abolished spontaneous contraction, data for the highest concentrations has been omitted from the figures. Both propofol and sevoflurane significantly decreased the amplitude of spontaneous activity of lymphatic vessels in a dose-dependent manner with or without endothelial function. There is no notable difference between the inhibitory effects with and without endothelial function in either the propofol group or sevoflurane group. On the other hand, sevoflurane reduced the frequency of lymphatic vessel activity with endothelial function in a dose-dependent manner, but propofol had no effect on the frequency of lymphatic vessel activity. When the endothelial function was eliminated, both of the anesthetics significantly decreased the frequency of spontaneous activity of lymphatic vessels.

To address the possibility that the anesthetics tested could not reach the intraluminal side, where endothelial cells exist, we exposed the intraluminal and extraluminal sides simultaneously to the anesthetics by perfusing solutions that had included each of the anesthetics. In the case of only extraluminal perfusion, the two anesthetics had similar inhibitory effects on lymphatic vessel activity, and there was no notable difference between the inhibitory effects with and without endothelial function in either the propofol or sevoflurane group (n = 3 each, data not shown).

We used Intralipid and EDTA as the solvent and vehicle for propofol, respectively. Intralipid/EDTA, the concentrations of which corresponded to those used as the solvent/vehicle for propofol ($1 \times 10^{-6} \sim 3 \times 10^{-5}$ M), did not affect any parameters tested in this in vitro study (n = 3 each, data not shown).

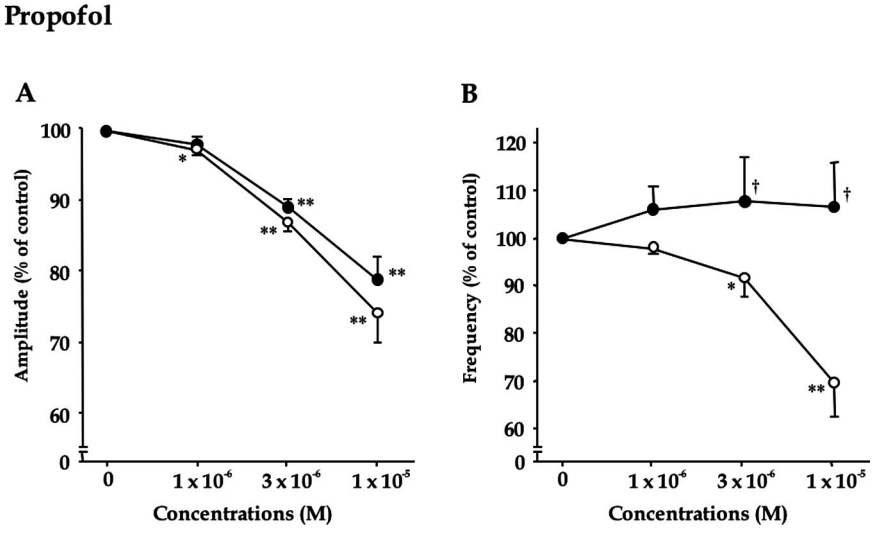

Fig. 5. Effect of propofol ($1 \times 10^{-6} \sim 1 \times 10^{-5}$ M) on the amplitude (*A*) and frequency (*B*) of spontaneous lymphatic vessel activity with and without endothelial function. Data are expressed as means ± SD (n = 14 and 13, respectively). *$P < 0.05$, **$P < 0.01$ *versus* control; †$P < 0.05$ *versus* data without endothelial function. *Solid circles* = data obtained with endothelial function; *open circles* = data obtained without endothelial function.

Sevoflurane

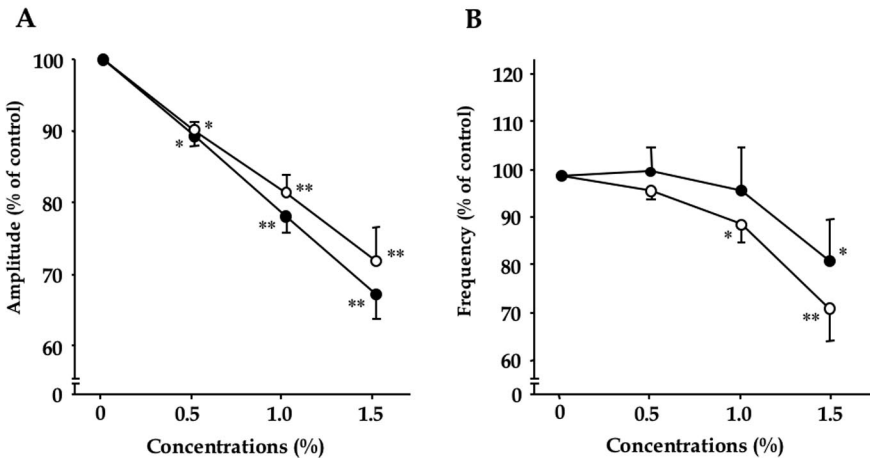

Fig. 6. Effect of sevoflurane (0.5∼1.5%) on the amplitude (*A*) and frequency (*B*) of spontaneous lymphatic vessel activity with and without endothelial function. Data are expressed as means ± SD (n = 8 and 7, respectively). *Solid circles* = data obtained with endothelial function; *open circles* = data obtained without endothelial function. *$P < 0.05$, **$P < 0.01$ *versus* control.

Discussion

In Vivo *Effects of the Anesthetics Propofol and Sevoflurane on Lymphatic Flow*

The salient finding of the current *in vivo* study is that the anesthetics tested at clinically relevant concentrations significantly inhibited lymphatic circulation. Lymphatic flow can be maintained by passive and active driving forces;[1,2] the passive forces are respiration, limb movements, external compression by muscle contraction, and blood vessel pulsation. Schad and Brechtelbauer[4] reported that halothane decreased lymph flow in dogs and they concluded that the inhibition was attributable mainly to the inhibition of extrinsic pumping in the immobilized animal. Whitwam *et al.*[7] also speculated on the basis of their results obtained by using halothane-anesthetized and artificially ventilated dogs that the inhibition of lymph flow was attributable to a decrease in arterial pressure. However, we successfully immobilized each of the animals tested in the current study in a specially designed plastic box, and the animals breathed spontaneously during the study. Although the anesthetics used in this study affected respiratory rate and volume to some extent, we used femoral and iliac portions, in which respiratory extrinsic force has little effect. Regarding arterial pressure, Whitwam *et al.*[7] demonstrated that comparable decreases in mean arterial pressures induced by sodium nitroprusside did not affect lymph flow. Furthermore, there is growing evidence that such extrinsic forces are not so important[5–7] and that the essential motor of lymph propulsion is the intrinsic spontaneous contractility of lymphatics themselves.[1,2] The current *in vivo* study demonstrated that the anesthetics propofol and sevoflurane inhibited lymphatic flow at clinically relevant concentrations and suggested that the anesthetics inhibited the active driving forces, that is, spontaneous lymphatic activity.

In Vitro *Study of the Effects of Anesthetics on Lymphatic Vessel Activity With or Without Endothelial Function*

One of the major findings in the case of intact endothelial function is that propofol and sevoflurane significantly and dose-dependently inhibited stretch-induced spontaneous contraction of lymph vessels in rats. The results for muscle force (amplitude) are consistent with those obtained by McHale and Thornbury[8] in a study on the effects of halothane and pentobarbital on the activity of mesenteric lymphatic vessels of cows. Similar direct inhibitory effects of propofol and sevoflurane have been observed on other types of smooth muscle, such as uterine,[22–24] vascular,[25–27] and airway smooth muscles.[28] Regarding the frequency of contraction of lymphatic vessels, sevoflurane inhibited the frequency of spontaneous lymphatic vessel contraction, but propofol had no effect on it up to a concentration of 1×10^{-5} M. McHale and Thornbury,[8] however, showed that halothane did not change contraction frequency and that pentobarbital increased contraction frequency in lymphatic vessels of cows. Clark and McCannell[29] reported that barbiturates increased the frequency of spontaneous mesenteric portal vein activity in rabbits. These discrepancies may result from the differences in tissue types and species or in the techniques employed in the studies. For example, both of them measured longitudinal muscle tension, and it was not determined whether endothelial cells, which are important for pacemaking,[10,12,13] were viable or not in their experiment.

To further investigate the effects of the anesthetics tested on lymphatic vessel activity, the effects of the anesthetics on the force and frequency of spontaneous contraction under an endothelium-denuded condition were tested. When endothelial function was eliminated by air perfusion, spontaneous contractile force *per se* of lymphatic vessels decreased, whereas contractile fre-

quency significantly increased in a resting condition. The decrease in force by eliminating endothelial function seems to be a result of the decrease in D_{max} from 580 to 500 μm. Nitric oxide, which is derived from endothelial cells, can relax lymphatic smooth muscles in a resting condition.[30] Because endothelial cells in lymphatic vessels play an important role in pacemaking for spontaneous contraction,[10,12] the cells seem to control, at least in part, the frequency of contraction. To be more concrete, endothelial cells can decrease the frequency of the spontaneous lymphatic vessel contraction by inducing hyperpolarization of cells.[13,31]

Without endothelial function, both of the anesthetics tested significantly and dose-dependently inhibited the force (amplitude) of lymphatic vessels as was found in the experiment with endothelial function. This means that the anesthetics tested had direct inhibitory effects on smooth muscle contractility of lymphatic vessels. As is the case for vascular smooth muscles, extracellular Ca^{2+} is important for lymphatic vessel contraction.[32] Many investigators have reported that in vascular,[33] airway,[34,35] and uterine[23,24] smooth muscle cells, propofol and sevoflurane inhibited voltage-dependent Ca^{2+} channel activity by which Ca^{2+} enters into cells, resulting in contraction. Therefore, the mechanism by which these anesthetics decreased the force of lymphatic vessels is thought to be, at least in part, inhibition of Ca^{2+} channel activity. Other possibilities that these anesthetics affected other membrane-associated channels (*e.g.*, K^+) or second messengers (*e.g.*, cyclic adenosine monophosphate) should also be considered.

We also found in this study that propofol significantly and dose-dependently decreased the frequency of the spontaneous lymphatic vessel activity without endothelial function but had no effect on the activity with endothelial function. The findings suggest that endothelial cells play a major role in regulation of the frequency of stretch-induced spontaneous lymphatic vessel activity and that propofol had a different effect on endothelial function, as compared with the effect of sevoflurane. Generally, the endothelium mainly produces nitric oxide,[12,13] resulting in a decrease in the frequency and increase in the amplitude of lymphatic vessel activity.[10,11,36] Although it has recently been revealed that some important channels exist on lymphatic vessels[31,37] and that some agents and hormones have effects on lymphatic vessel activity,[38–40] the endothelial cells have an important role in the control of spontaneous lymphatic activity.[1,2,9–13] Yamashita *et al.*[41] reported that 10^{-5} M propofol inhibited 10^{-6} M ACh-induced, endothelium-dependent relaxation. Other investigators also investigated that sevoflurane also affected endothelial function in rat[42] and rabbit[43] mesenteric arteries. Further studies are needed to clarify the precise role of endothelial cells and different effect of the anesthetics tested in this study in spontaneous activity of lymphatic vessels.

Concentration Dependence and Clinical Relevance

We used a commercially available propofol, 1% Diprivan®, for this study. Because propofol is not soluble in water, Diprivan® includes Intralipid® (10% vol/vol soybean oil, 2.5% glycerol, and 1.2% purified egg lecithin). Diprivan also includes 0.005% EDTA for bacteriostasis, and EDTA can chelate with free Ca^{2+} in a bath solution. However, Intralipid and low concentrations of EDTA corresponding to the concentrations of propofol tested did not have any effect on lymphatic vessel activity with or without endothelial function. Propofol concentrations of $1 \times 10^{-5} \sim 3 \times 10^{-5}$ M *in vitro*, which have significant effects on vessel activity, are comparable to 1∼10 μg/ml propofol *in vivo*.[27] Because the therapeutic range of plasma propofol concentrations is 2∼9 μg/ml[1,44] the effective propofol concentrations tested in this *in vitro* study were rather higher than the free concentrations observed clinically in serum. The significant effect was seen in the *in vivo* study, in which relatively low concentrations of anesthetics were used. Although there is no direct evidence that anesthetic-induced inhibition of lymphatic activity can cause lymphedema during surgery, we sometimes encounter heavy edema (*e.g.*, eyelid or face) after the anesthesia with extreme position. Because propofol and sevoflurane *per se* have high lipid affinity, they can easily enter into the endothelial portion in the experimental design (extraluminal exposure). The validity of the experimental design is supported by the similar effects of these anesthetics on vessel activity when the intraluminal and extraluminal sides were simultaneously exposed to the anesthetics.

In *in vitro* studies, sevoflurane seems to have a greater inhibitory effect than propofol on lymphatic activities. However, we cannot compare the effects of these two anesthetics because sevoflurane is a volatile anesthetic and propofol is an intravenous one. Practically, there was not a significant difference between propofol and sevoflurane in the *in vivo* staining study.

In summary, both propofol and sevoflurane significantly and dose-dependently inhibited the spontaneous contractility of lymphatic vessels of the rat thoracic duct. Sevoflurane also dose-dependently decreased the frequency of the vessel activity but propofol had no effect on it in an endothelium-intact condition. In an endothelium-denuded condition, both propofol and sevoflurane had inhibitory effects on the frequency. These anesthetics tested had somewhat different effects on endothelial function, which regulates the pacemaking of spontaneous contraction of vessels.

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