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Portal blood flow-dependent NO-mediated lymph formation in rat jejunum

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Abbreviations: physiological saline solution, PSS; phosphate buffered saline, PBS; nitric oxide, NO; N^G-Nitro-L-arginine methyl ester hydrochloride, L-NAME; 1-(5-Iodonaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepinehydrochloride, ML-7; enzyme-linked immunosorbent assay, ELISA; myosin light chain kinase, MLCK; standard errors of the mean, SEM; not significant, NS

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Abstract

The higher permeability of the venules in jejunal microcirculation to albumin contributes to the increased mesenteric lymph formation. Recently, we demonstrated that water intake induced serotonin release from enterochromaffin cells in rat jejunum, serotonin of which circulated through the portal vein into blood circulation and then increased the mesenteric lymph formation. The mode of action of serotonin remains unclear. Therefore, we aimed to clarify the mechanisms involved in the regulation of the jejunal lymph formation with permeant albumin in *in vivo* rat experiments. We investigated the effects of intravenous administration of serotonin or water intake on the jejunal-originated lymph volume and the concentration of albumin in the lymph in the presence or absence of L-NAME. The effects of intravenous administration of L-NAME, nicardipine, A23187, and ML-7 on the lymph formation with permeant albumin were also evaluated. Serotonin or water intake significantly increased the mesenteric lymph volume with permeant albumin in the jejunal

microcirculation. The serotonin- and water intake-mediated responses were significantly reduced by the pretreatment with intravenous administration of L-NAME. Intravenous administration of L-NAME itself also decreased significantly the jejunal lymph formation. Administration of A23187 and ML-7 significantly reduced the jejunal lymph formation with permeant albumin. In contrast, administration of nicardipine significantly increased the lymph formation. In conclusion, portal venous blood flow- or serotonin-mediated NO release from venular endothelial cells play physiologically key roles in the lymph formation in rat jejunum via the extrusion of calcium ions and inactivation of MLCK in endothelial cells.

Introduction

The jejunal microcirculation, compared to those in other organs, has specific properties, such as the movement of large amounts of albumin through the venular walls into the interstitial tissues and the resulting higher tissue osmotic pressure at the venular side (3,4,20,23). Jejunum-originated mesenteric lymph flow is also known to be larger than those in other organs (28). Consistent with these properties, mesenteric collecting lymph vessels show heart-like spontaneous contractions (15-17,19), helping to actively transport such large amounts of lymph produced by the higher tissue osmotic pressure, gastrointestinal peristalsis, and abdominal respiration (8,19,28). Thus, the permeability of the venular walls to macromolecules under physiological conditions is known to be regulated by microvascular pressure, segmental permeability variations, pericyte activity, barrier function of the basement membrane, myosin light chain kinase-dependent contractility of venular cells, and endothelial gap formation (12,20,23,31-33).

On this basis, to clarify such mechanisms of larger lymph formation with permeant albumin in the jejunum with *in vivo* animal experiments, we developed a new *in vivo* rat and rabbit preparations to measure the jejunal-originated mesenteric lymph volume and the concentration of albumin in the lymph for evaluating the jejunal lymph formation (10,14). With the new rabbit preparation, we demonstrated that the intravenously injected Evans blue dye was rapidly transported into the mesenteric lymph vessels and cisterna chyli. Water intake in the rabbits accelerated the rate of appearance for Evans blue dye in the mesenteric lymph vessels (14). Thus, the higher permeability of the jejunal microcirculation to albumin plays key a role in the transport of consumed water through lacteal vessels in the lamina propria in jejunal villi (14).

In addition, with the new rat *in vivo* preparation, we also demonstrated that water intake accelerated serotonin release from enterochromaffin cells in the jejunal villi (10). Serotonin is transported mainly through the portal vein into systemic blood circulation. Intravenous

administration of serotonin increased mesenteric lymph volume and the concentration of albumin in the lymph, suggesting that serotonin circulated through blood regulates physiologically the mesenteric lymph formation (10). However, the mechanisms of the serotonin-mediated increase in lymph formation of rat jejunum remains unknown.

Therefore, in the present experiments we aimed to clarify the mechanisms of the serotonin-mediated increase in the lymph formation in rat jejunum and the roles of portal blood flow in the jejunal lymph formation with permeant albumin using rat *in vivo* experiments with pharmacological tools such as N^{G}-Nitro-L-arginine methyl ester hydrochloride (L-NAME), a part inhibitor of NO synthase (NOS); nicardipine, a calcium antagonist; A23187, a calcium ionophore; and 1-(5-Iodonaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepinehydrochloride (ML-7), a selective myosin light chain kinase (MLCK) inhibitor.

Materials and Methods

Ethics approval

This study and all experimental protocols were approved by the Institutional Animal Care and Use Committee of Shinshu University.

In Vivo Rat Experiments

Male Sprague–Dawley rats (10–12-week-old; Japan SLC, Tokyo, Japan) were fed a standard pellet diet (MF, Oriental Yeast, Tokyo, Japan) and water ad libitum. The animals were fasted overnight to reduce the effect of feeding on mesenteric lymph flow. The rats were anesthetized with isoflurane and then placed on the operating table in a supine position. A catheter was inserted into the femoral vein to inject physiological saline solution (PSS; Otsuka Pharma, Tokyo, Japan) or drugs used in the experiments. The intravenous administration of PSS was used to keep normal physiological condition in the rats before starting the experiments. To minimize hemodynamic

changes in the jejunal microcirculation, the intravenous infusion of PSS was stopped during the experiments because we had confirmed with preliminary experiments that the intravenous infusion of PSS causes a significant increase in rat mesenteric lymph flow.

To collect the lymph from jejunal-originated mesenteric lymph vessels, the abdomen was opened by cutting along the midline, and mesenteric adipose and connective tissues were removed to expose the mesenteric lymph node located outside of the jejunum and the efferent lymph vessel. A heparinized small polyethylene catheter (0.5–0.6 mm) was inserted centrifugally into an efferent lymph vessel. However, there exists a little variation of the collected lymph volume in the control condition with each experiment because of stopping for the intravenous infusion of PSS during the experiments.

To evaluate the effect of water intake on the jejunal-originated mesenteric lymph volume and the concentration of albumin in the lymph, distilled water (3 ml) was administered through a needle catheter inserted into the stomach through the mouth according the previous study (14). Distilled water was selected because a previous study (22) demonstrated that distilled water (5 ml), but not PSS, injected into rat stomach produced a significant increase in the lymph flow through the thoracic duct. The lymph was collected over a 15 min interval over the course of an hour, and the volume was measured.

Other *in vivo* rat experiments have been conducted on the effects of 0.3 ml intravenous one-shot administration of 10^{-6} M serotonin (10), 10^{-6} M nicardipine (11,26), 10^{-6} M A-23187 (24), 10^{-6} M ML-7 (30) through the femoral vein in the presence or absence of 10^{-6} M L-NAME (18,21) on the mesenteric lymph volume collected over each 15 min interval and the concentration of albumin in the lymph. Each drug was injected into the femoral vein during several seconds. After the injection, the same volume of heparinized PSS (0.3 ml) was administered into the vein to wash out the drug int the needle. In addition, the effects of intravenous administration of 10^{-6} M L-NAME (0.3 ml) itself on the lymph volume and the concentration of albumin were also evaluated. The concentrations of these drugs used in the present experiments are known to exhibit selective pharmacological action (10,11,18,21,24,26). A-23187 and ML-7 were diluted with ethanol hydrochloride (Wako, Tokyo, Japan). Thus, we firstly made the 10^{-2} M solution with 100% ethanol and distilled water (1:1) to dissolve the drugs. Next, the solution contained with each drug was diluted by PSS to use for the experiments. The intravenous one-shot administration of the 10^{-2} M ethanol (0.3 ml) produced no significant effect on the mesenteric lymph volume. The data are shown in the Results.

Measurement of albumin concentration in the mesenteric lymph

First, the collected mesenteric lymph was centrifuged, and the supernatant was used to measure the concentration of albumin. The concentration of albumin in the lymph was measured using enzyme-linked immunosorbent assay (ELISA) kits-a rat albumin ELISA quantitative kit (Catalog number E111-125, Bethyl Laboratories, Montgomery, TX, USA) was used.

Drugs

All salts were obtained from Wako (Tokyo, Japan). Heparin sulfate was purchased from Mochida Pharmaceutical Co. (Tokyo, Japan). Serotonin hydrochloride, nicardipine hydrochloride, and ML-7 were purchased from Sigma-Aldrich (St Louis, MO, USA). A-23187 was purchased from Fuji Film Wako (Tokyo, Japan). Drug concentrations were described as the final concentrations in phosphate-buffered saline (PBS).

Statistical analysis

All results are expressed as the mean ± SE. Statistical analyses were performed using Student's *t*-test for paired or unpaired results or one-way analysis of variance, followed by Duncan's post-hoc test, as appropriate. A value of $p < 0.05$ was considered statistically significant.

Results

Effects of L-NAME on serotonin-mediated increase in mesenteric lymph volume and the concentration of albumin in the lymph

To evaluate the role of NO in the serotonin-mediated increase in mesenteric lymph volume with permeant albumin, we examined the effects of the pretreatment with intravenous one-shot administration of L-NAME (10^{-6} M, 0.3 ml, several seconds) on the intravenous one-shot administration of serotonin (10^{-6} M, 0.3 ml, several seconds)-mediated increase in the jejunal-originated mesenteric lymph volume with permeant albumin.

Figure $1A_1$ and A_2 show representative recordings of the effects of serotonin on the jejunal-originated mesenteric lymph volume collected over 15 min interval in the absence (Fig $1.A_1$) or presence of L-NAME (10^{-6} M, Fig $1A_2$). Serotonin increased the mesenteric lymph volume collected at 0-15 min and 15-30 min periods after the administration. These data are summarized in Figure

$1B_1$. All values were normalized with the measured control lymph volume. The effects of serotonin on the concentration of albumin in the lymph were summarized in Figure $1C_1$. All values were normalized with the concentration of albumin in the control lymph.

Surprisingly, the pretreatment with one-shot intravenous administration of L-NAME (10^{-6} M, 0.3 ml, several seconds) markedly decreased mesenteric lymph volume (Fig. $1A_2$). In addition, the pretreatment with L-NAME also inhibited the serotonin-mediated increase in the lymph volume. The data are presented Figure $1B_2$. Thus, the L-NAME produced a significant reduction of the lymph volume collected during 15-60 min after the administration of L-NAME.

Consistent with changes in the lymph volume, the L-NAME also significantly decreased the concentration of albumin in the lymph collected during 0-15 min after the administration of L-NAME (Fig. $1C_2$). In contrast, under the pretreatment with L-NAME, serotonin produced no significant increase in the concentration of albumin in the lymph. All values were normalized with the control concentration of albumin in the lymph (Fig. $1C_2$).

L-NAME produced the reduction of jejunal-originated lymph volume and the concentration of albumin in the lymph

To evaluate the roles of endogenous NO in lymph formation with permeant albumin at normal physiological condition in the jejunum, the effects of intravenous one-shot administration of L-NAME (10^{-6} M, 0.3 ml, several seconds) on the mesenteric lymph volume collected over each 15 min interval and the concentration of albumin in the lymph were investigated. Figure $2A_1$ shows a representative recording of the effect of L-NAME on the mesenteric lymph volume. Intravenous administration of L-NAME caused a marked reduction in the lymph volume collected during 0-60 min after the administration of L-NAME. The data are summarized in Figure $2B_1$. The intravenous one-shot administration of 10^{-6} M L-NAME produced a significant reduction of the mesenteric lymph volume. Consistent with the response of lymph volume, the concentration of albumin in the lymph was also significantly reduced following the intravenous administration of L-NAME (10^{-6} M, 0.3 ml, several seconds). The data are summarized in Figure $2C_1$. In this experiment only, the number of experiments (n=6) was increased in order to confirm the most important finding of L-NAME.

In contrast, in absence of L-NAME no significant changes in the mesenteric lymph volume and the concentration of albumin in the lymph were observed. Figure $2A_2$ demonstrates a representative recording of the mesenteric lymph volume collected over each 15 min. The summarized data of the lymph volume is shown in Fig. $2B_2$. Fig. $2C_2$ is the summarized data of the concentration of albumin.

Effects of L-NAME on water intake-mediated increase in mesenteric lymph volume and the concentration of albumin in the lymph

Basis on the above findings on the effects of the intravenous one-shot administration of L-NAME, we next aimed to evaluate the effects of changes in blood flow through the portal vein on the mesenteric lymph volume and the concentration of albumin in the lymph. Because there are the well-

known findings that blood and lymph flow-mediated shear stress stimulation on the endothelial cells regulate the production and release of NO from the cells (1,9,18). Additionally, we currently demonstrated that water intake (3 ml) significantly increased rat portal venous blood and jejunal-originated mesenteric lymph volume (10). Thus, we investigated to evaluate the effects of intravenous pre-treatment with L-NAME (10^{-6} M, 0.3 ml, several seconds) on water intake-mediated increase in mesenteric lymph volume and the concentration of albumin in the lymph were investigated.

Figure $3A_1$ and A_2 show representative recordings of the effects of water intake (3 ml) on mesenteric lymph volume collected over each 15 min interval in the presence (Fig $3A_2$) or absence (Fig. $3A_1$) of L-NAME (10^{-6} M, 0.3 ml, several seconds). The lymph volume collected during the first 15 min after water intake increased markedly and then returned to the control volume approximately 15-30 min after water intake. The summarized data is shown in Figure $3B_1$. Consistent with the findings in lymph volume, the concentration of albumin in the lymph increased only during the 0-15 min after the water intake (Fig. $3C_1$).

In agreement with Figs. $1A_2$ and 2A, L-NAME markedly decreased the mesenteric lymph volume (Fig. $3A_2$). The data are summarized in Figure $3B_2$. Thus, the intravenous administration of L-NAME produced a significant reduction of the mesenteric lymph volume collected during 0-15 min after the administration of L-NAME. Consistent with changes in the lymph volume, the L-NAME also significantly deceased the concentration of albumin in the lymph (Fig. $3C_2$).

In addition, pretreatment with L-NAME produced a significant decrease in the water intake-mediated responses (Fig. 3 A_2 and B_2). Thus, L-NAME reduced completely the water intake-mediated increase in lymph volume. All values were normalized with the control lymph volume collected over 15 min interval. The water intake-mediated increase in the concentration of albumin was also inhibited by the pretreatment with L-NAME. (Fig. $3C_2$).

Effects of intravenous administration of a Ca^{2+} ionophore, A23187 on the mesenteric lymph volume and the concentration of albumin in the lymph

Endogenous NO produces the relaxation of smooth muscle cells through the activation of cyclic GMP and the resulting extrusion of calcium ions from the cells (1,9,18). Based on this evidence, we examined the effects of intravenous one-shot administration of A23187 (10^{-6} M, 0.3ml, several seconds) on the mesenteric lymph volume and the concentration of albumin in the lymph. Figure $4A_1$ shows a representative recording of the effects of intravenous one-shot administration of A23187 in mesenteric lymph volume collected at each 15 min interval. A23187 produced a marked reduction in the mesenteric lymph volume during the 0-45 min after the administration. The data are summarized in Figure $4B_1$. Consistent with the findings in lymph volume, the concentration of albumin in the lymph was also significantly reduced only at 0-15 min after the administration of A23187. The data are summarized in Figure $4C_1$.

The intravenous one-shot administration of ethanol (10^{-6} M, 0.3 ml, several seconds) produced no significant effect on the mesenteric lymph volume. The lymph volume is 118.2 ± 19.6 µl/15 min in the control. The lymph volume obtained during 0-15, 15-30, 30-45 and 45-60 min after the intravenous administration of ethanol are 108.0 ± 5.6%, 100.4 ± 9.5%, 98.6 ± 6.6%, and 101.2 ± 4.6% of the control, respectively (NS vs the control, n=4 in each case).

Effects of intravenous administration of a Ca^{2+} antagonist, nicardipine on the mesenteric lymph volume and the concentration of albumin in the lymph

To confirm the above-mentioned findings of the effects of A23187, we examined the effects of intravenous one-shot administration of nicardipine on the mesenteric lymph volume collected over each 15 min interval and the concentration of albumin in the lymph. Figure $4A_2$ shows a representative recording of the effects of the intravenous administration of nicardipine (10^{-6} M, 0.3 ml, several seconds) on mesenteric lymph volume. In contrast to the A23187 response, the nicardipine markedly increased the mesenteric lymph volume during the 0-45 min after the administration of nicardipine. The data are summarized in Figure $4B_2$. Consistent with the findings of lymph volume, the concentration of albumin in the lymph increased significantly only during the 0-15 min after the administration of nicardipine. However, the albumin concentration in the lymph volume collected during the 15-45 min after the administration of nicardipine significantly decreased. The data are summarized in Figure $4C_2$.

Effects of intravenous administration of a selective inhibitor of MLCK, ML-7 on the mesenteric lymph volume and the concentration of albumin in the lymph

To evaluate the role of Ca^{2+} ions and MLCK in the venular endothelial cells, we investigated the effects of intravenous one-shot administration of ML-7 (10^{-6} M, 0.3 ml, several seconds) on the lymph volume and the concentration of albumin in the lymph. Figure 5A shows a representative recording of the effects of ML-7 on mesenteric lymph volume collected over each 15 min interval. Figure 5B shows the summarized data. Consistent with the findings in lymph volume, the concentration of albumin in the lymph significantly decreased during the 0-30 min after the administration of ML-7. The data are summarized in Figure 5C.

Discussion

Nitric oxide plays a key role in the serotonin-mediated lymph formation in rat jejunal microcirculation

Previously, we demonstrated that water intake produced serotonin release from enterochromaffin cells in rat jejunum, which is mainly transported through the portal vein into systemic blood circulation. Serotonin in blood contributes to increased mesenteric lymph formation with permeant albumin in rat jejunal villi (10). In the present study, the similar findings were confirmed that the intravenous one-shot administration of serotonin (10^{-6} M, 0.3 ml, several seconds) increased tentatively the jejunal-originated mesenteric lymph volume and the concentration of albumin approximately 0-15 min after the administration (Fig. 1). Michel et al. (12) estimated that 0.26 mM serotonin increased the permeability of water-soluble macromolecules by opening in the venular endothelium of the microcirculation in the small intestine; the estimation was conducted using the theoretical value of hydraulic permeability and oncotic pressure in rat mesenteric venules. However, the mechanisms of serotonin-mediated increase in mesenteric lymph volume and permeant albumin in the jejunal microcirculation remains unknown. In the present study, the intravenous one-shot administration of L-NAME (10^{-6} M, 0.3 ml, several seconds) significantly suppressed the serotonin-mediated increase in mesenteric lymph volume with permeant albumin (Fig. 1). These findings suggest that the endogenous NO may contribute partly to the serotonin-mediated increase in lymph formation in the rat jejunal microcirculation. In fact, serotonin has been shown to produce an endogenous NO-dependent relaxation of the smooth muscles in rat lymph nodes (13). On the other hand, serotonin is one of most sensitive vasoconstrictor substances for the smooth muscles in rat mesenteric lymph vessels, resulting in inhibition of mesenteric lymph transport with 10^{-6} M serotonin (15,17).

Portal blood flow-dependent endogenous NO release physiologically contributes to the lymph formation in rat jejunum

It is noteworthy that the intravenous one-shot pretreatment with L-NAME (10^{-6} M, 0.3 ml, several seconds) significantly decreased the jejunal-originated lymph volume with permeant albumin under physiological conditions (Fig. 2). In addition, no or few changes in the jejunal-originated lymph volume collected over each 15 min interval were observed in the control condition with no drug. The finding may be the first demonstration in the field of jejunal microcirculation. In addition, water intake, which significantly increases the portal venous blood flow several times around 0-15 min after the intake (10), and produces simultaneously the increase of the jejunal-originated lymph volume (10). In the present experiment, the water intake-mediated increase in jejunal lymph volume with permeant albumin was significantly reduced by the intravenous one-shot administration of L-NAME (10^{-6} M, 0.3 ml, Fig. 3). These findings suggest that the portal blood flow may

physiologically regulate lymph formation with permeant albumin in rat jejunum through the action of the endogenous NO released from venular endothelial cells.

In fact, the shear stress stimulation on the endothelial cells produced by blood or lymph flow is well known to release the endogenous NO from these cells via the activation of endothelial constitutive NOS (ecNOS, 1,9,18,21,25,27). In particular, the lymphatic endothelial cells release the endogenous NO at lower levels of shear stress stimulation compared to arterial blood flow (2,18). Thus, mesenteric lymph endothelial cells are sensitive to small amounts of lymph flow induced by spontaneous heart-like contractions *in vivo* rat experiment (21). In addition, the shear stress stimulation with 0.5-2 dyn/cm^2 accelerates the expression of ecNOS in the cultured human lymphatic endothelial cells (9). However, there is no or few studies have evaluated the *in vivo* effects of shear stress stimulation on venular endothelial cells in jejunal microcirculation. Further investigation is needed to evaluate the effects of shear stress stimulation on venular endothelial cells in the jejunum.

Over all, it is well known in lymphatic system that the endothelial cells produce and release NO physiologically through lymph flow stimulation or vasoactive substances such as acetylcholine and serotonin, which results in the relaxation of lymphatic smooth muscles via the activation of cyclic GMP-dependent Ca2+ extrusion from lymphatic smooth muscles (13,18,19). In addition, the slow inward current-dependent Ca^{2+} influx contributes to appearance of spontaneous contractions of lymphatic smooth muscles. Thus, the calcium antagonist, nicardipine inhibits the appearance of spontaneous contractions (19).

Endogenous NO, calcium ions, and myosin light chain kinase in venular endothelial cells physiologically contribute to lymph formation with permeant albumin in rat jejunum

Another important aspect of the present study is that the calcium ionophore, A23187 and the myosin light chain inhibitor, ML-7 significantly decreased the jejunal-originated lymph volume with temporary reduction of albumin concentration in the lymph (Figs. 4&5). In contrast, the calcium antagonist, nicardipine significantly increased mesenteric lymph volume and temporarily increased the concentration of albumin in the lymph. The finding of ML-7 is consistent with the classical studies that suggest myosin light chain phosphorylation contributes to the modulation of basal and agonist-stimulated coronary venular permeability (32) and the neutrophil-stimulated coronary microvascular leakage (30). In addition, an important study was reported that myosin light-chain activation-mediated myosin light-chain phosphorylation can induce the contraction of venular endothelial cells (29). However, there are no or few studies which evaluate the effects of A23187 and nicardipine on the jejunal lymph formation with permeant albumin in rat *in vivo* experiments. In contrast, calcium ions and nicardipine are well known to modulate the contractility of vascular and lymphatic smooth muscle cells (11,19,26,29). Ultrastructural studies have also suggested that leukotriene E_4 selectively increases vascular permeability by producing the contraction of endothelial cells selectively, in the postcapillary venules (7). Another key study has demonstrated that the

elevation of endothelial cytosolic calcium is an early signaling event preceding NO synthesis in the transduction pathway of endothelial hyperpermeability (6). The findings with these classical studies may support the conclusion in the present experiments that the endogenous NO and activation of cyclic GMP-dependent extrusion of calcium ions from the endothelial cells in the venules of jejunal villi contributes to the regulation of the contractility of the endothelial cells, and then an increase in jejunal lymph formation with permeant albumin. Surprisingly, the intravenous administration of nicardipine significantly increased the jejunal-originated mesenteric lymph volume and the concentration of albumin in the lymph at only 0-15 min after the administration, but the administration of nicardipine oppositely produced a significant decrease in the concentration of albumin in the lymph between 15 and 45 min after the administration. The concentration of albumin in the lymph was measured using the mesenteric lymph volume collected over each 15 min interval. Thus, the nicardipine-mediated large amounts of increase in lymph volume may contribute to the decreased concentration of albumin during the 15-45 min. We should consider that the concentration of albumin in the present experiments may be related to both the permeability of albumin through venular endothelial cell layers and the lymph volume collected over the 15 min interval.

In the present study, it is summarized that portal venous blood flow-dependent or serotonin in blood circulation-mediated NO release from venular endothelial cells play physiologically key roles in the lymph formation with permeant albumin in rat jejunum. In addition, except for the present study, no study has evaluated the lymph formation with permeant albumin by *in vivo* direct measurement of jejunal-originated lymph volume and the concentration of albumin in the lymph.

In conclusion, the intravenous one-shot administration of serotonin and water intake-mediated increase in portal blood flow significantly increased the mesenteric lymph volume with permeant albumin in rat jejunal microcirculation. Jejunal lymph formation was significantly reduced by the intravenous pretreatment with L-NAME. Intravenous one-shot administration of L-NAME itself decreased the jejunal lymph formation physiologically. Intravenous administration of A23187 and ML-7 significantly reduced jejunal lymph formation. In contrast, the intravenous administration of nicardipine significantly increased the lymph formation. Thus, portal venous blood flow-dependent or serotonin circulated through blood-mediated NO release from venular endothelial cells play physiologically key roles in the lymph formation with permeant albumin in rat jejunum via the extrusion of calcium ions and inactivation of MLCK in the endothelial cells. The graphical abstract (Fig. 6) summarizes the conclusions of these experiments. However, no or few studies to evaluate the effects of NO, A23187, nicardipine, and ML-7 on the contractility of pericyte. Further investigation will be needed in the future to investigate the contractile properties of pericytes.

AUTHOR CONTRIBUTION

T.O. wrote the manuscript, designed the experiments, and analyzed the data. Y.K., M.H., and T-W-A. designed the experiments, analyzed the data, and revised manuscript. K.A., R.K., N.A., K.A., D.M., Y.Y., and M.K., performed the experiments, and analyzed data. All authors approved the final version of the manuscript and accepted for the publication of this manuscript.

DATA AVAILABILITY

All relevant data are available from the corresponding author on request.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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Figure legends

Figure 1

A_1: A representative photomicrograph of the effect of intravenous administration of serotonin (10^{-6} M, 0.3 ml) in rat jejunal-originated mesenteric lymph volume collected over 15 min intervals. B_1: The summarized data of the effect of serotonin on the mesenteric lymph volume. All values are normalized with the control lymph volume (100% = 112.5 $\pm$ 14.8 μl/15 min, n=4). **p < 0.01; NS, not significant.

C_1: The summarized data of the effect of serotonin on the concentration of albumin in the collected lymph volume. All values are normalized with the control concentration of albumin in collected

lymph volume (100% = 10.0 ± 0.7 mg/ml, n=4). *p<0.05; NS, not significant.

A_2: A representative photomicrograph of the effect of intravenous administration of L-NAME (10^{-6}M, 0.3 ml) on the serotonin-mediated responses of the lymph volume collected over15 min intervals. B_2: The summarized data of the effect of L-NAME on the serotonin-mediated responses of lymph volume. The ordinate is the same one as B_1 (100% = 112.5 ± 14.8 µl/15 min, n=4). **p < 0.01; NS, not significant.

C2: The summarized data of L-NAME on the serotonin-mediated responses of the concentration of albumin in collected lymph volume. The ordinate is the same one as C_1 (100% = 10.0 ± 0.7 mg/ml, n=4). **p < 0.01; NS, not significant.

Figure 2

$2A_1$: A representative photomicrograph of the effect of intravenous administration of L-NAME (10^{-6} M, 0.3 ml) on the mesenteric lymph volume collected over 15 min intervals. $2B_1$: The summarized data of the effect of L-NAME on the mesenteric lymph volume. All values are normalized with the control lymph volume (100% = 118.9 ± 18.6 µl/15 min, n=6). **p<0.01. $2C_1$: The summarized data of the effect of L-NAME on the concentration of albumin in collected lymph volume (100% = 11.6 ± 0.4 mg/ml n=6). **p<0.01, *p<0.05.

$2A_2$: A representative photomicrograph of the mesenteric lymph volume collected over each 15 min in the absence of L-NAME. $2B_2$: The summarized data of the lymph volume. (n=4 in each) in the absence of L-NAE. $2C_2$: The summarized data of the concentration of albumin in the lymph (n=4 in each).

Figure 3

A_1: A representative photomicrograph of the effect of intragastric administration of distilled water (DW, water intake, 3 ml) on the mesenteric lymph volume collected over 15 min intervals. B_1: The summarized data of the effect of DW (water intake) on the mesenteric lymph volume. All values are normalized with control lymph volume (100% = 97.5 ± 11.3 µl/15 min, n=4). *p<0.05; NS, not significant. C_1: The summarized data of the effect of DW on the concentration of albumin in collected lymph volume (100% = 10.7 ± 0.3 mg/ml n=4). *p<0.05; NS, not significant.

A_2: A representative photomicrograph of the effect of intravenous administration of L-NAME (10^{-6} M, 0.3 ml) on the DW-mediated responses of the lymph volume collected over 15 min intervals. B_2: The summarized data of the effect of L-NAME on the DW-mediated responses of the lymph volume. All values are normalized with control lymph volume (100% = 116.3 ± 13.3 µl/15 min, n=4). **p<0.01; NS, not significant. C_2: The summarized data of the effect of L-NAME on the DW-mediated responses of the concentration of albumin in the collected lymph volume (100% = 9.3 ± 0.3 mg/ml n=4). **p<0.01; NS, not significant.

Figure 4

A_1: A representative photomicrograph of the effect of intravenous administration of a Ca^{2+}

ionophore, A23187 (10^{-6} M, 0.3 ml) on the mesenteric lymph volume collected over 15 min intervals. B_1: The summarized data of the effect of A23187 on the mesenteric lymph volume. All values are normalized with control lymph volume (100% = 116.9 $\pm$ 20.6 µl/15 min, n=4). **p<0.01. C_1: The summarized data of the effect of A23187 on the concentration of albumin in the collected lymph volume (100% = 12.6 $\pm$ 0.6 mg/ml n=4). **p<0.01; NS, not significant.

A_2: A representative photomicrograph of the effect of intravenous administration of Ca^{2+} antagonist, nicardipine (10^{-6} M, 0.3 ml) on the mesenteric lymph volume collected over 15 min intervals. B_2: The summarized data of the effect of nicardipine on the mesenteric lymph volume. All values are normalized with control lymph volume (100% = 101.3 $\pm$ 12.9 µl/15 min, n=4). **p<0.01. C2: The summarized data of the effect of nicardipine on the concentration of albumin in the collected lymph volume (100% = 9.6 $\pm$ 0.4 mg/ml n=4). **p<0.01; *p<0.05.

Figure 5

A: A representative photomicrograph of the effect of an inhibitor of myosin light chain kinase, ML-7 on the mesenteric lymph volume collected over 15 min intervals. B: The summarized data of the effect of ML-7 on the mesenteric lymph volume. All values are normalized with control lymph volume (100% = 112.9 $\pm$ 18. µl/15 min, n=4). **p<0.01. C: The summarized data of the effect of ML-7 on the concentration of albumin in the collected lymph volume (100% = 11.6 $\pm$ 0.8 mg/ml n=4). **p<0.01; NS, not significant.

Figure 6

The graphical abstract summarizes the conclusions of these experiments.

Fig1

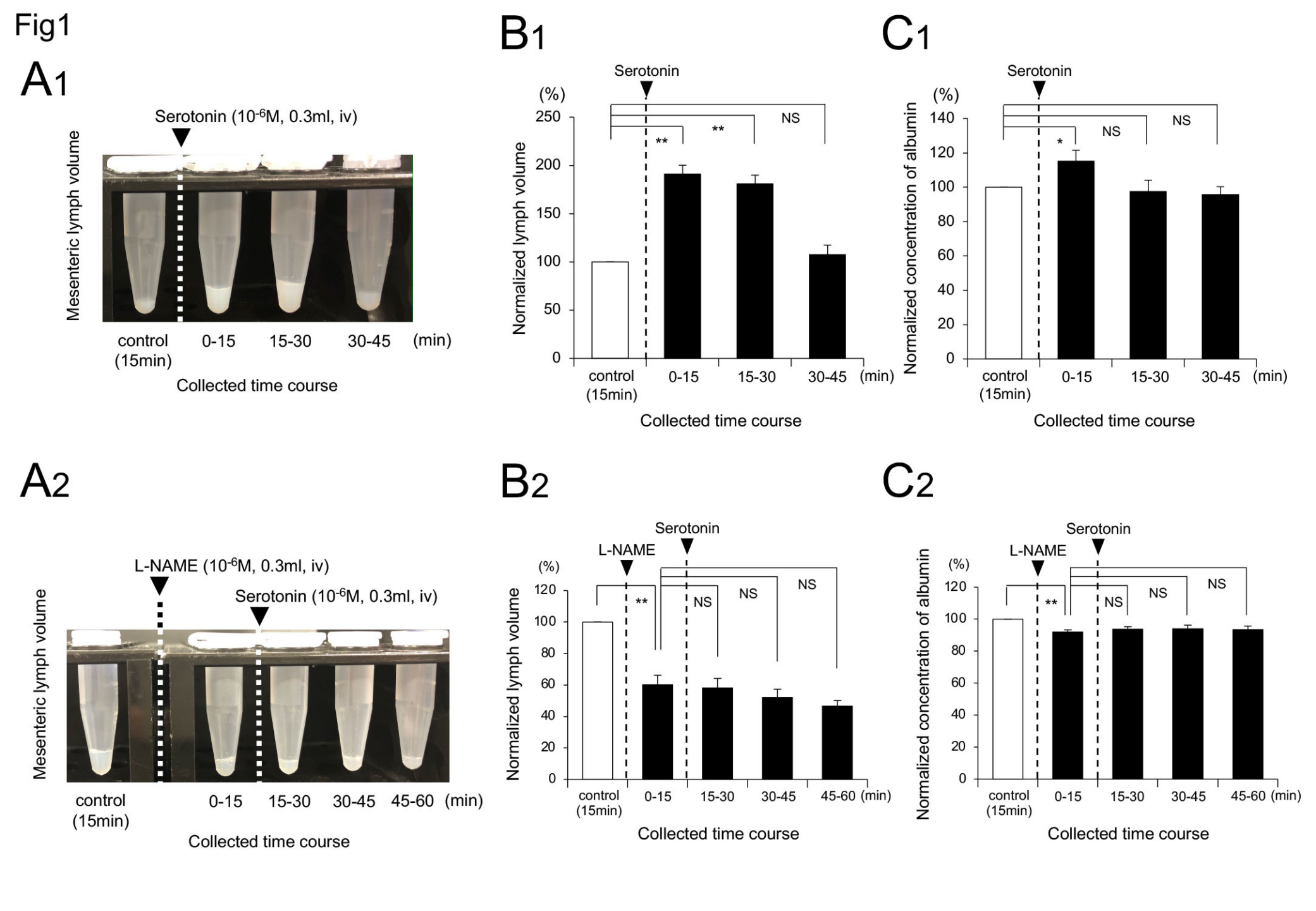
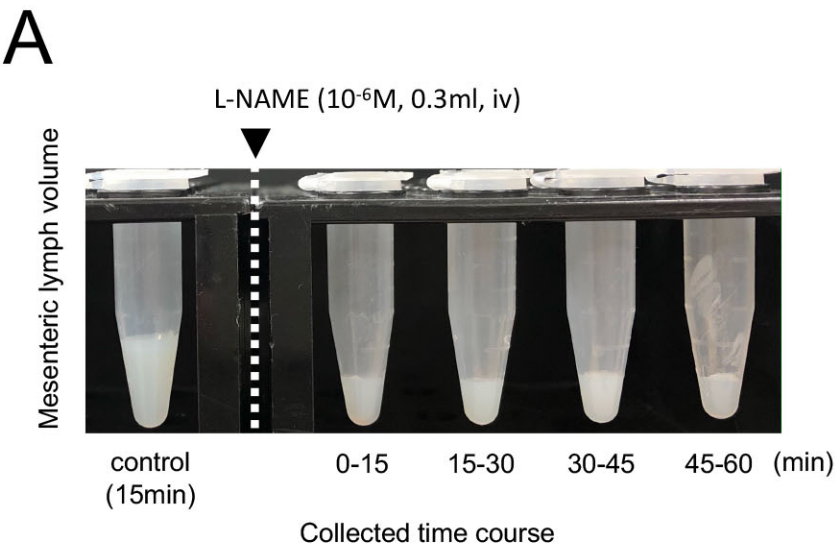
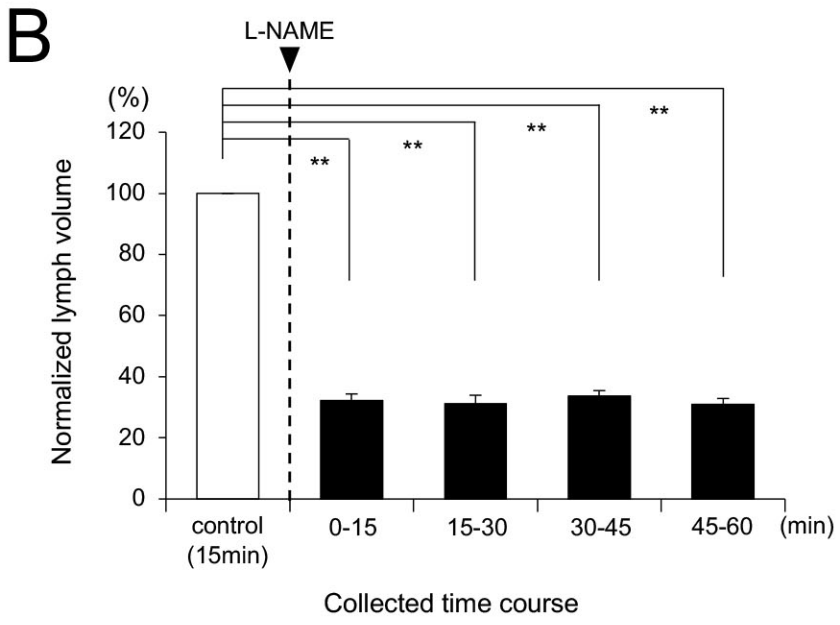

A1
Serotonin (10-6M, 0.3ml, iv)
Mesenteric lymph volume
control (15min)
0-15
15-30
30-45
(min)
Collected time course
B1
Serotonin
(%)
Normalized lymph volume
250
200
150
100
50
0
**
**
NS
control (15min)
0-15
15-30
30-45
(min)
Collected time course
C1
Serotonin
(%)
Normalized concentration of albumin
140
120
100
80
60
40
20
0
*
NS
NS
control (15min)
0-15
15-30
30-45
(min)
Collected time course
A2
L-NAME (10-6M, 0.3ml, iv)
Serotonin (10-6M, 0.3ml, iv)
Mesenteric lymph volume
control (15min)
0-15
15-30
30-45
45-60
(min)
Collected time course
B2
L-NAME
Serotonin
(%)
Normalized lymph volume
120
100
80
60
40
20
0
**
NS
NS
NS
control (15min)
0-15
15-30
30-45
45-60
(min)
Collected time course
C2
L-NAME
Serotonin
(%)
Normalized concentration of albumin
120
100
80
60
40
20
0
**
NS
NS
NS
control (15min)
0-15
15-30
30-45
45-60
(min)
Collected time course

Fig2


A
L-NAME (10-6M, 0.3ml, iv)
Mesenteric lymph volume
control
(15min)
0-15
15-30
30-45
45-60
(min)
Collected time course


B
L-NAME
(%)
120
100
80
60
40
20
0
Normalized lymph volume
**
**
**
**
control
(15min)
0-15
15-30
30-45
45-60
(min)
Collected time course

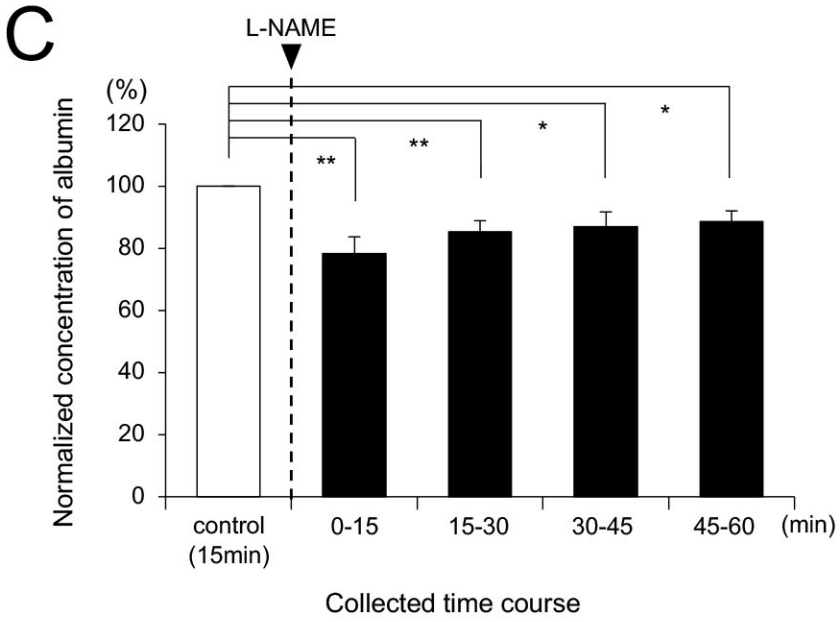

C
L-NAME
(%)
120
100
80
60
40
20
0
Normalized concentration of albumin
**
**
*
*
control
(15min)
0-15
15-30
30-45
45-60
(min)
Collected time course

Fig3

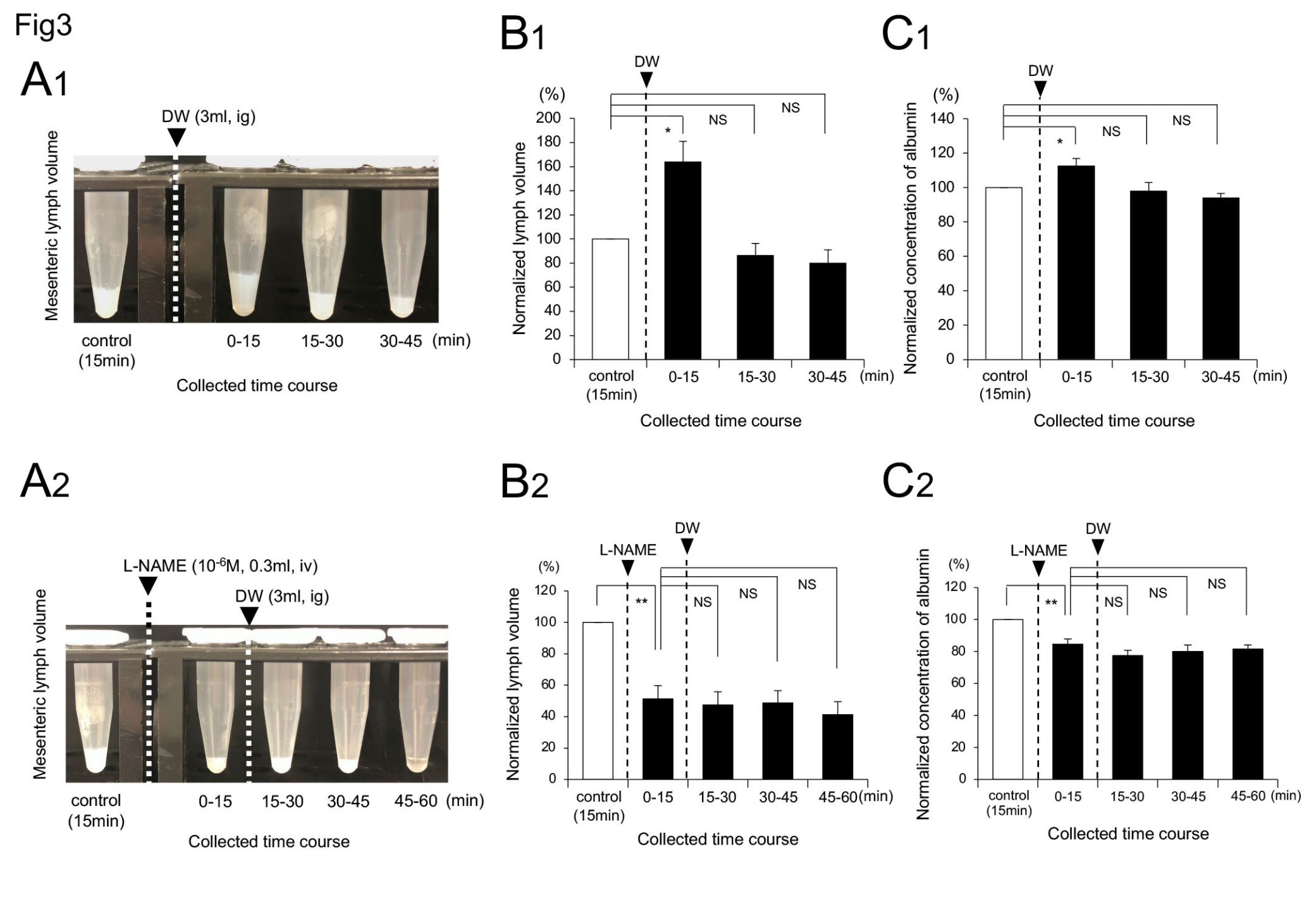
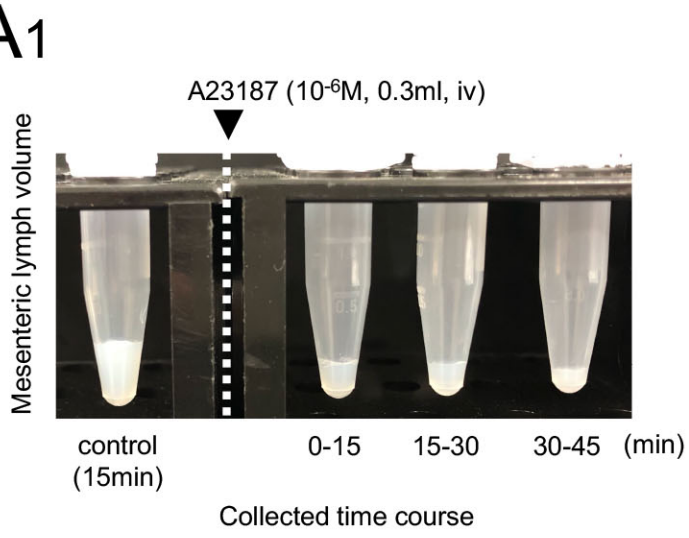
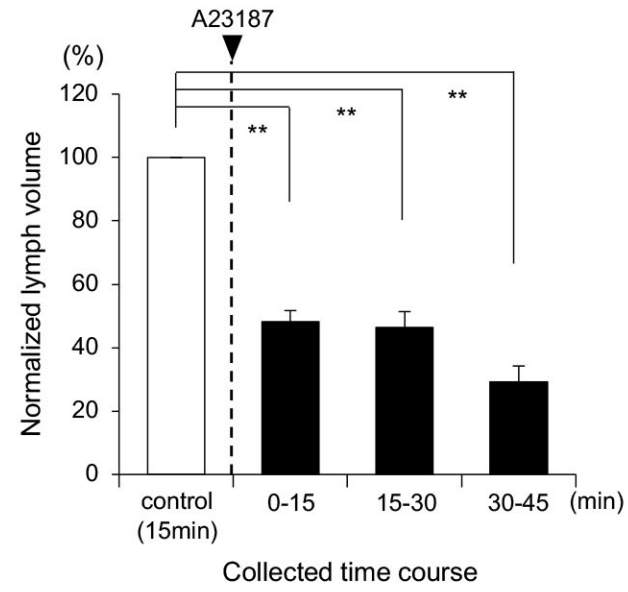
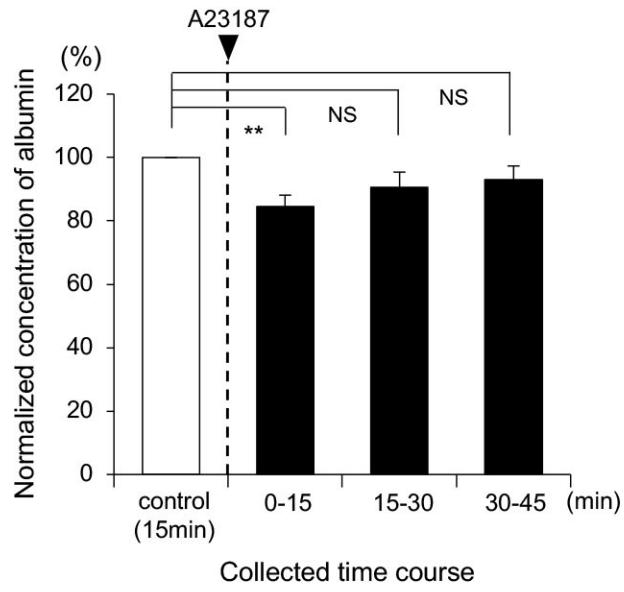
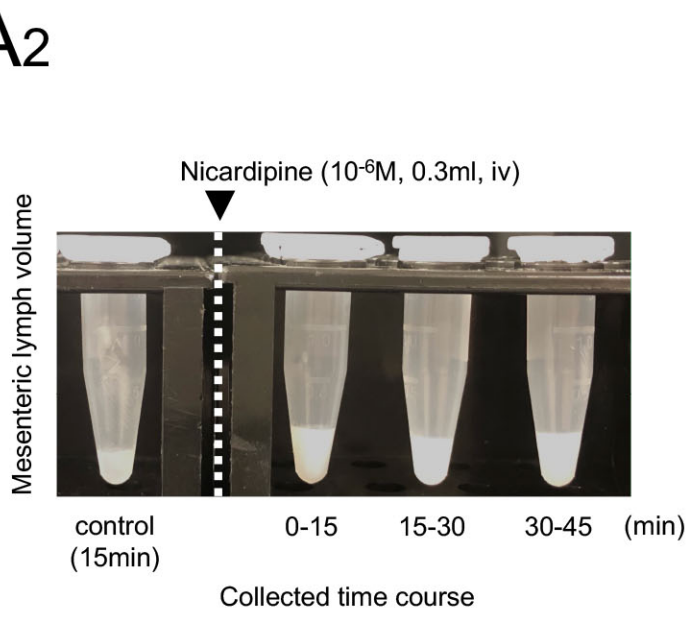

A1
DW (3ml, ig)
Mesenteric lymph volume
control (15min)
0-15
15-30
30-45 (min)
Collected time course
B1
DW
(%)
Normalized lymph volume
NS
*
control (15min)
0-15
15-30
30-45 (min)
Collected time course
C1
DW
(%)
Normalized concentration of albumin
NS
*
control (15min)
0-15
15-30
30-45 (min)
Collected time course
A2
L-NAME (10-6M, 0.3ml, iv)
DW (3ml, ig)
Mesenteric lymph volume
control (15min)
0-15
15-30
30-45
45-60 (min)
Collected time course
B2
L-NAME
DW
(%)
Normalized lymph volume
**
NS
control (15min)
0-15
15-30
30-45
45-60(min)
Collected time course
C2
L-NAME
DW
(%)
Normalized concentration of albumin
**
NS
control (15min)
0-15
15-30
30-45
45-60 (min)
Collected time course

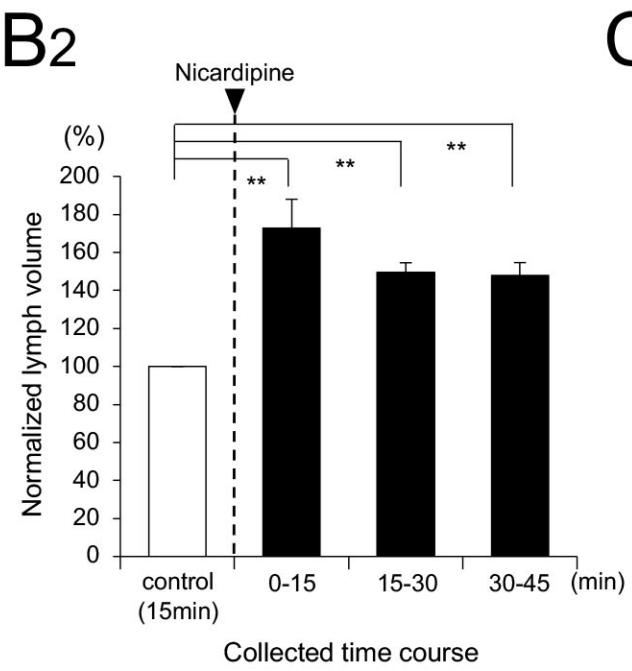
Fig4


A1
A23187 (10-6M, 0.3ml, iv)
Mesenteric lymph volume
control (15min)
0-15
15-30
30-45
(min)
Collected time course


B1
A23187
(%)
Normalized lymph volume
**
control (15min)
0-15
15-30
30-45
(min)
Collected time course


C1
A23187
(%)
Normalized concentration of albumin
**
NS
control (15min)
0-15
15-30
30-45
(min)
Collected time course


A2
Nicardipine (10-6M, 0.3ml, iv)
Mesenteric lymph volume
control (15min)
0-15
15-30
30-45
(min)
Collected time course


B2
Nicardipine
(%)
Normalized lymph volume
**
control (15min)
0-15
15-30
30-45
(min)
Collected time course

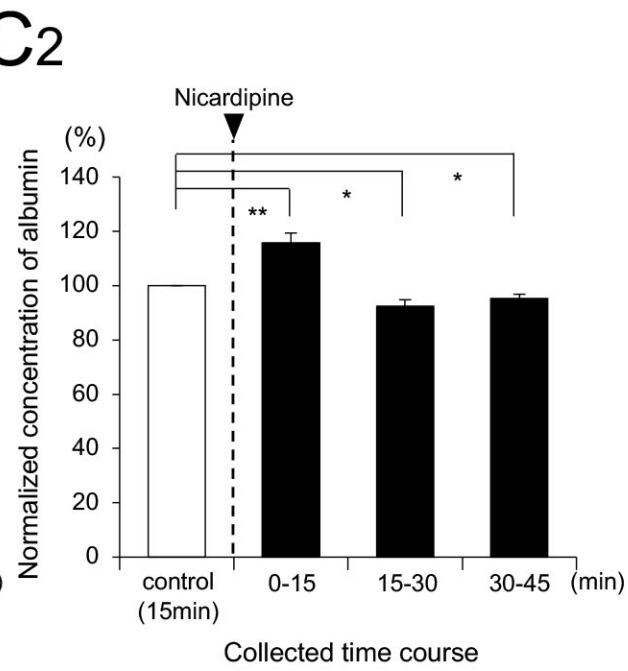

C2
Nicardipine
(%)
Normalized concentration of albumin
**
*
control (15min)
0-15
15-30
30-45
(min)
Collected time course

Fig5

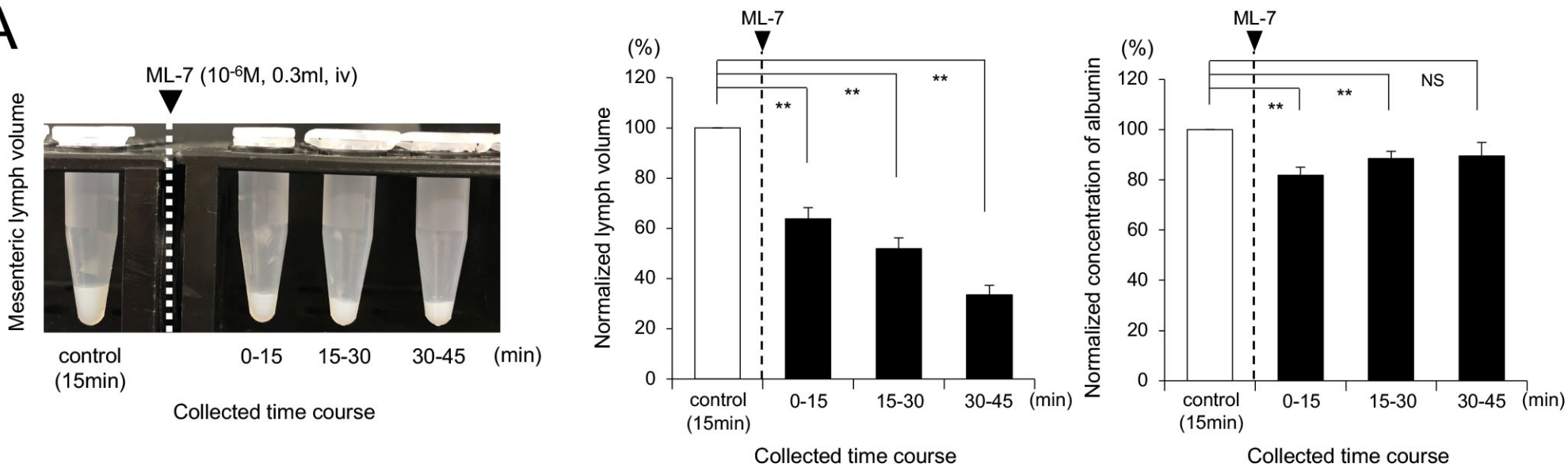
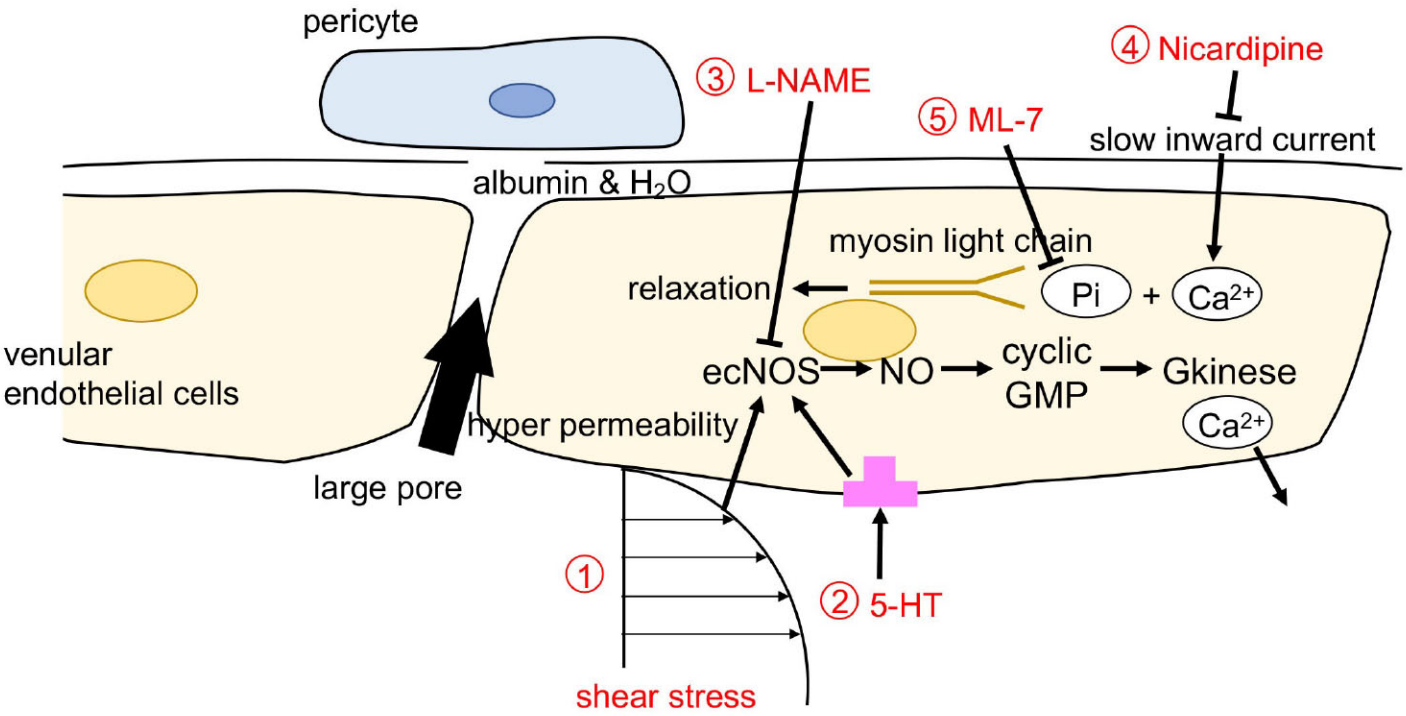

A
ML-7 (10-6M, 0.3ml, iv)
Mesenteric lymph volume
control (15min)
0-15
15-30
30-45
(min)
Collected time course
B
ML-7
(%)
Normalized lymph volume
120
100
80
60
40
20
0
**
**
**
control (15min)
0-15
15-30
30-45
(min)
Collected time course
C
ML-7
(%)
Normalized concentration of albumin
120
100
80
60
40
20
0
**
**
NS
control (15min)
0-15
15-30
30-45
(min)
Collected time course

Fig6

A Hyper permeability for albumin in jejunum



ecNOS : endothelial constitutive NO syntase

NO : nitric oxide

B Reduced permeability for albumin in jejunum