The main physiological role of the veins is to adjust the capacity of the vascular system, ensuring an appropriate return of blood to the heart. The veins are also a determinant of capillary pressure, ensuring a favorable internal environment for cells of the body. Thus, the veins can behave as a passive reservoir due to the visco-elastic components of their wall and the presence of valves in the system [1, 2]. Venous smooth muscle tone plays a crucial role in the visco-elastic behavior of the wall, thereby significantly affecting the amount of blood that is passively mobilized from the reservoir towards the heart [3]. Venous smooth muscle tone is modified by nerves as well as humoral factors [2, 4]. Eicosanoids are especially important regulatory factors in normal venous circulation and in the response of veins to thrombotic diseases [5].

It is well known that the addition of plasma to the physiological salt solution in which helical strips of arterial smooth muscles are suspended causes the muscle to contract. In addition, plasma concentrations ranging from 2 to 30% produce a marked potentiation of noradrenaline (NA)-induced contraction in arterial strips [6, 7]. Numerous investigators have shown that...
lysophosphatidylcholine (lysoPC) is one of the agents responsible for plasma-induced arterial contraction. For example, the lysoPC in oxidized low-density lipoprotein (LDL) elicits vasoconstriction and inhibits endothelium-dependent relaxation [8]. Little information, however, exists regarding the potential effect of plasma or lysoPC on the mechanical activity of venous smooth muscles. In the present study, we examined whether or not normal plasma can enhance the contractility of venous smooth muscles, cardiac muscles, and arterial smooth muscles. The second goal of the study was to test the hypothesis that lysoPC, similar to normal plasma, may contribute to the regulation of contractile responses of venous smooth muscles to eicosanoids.

**MATERIALS AND METHODS**

**Tissue preparation.** Seventeen adult dogs of either sex, weighing 6.0–17.0 kg were anaesthetized with pentobarbital sodium (25 mg/kg, i.v.) and exsanguinated from the femoral vein. Before exsanguination, heparin (500 U/kg) was intravenously administered to all animals. External jugular veins were rapidly dissected out and cleaned of surrounding tissues. Ring segments (3 mm in width) were cut from the middle portion of the external jugular veins. The other tissues from these animals were used in other studies. All animals were obtained from a licensed source (Nihon-Nousan-Kougyou, Yokohama, Japan). The experimental protocols were approved by the Animal Ethics Committee, Shinshu University School of Medicine, in accordance with Physiological Sciences of the Physiological Society of Japan.

**Measurement of mechanical activity.** Each segment was suspended vertically in a 10-ml organ bath and perfused at a constant rate (4 ml/min) with Krebs-bicarbonate solution, which was maintained at 37.5±0.5°C and aerated with a gas mixture of 95% O₂ and 5% CO₂ to give a pH of 7.40±0.05. The composition of the solution (in mM) was as follows: NaCl 120.0, KCl 5.9, NaHCO₃ 25.0, NaH₂PO₄ 1.2, CaCl₂ 2.5, MgCl₂ 1.2, and glucose 5.5. A high-potassium Krebs-bicarbonate solution (80 mM K⁺) was prepared by replacing NaCl with equimolar amounts of KCl. The 80 mM high-potassium solution was loaded repeatedly for 15 min to obtain maximal contraction in each venous segment before starting the experiments. The short-time loading of the high-potassium solution was confirmed to produce no chemical denudation of the venous endothelial cells, and there was no significant effect on mechanical responses of the venous segments due to drugs used in the experiments [9].

Two thin silk strings were run through the lumen of a ring segment and formed into two rings. The lower end of the segment was connected to the bottom of the bath. The upper end was connected to the lever of a force-displacement transducer (UL-10, Shinko Tsushin, Tokyo, Japan). The circumferential isometric tension detected by the transducer was amplified and recorded on a direct-writing oscillograph (8K, Sanei Sokki, Tokyo, Japan). The resting tension was set at 0.5 g throughout the experiment, which was optimal for obtaining maximum contractile response in each venous segment to the 80 mM high-potassium Krebs-bicarbonate solution. All segments were allowed to equilibrate for 60 min in the oxygenated bathing medium before the start of the experiment.

**Plasma preparation.** Plasma was obtained from the same experimental dogs with the administration of heparin (500 U/kg), usually fasted for 18 h to avoid a milky plasma. Polyethylene tubing (PE-190, Natume, Japan) was siliconized and inserted in the femoral vein of a dog. Venous blood was allowed to flow freely into siliconized polyethylene test tubes, immediately cooled to 0°C, and centrifuged at 4°C at high speeds (3,000 rpm for 15 min) to remove formed elements, including platelets. The plasma was stored at 0°C in chilled ice.

**Protocols.** After we obtained stable responses to repetitive administration of the high-potassium (80 mM) Krebs-bicarbonate solution, a cumulative concentration-contractile response curve for U46619, a thromboxane A₂ analog, was constructed for each segment by adding the agonist directly to the organ bath via a stepwise increase in concentration of a factor of 10. Ten minutes after perfusion of Krebs-bicarbonate solution containing plasma (1.0 or 0.1% vol/vol), another contractile–response curve for U46619 in the presence of plasma was obtained with the same venous segment in a similar manner. At the end of each experiment, the contractile response to 80 mM high-potassium Krebs-bicarbonate solution was also evaluated in the presence of plasma. The contractile responses to U46619 were also examined in some venous segments from which the endothelial cells had been removed mechanically by passing a silk string through the lumen. The absence of endothelial cells was confirmed histologically by silver staining and pharmacologically with acetylcholine- and histamine-induced vasodilations. Control experiments were done, and concentration–contractile response curves for U46619 were produced in some venous segments before and 10 min after perfusion of the normal (with no plasma) Krebs-bicarbonate solution containing 1,000 U/l heparin and 2×10⁻⁵ M lysoPC. In the other
venous segments, the concentration–contractile response curves for U46619 were achieved in a similar manner before and 10 min after perfusion of Krebs-bicarbonate solution containing 1.0% plasma + $10^{-4}$ M sodium L-ascorbate or 1.0% plasma + $10^{-4}$ M $\alpha$-tocopherol. The degree of contraction induced by U46619 was expressed as a percentage of the high-potassium (80 mM) contraction in each segment. Control experiments were always run without plasma to examine time-dependent changes in the sensitivity of the venous segments to U46619.

The concentration–contractile response curves for NA in the other venous segments with intact endothelium were also constructed in a similar manner to those obtained with U46619 before and 10 min after the perfusion of Krebs-bicarbonate solution containing plasma (1.0% or 0.1% vol/vol). Time-dependent controls for NA-induced contraction were always induced in the absence of plasma.

The concentration–contractile response curves for NA and the contractile responses to 80 mM high-potassium solution were obtained in the presence or absence of normal (with no plasma) Krebs-bicarbonate solution (10-min perfusion) containing 1,000 U/l heparin and $2\times10^{-5}$ M lysoPC.

**Drugs.** The following drugs were used in the present study: 9,11-dideoxy-11$\alpha$,9$\alpha$-epoxymethano prostaglandin F$_{2\alpha}$ (U46619), noradrenaline hydrochloride, sodium L-ascorbate, $\alpha$-tocopherol, and lysoPC (Sigma, St. Louis, MO, USA). The concentrations of the drugs are expressed in terms of the base and at the final organ bath concentrations.

**Statistics.** Experimental values in the text and figures are expressed as the mean±SE. The negative logarithm of ED$_{50}$, the concentration of agonists causing one half of the maximal contraction produced by the agonists ($E_{\text{max}}$), is expressed as the $pD_2$ value. The ED$_{50}$ values were measured in each concentration–response curve with the corresponding $E_{\text{max}}$ values. Firstly, comparisons of concentration–response curves under different treatment were made using two-way analysis of variance (ANOVA) for repeated measurements. Next, for comparison of statistical differences between two groups, such as $pD_2$ and $E_{\text{max}}$ values, Student’s paired or unpaired t-test was used. The difference in means was considered significant at $p<0.05$.

**RESULTS**

**Effects of plasma on U46619-, 80 mM K$^+$–, and NA-induced contractions in dog external jugular veins**

The addition of U46619 in concentrations ranging from $10^{-10}$ to $10^{-6}$ M caused a dose-related contraction in dog isolated external jugular vein with intact endothelium (Fig. 1A). As shown in Fig. 1A, the perfusion of Krebs-bicarbonate solution containing 1.0% plasma caused no significant effect to the basal tone of the venous smooth muscles. In the presence of 1.0% plasma, addition of $10^{-13}$ M U46619 produced a slight contraction of the same venous segment. The $10^{-6}$ M U46619–induced contraction was significantly larger than that produced in the control group (without 1.0% plasma, Fig. 1A). No time-dependent changes in the sensitivity of the venous smooth muscle to U46619 were observed ($n=4$).

In contrast, the perfusion of high-potassium Krebs-bicarbonate solution containing 1.0% plasma caused no significant effect on the 80 mM K$^+$–mediated contraction in the venous segments (Fig. 1B; 2.20±0.44 g in the control group ($n=4$) vs. 2.25±0.43 g with plasma ($n=4$); not significant). One hour after the reperfusion of normal Krebs-bicarbonate solution containing no plasma, administration of the 80 mM K$^+$ solution produced a quite similar contraction to that induced in the control group (Fig. 1B; 2.20±0.44 g in the control group ($n=4$) vs. 2.40±0.40 g with reperfusion ($n=4$); not significant).

Figure 2 summarizes the effects of plasma on the
U46619- or NA-induced contractions in dog isolated external jugular veins with or without intact endothelium. The degree of contraction induced by U46619 or NA is expressed as a percentage of the high-potassium (80 mM) contraction in each segment. As shown in Fig. 2A, pretreatment with 1.0% plasma caused a significant left and upward shift in the concentration–contractile response curve for U46619. Thus, the threshold concentration and ED₅₀ value, the concentration of agonist causing 50% of each maximum response, of the concentration–response curve for U46619 was found in the presence of 1.0% plasma was significantly greater than those produced in the control group (pD₂ value, 7.72 ± 0.10 in the control group (n=4) vs. 8.39 ± 0.20 with plasma (n=4); p<0.05). The maximum response of 10⁻⁶ M U46619–induced contraction in the presence of 1.0% plasma was significantly lower than those produced in the control group (Eₘₐₓ, 137.9 ± 4.0% in the control group (n=4) vs. 165.1 ± 8.5% with plasma (n=4); p<0.01) (100%=2.33 ± 0.29 g, n=4).

A similar left and upward shift of the concentration–contractile response curve for U46619 was found in the venous segments with intact endothelium (Fig. 2B; pD₂ value, 8.10 ± 0.13 in the control group (n=4) vs. 8.49 ± 0.13 with plasma (n=4); p<0.05; Eₘₐₓ, 133.7 ± 4.3% in the control group (n=4) vs. 147.6 ± 5.1% with plasma (n=4); p<0.05) (100%=0.98 ± 0.11 g, n=4).

In contrast, pretreatment with 0.1% plasma caused no significant effect on the concentration–contractile response curve in the venous segment with intact endothelium (Fig. 2C; pD₂ value, 8.23 ± 0.04 in the control group (n=4) vs. 8.25 ± 0.08 with plasma (n=4); not significant; Eₘₐₓ, 126.7 ± 8.9% in the control group (n=4) vs. 119.3 ± 7.1% with plasma (n=4); not significant) (100%=2.66 ± 0.36 g, n=4).

Pretreatment with 1.0% plasma caused no significant effect on the NA-induced contractions in the venous segments with intact endothelium (Fig. 2D; pD₂ value, 5.69 ± 0.12 in the control group (n=4) vs. 5.72 ± 0.08 with plasma (n=4); not significant; Eₘₐₓ, 189 ± 9.0 in the control group (n=4) vs. 197.9 ± 6.7% with plasma (n=4); not significant) (100%=3.04 ± 0.49 g, n=4).

**Effects of lysophosphatidylcholine on U46619-, 80 mM K⁺-, and NA-induced contractions in dog external jugular veins**

Administration with lysoPC in the absence of 1.0% plasma produced no significant effect on the basal mechanical tone of dog isolated external jugular veins with intact endothelium precontracted or not precontracted by 10⁻⁵ M phenylephrine (Fig. 3). In the same venous strip, acetylcholine (ACh) ranging from 10⁻⁸ to 10⁻⁵ M produced a dose-related relaxation and 10⁻⁴ M sodium nitroprusside (SNP) caused a maximal relaxation that was similar to that induced by 10⁻⁵ M ACh.

Figure 4 summarizes the effects of lysoPC on the concentration–contractile response curve for U46619 in the venous segments with intact endothelium. Simi-
lar to the action of plasma, pretreatment with $2 \times 10^{-5}$ M lysoPC caused a significant left and upward shift of the concentration–contractile response curve for U46619. Thus, the pD$_2$ value, $-\log[ED_{50}]$, of the curve in the presence of $2 \times 10^{-5}$ M lysoPC was significantly greater than that obtained in the control group (pD$_2$ value, 8.29±0.16 in the control group vs. 8.66±0.27 with lysoPC (n=4); p<0.05). The maximal U46619 ($10^{-6}$ M)-induced contraction was significantly larger than that obtained in the control group ($E_{\text{max}}$, 161.5±6.0% in the control group (n=4) vs. 179.5±10.2% with lysoPC (n=4); p<0.05) (100%=2.71±0.26 g, n=4).

The same lysoPC-mediated potentiation of the U46619-induced vasoconstriction was observed in the venous segments without intact endothelium (pD$_2$ value, 7.66±0.24 in the control group (n=4) vs. 8.51±0.21 with plasma (n=4); p<0.05; $E_{\text{max}}$, 100.8±2.7 in the control group (n=4) vs. 107.0±2.9% with plasma (n=4); p<0.05 (100%=2.93±0.30 g, n=4).

Pretreatment with 1,000 U/l heparin only, however, produced no significant effect on the concentration–contractile response curve for U46619 in the venous segments with intact endothelium.

In contrast, the administration of $2 \times 10^{-5}$ M lysoPC caused no significant effect on the 80 mM K$^+$-induced contractions in the venous segments with intact endothelium (Fig. 5A). One hour after the reperfusion of normal Krebs-bicarbonate solution containing no lysoPC, another administration of 80 mM K$^+$ solution produced a quite similar contraction to that produced in the control group (Fig. 5A).

Pretreatment with $2 \times 10^{-5}$ M lysoPC caused no significant effect on the NA-induced contractions in the venous segments with intact endothelium (Fig. 5B).

**Effects of L-ascorbate or $\alpha$-tocopherol on U46619-induced contractions in the presence of 1.0% plasma**

The administration of $10^{-4}$ M L-ascorbate or 0.1 mg/ml $\alpha$-tocopherol in the presence of 1.0% plasma caused no significant effect on the basal mechanical tone of dog isolated external jugular veins. Figure 6 summarizes the effects of L-ascorbate or $\alpha$-tocopherol on 1.0% plasma–mediated potentiation of the concentration–contractile response curve for U46619 in the venous segments. Pretreatment with $10^{-4}$ M L-ascorbate (Fig. 6A) or 0.1 mg/ml $\alpha$-tocopherol (Fig. 6B) caused a complete reduction of the plasma-mediated left and upward shift in the concentration–contractile response curve for U46619. Thus, the pD$_2$ and $E_{\text{max}}$ values of the curve obtained in the presence of 1.0% plasma+$10^{-4}$ M L-ascorbate or 1.0% plasma+0.1
Potentiating effect of plasma on U46619-induced contraction in dog external jugular veins. The reasons why dog external jugular vein was used in the present experiment are as follows: (1) The vein is easy to use in in vitro experiments because middle-sized veins without branches can be easily isolated with their long length. (2) It has a favorable amount of smooth muscle layers in the wall, which is suitable for examining the effects of activation of venous smooth muscle cells on the mechanical properties of veins. (3) Its value of incremental volume elasticity in the physiological range of transmural pressure (0–2 mmHg) is known to average the values obtained with dog extremity and trunk veins [3]. The vein may be regarded as having characteristic mechanical and histological properties of middle- or large-sized veins, which seems to play an important role in the regulation of venous return [3].

Our major findings in this study are summarized as follows: (1) Pretreatment with 1.0% plasma in dog isolated external jugular veins with or without intact endothelium caused significant potentiation of U46619-induced contraction, but no effect on the 80 mM K¹⁺- and NA-mediated contractions in venous segments with intact endothelium. (2) Pretreatment with 1.0% plasma produced a significant left and upward shift in the concentration–contractile response curve for U46619. (3) In contrast, treatment with 0.1% plasma induced no significant effect on U46619-mediated contraction in the venous segments with intact endothelium. In conclusion, normal plasma (1.0%) has the ability to selectively enhance the contractile response of venous smooth muscles to a thromboxane A₂ analog, U46619. Enhancement of the venous segments is independent of the biological properties of the endothelial cells. Thus, endothelium-derived relaxing or contractile factors may not be involved with the plasma-mediated potentiation of U46619-induced vasoconstriction in the venous segments.

From a pharmacological point of view with analysis of the concentration–response curve, an increase in efficacy and sensitivity is involved in the plasma-mediated potentiation of contractile response to U46619. Thus, changes in the sensitivity of the thromboxane A₂ receptor and the intracellular calcium handling of venous smooth muscles seem to contribute to the plasma-mediated potentiation of contractile response to U46619.

On the other hand, in the present experiment, pretreatment with 1.0% plasma caused no significant effect on the 80 mM K¹⁺- and NA-mediated contractions of dog isolated external jugular veins with intact endothelium. In rabbit isolated aorta, pretreatment with plasma ranging from 1 to 8% of the final bath volume caused a significant potentiation of adrenaline- and angiotensin-mediated contractions [6, 10]. There is a marked difference in the plasma-induced potentiation...
of the NA-mediated contraction between the aorta and the vein. One of the reasons causing the difference between the aorta and vein may be, in part, the concentration of plasma used in the experiments. Further investigation will be needed to evaluate the reasons why a low concentration of plasma (1.0% plasma) is able to selectively enhance contractile response of the vein to U46619.

**Mechanisms of the potentiating effect of plasma on U46619-induced contraction in dog external jugular vein.** Another important aspect of the present study is that lysoPC (2×10⁻⁵M) produced a mimic enhancement, similar to plasma, of the U46619-induced contractions in dog isolated external jugular veins with or without intact endothelium. The present experiments also demonstrated that pretreatment with lysoPC (2×10⁻⁵M) had no significant effect on the 80 mM K⁺– and NA-mediated contractions in the venous segments with intact endothelium. In addition, treatment with 10⁻⁴M L-ascorbate or 0.1 mg/ml α-tocopherol, being an antioxidant vitamin, in the presence of 1.0% plasma caused a complete reduction in the plasma-mediated enhancement of U46619-induced contractions in the venous segments with intact endothelium.

The elevation of extracellular and intracellular levels of lysoPC has been suggested to play a role in muscle malfunction under conditions such as myocardial arrhythmia [11] and atherosclerosis [12]. Thus, one effect of lysoPC is known muscle hypercontractility [13]. Increased Ca²⁺ sensitivity through a G-protein independent, but protein kinase C–dependent mechanism contributes to lysoPC-mediated hypercontractility of vascular smooth muscles [13].

It is worth noting that the lysoPC-mediated mechanism of muscle hypercontractility [14] seems to contribute, in part, to lysoPC-induced potentiation of U46619-mediated contraction of the isolated veins in the present experiment. In addition, lysoPC is known to dose-dependently produce a sustained increase in intracellular free calcium in cultured vascular smooth muscle cells [15], and to then directly stimulate the activity of protein kinase C in the cells [16]. The lysoPC-mediated increase of intracellular calcium ions in the stores and other mechanisms may contribute to lysoPC-induced potentiation of U46619-mediated contraction of the isolated veins in the present experiment.

Recently, several lines of evidence have indicated that lysoPC, as a prominent phospholipid component of oxidized low-density lipoprotein (OxLDL), may be responsible for various biological activities of OxLDL [17]. On the other hand, L-ascorbate and α-tocopherol are well known to limit LDL oxidation [18, 19]. Moreover, α-tocopherol is known to inhibit protein kinase C in vascular smooth muscle cells [20], and protein kinase C activation has been implicated in vascular diseases due to OxLDL [21]. It is attractive to speculate that lysoPC produced by the oxidation of LDL in normal plasma seems to contribute mainly to the plasma-mediated potentiation of U46619-induced contractions in dog isolated external jugular veins, and that treatment with L-ascorbate or α-tocopherol selectively inhibits the plasma-mediated potentiation of U46619-induced contraction because of selective reduction of the vitamins C and E against oxidative reaction in normal plasma. In the present experiment, Krebs-bicarbonate solution containing 1.0% plasma was always used for oxygenation with 95% O₂ and 5% CO₂. Oxygenation may biochemically enhance oxidation of LDL in the plasma, resulting in the production of lysoPC in the oxygenized Krebs-bicarbonate solution containing plasma. In fact, Wallentin et al. [22] reported that the concentration of lysoPC in the plasma was increased by 6-h incubation in air (20% oxygen) at 37°C. This finding supports our hypothesis that the concentration of lysoPC may have increased in the oxygenated Krebs-bicarbonate solution containing plasma in the present experiments. Further investigations will be needed in the future to biochemically evaluate the concentrations of lysoPC in the present experiments and the cellular mechanisms of plasma-mediated potentiation of U46619-induced vasoconstriction in the venous segments.

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