



Acute Effects of Intravenous Administration of Polyunsaturated Fatty Acids on Blood Pressure and Heart Rate in U46619- and Noradrenaline-infused Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author DC designed the study. Authors DC, SY, YS and FH performed the experiments. Authors DC, KS, KO and YT wrote the first draft of the manuscript. All authors managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Experimental studies and epidemiological surveys have indicated that chronic oral administration of the n-3 polyunsaturated fatty acids (PUFAs) docosahexaenoic (DHA) or eicosapentaenoic (EPA) acids reduces blood pressure in hypertensive patients. However, few reports have described the acute blood pressure lowering effects of these PUFAs. In this study, we determined the acute effects of DHA and EPA on blood pressure of rats with increased blood pressure resulting from continuous injection of pressor substances. U46619 (a TXA₂ receptor agonist) and noradrenaline (NA) were continuously infused (500 µg/kg/h each) into urethane-anesthetized male Wistar rats and produced sustained elevated mean blood pressure (MBP). In both U46619- and NA-infused rats, bolus administration of DHA (3–30 mg/kg, i.v.) reduced blood pressure in a dose-dependent manner, although the MBP reduction was greater in U46619-infused rats. Similarly, administration

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of EPA (3–30 mg/kg, i.v.) also induced a greater reduction in MBP of U46619-infused rats. In contrast, bolus administration of linoleic acid (3–30 mg/kg, i.v.), an n-3 type unsaturated fatty acid, failed to reduce blood pressure in all drug-infused rats. Finally, administration of the nitric oxide donor sodium nitroprusside (0.3–100 µg/kg, i.v.) showed a similar blood pressure drop in all drug-infused rats. These findings clearly indicate that both DHA and EPA induce acute blood pressure reduction in anesthetized rats, and suggest that the blood pressure drop is mediated via the TXA₂ receptor. These characteristic blood pressure lowering effects of these PUFAs are likely to be useful for prevention and treatment of hypertension.

Keywords: Docosahexaenoic acid; eicosapentaenoic acid; linoleic acid; TXA₂ receptor; acute blood pressure changes; polyunsaturated fatty acids.

1. INTRODUCTION

Docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids are representative n-3 polyunsaturated fatty acids (PUFAs). Many pharmacological studies carried out so far show that both DHA and EPA have various pharmacological effects, and their intake is effective for the prevention and amelioration of various diseases. The observed pharmacological effects of DHA and EPA include platelet aggregation inhibition [1], vasodilation [2–6], blood pressure reduction [7–9], cholesterol/neutral fat level reduction [10, 11], anti-inflammatory effect [12, 13], hypoglycemic effect [14], nerve cell activation/differentiation [15], and antioxidant action [16]. These effects are postulated to be the basis for the prevention and amelioration by DHA and EPA of cardiovascular diseases such as hypertension, coronary artery disease, atherosclerosis, and stroke [13, 17, 18], metabolic diseases such as dyslipidemia [19, 20], inflammatory diseases such as ulcerative colitis and Crohn's disease [13], and central nervous system diseases such as Alzheimer-type dementia and depression [21, 22]. However, in addition to the many studies on the effects of chronic administration of these n-3 PUFAs, few studies described the acute effects. For example, many reports have described the inhibitory effects of chronic administration of DHA and EPA on blood pressure elevation, but few describe the acute effects on blood pressure reduction [23]. Furthermore, in the studies examining the effects of DHA and EPA on the isolated blood vessels, the effects are small even though relatively high concentrations of PUFAs are applied.

In this context, we have focused on the vasodilation effects of DHA and EPA in isolated arteries. We found that both PUFAs selectively and strongly inhibit the TXA₂ receptor (TP receptor) and PGF_{2α} receptor (FP receptor)-

mediated contraction in rat thoracic aorta [4] and mesenteric artery [6] using physiologically relevant concentrations, indicating that DHA and EPA exert their vasodilation effects through prostanoid receptors. It should be emphasized that the production of TXA₂, an endogenous mediator that induces marked platelet aggregation and blood vessel contraction that cause abnormal narrowing of blood vessels, is increased in hypertension [24, 25] and is thus a causative mediator of hypertension.

In the present study, we investigated whether the TP receptor-dependent arterial relaxant effects of DHA and EPA lead to reduced blood pressure in vivo. Rats with sustainably elevated blood pressure using U46619 (a TP receptor agonist) or noradrenaline (NA) were treated with bolus injection of DHA or EPA and tested for blood pressure lowering effects.

2. MATERIALS AND METHODS

Eight to nine weeks old male Wistar rats (weighing 180–230 g, Sankyo Labo Service, Tokyo, Japan) were housed under controlled conditions (temperature 21–22°C, relative air humidity 50 ± 5%, fixed 12-h light (08:00 to 20:00)/12-h dark cycle). Food (CLEA Japan, Tokyo) and water were available ad libitum. This study was approved by the Toho University Animal Care and User Committee (approval number 15-51-294, accredited on May 22, 2015), and conducted in accordance with the User's Guideline to the Laboratory Animal Center of Faculty of Pharmaceutical Sciences, Toho University.

2.1 Blood Pressure Determination in Anesthetized Rats

Rats were anesthetized with urethane (1.4–2.0 g/kg, i.p.) and were fixed in supine position after disappearance of the forward reflex. Next, the

neck (throat area) was incised and a polyethylene cannula was inserted into the trachea to secure spontaneous breathing. The left external jugular vein and the femoral vein were then exposed and polyethylene cannulas were inserted for drug administrations. A polyethylene cannula was also inserted into the right common carotid artery to measure blood pressure and heart rate. Changes in blood pressure and heart rate were recorded with data acquisition software (Chart, version 5.0 for Windows; AD Instruments, Australia) via a pressure transducer (MLT0380 Reusable BP Transducer; AD Instruments, Australia) and an A/D converter (PowerLab/4SP; AD Instruments, Australia). After the operation, experiments were started with a stabilization time of at least 30 min. Recordings were carried out at $24 \pm 1^\circ\text{C}$.

2.2 Administration of DHA, EPA, and Pressor Substances U46619 and NA

After surgery, rats received continuous infusion of U46619 (500 $\mu\text{g}/\text{kg}/\text{h}$) or NA (500 $\mu\text{g}/\text{kg}/\text{h}$) from the jugular vein to produce sustained blood pressure elevation. Rats with normal blood pressure that received continuous administration of physiological saline (5 mL/kg/h) were used as control. Blood pressure and heart rate stabilized after 30 min of continuous administration of U46619, NA, or saline. Vehicle (physiological saline containing 0.5% Tween 20), DHA (3–30 mg/kg, i.v.), EPA (3–30 mg/kg i.v.), linoleic acid (LA) (3–30 mg/kg i.v.), or sodium nitroprusside (SNP) (0.3–100 $\mu\text{g}/\text{kg}$ i.v.) were then administered, and changes in blood pressure and heart rate were recorded.

2.3 Drugs and Chemicals

DHA, EPA, and LA were obtained from Sigma-Aldrich (St. Louis, MO, USA), U46619 from Cayman Chemical (Ann Arbor, MI, USA), and SNP and (*R*)-(-)-norepinephrine hydrogen tartrate monohydrate from Wako Pure Chemical (Osaka, Japan). DHA, EPA, and LA were stored at -20°C and diluted to the desired concentrations with 0.5% Tween 20-containing physiological saline solution (0.9% NaCl) on the day of the experiment. The remaining drugs were diluted to the desired concentrations with physiological saline on the day of the experiment.

2.4 Statistical Analysis

Changes in mean blood pressure and heart rate in response to drug administration were

evaluated based on the difference between the levels before and the highest levels after drug administration. Results are expressed as mean values \pm SEM. The significance of the differences between mean values was evaluated by one-way or two-way ANOVA followed by Dunnett's or Tukey's multiple comparison tests using GraphPad Prism (Version 4.0; GraphPad Software, San Diego, C.A., USA). *P* values of less than 0.05 were considered statistically significant.

3. RESULTS

3.1 Effects of Continuous Infusion of U46619 and NA on Blood Pressure and Heart Rate of Urethane Anesthetized Rats

We first determined the effect of continuous infusion of saline, U46619, and NA on blood pressure and heart rate of urethane anesthetized male Wistar rats (Fig. 1). Saline infused from the jugular vein at 5 mL/kg/h did not have a marked effect on blood pressure and heart rate (Fig. 1A and 1D). In contrast, continuous infusions of U46619 (500 $\mu\text{g}/\text{kg}/\text{h}$) or NA (500 $\mu\text{g}/\text{kg}/\text{h}$) produced a sustained blood pressure increase (Fig. 1B and 1C) that persisted until infusion was interrupted (data not shown). In addition, the increase in blood pressure was stable and was observed after 20 min of infusion (Fig. 1D). The blood pressure elevation induced by U46619 was restored to original levels by seratrodist (1 mg/kg), which did not affect the blood pressure of rats that received continuous infusion of NA or saline (data not shown). Both U46619 and NA increased the heart rate, with NA infusion showing the strongest effect (Fig. 1E).

3.2 Effects of DHA, EPA, and LA on Blood Pressure and Heart Rate of Rats with Continuous Infusion of U46619 or NA

We next determined the effects of DHA on blood pressure and heart rate of rats into which U46619 or NA was continuously infused (Fig. 2). In U46619-infused rats, DHA (30 mg/kg, i.v.) produced a blood pressure decrease, the highest level of which was attained about 45 s (0.7 min) after injection. The lowered blood pressure recovered gradually and returned to original levels 2 min after DHA injection (Fig. 2A). DHA also decreased the heart rate, which returned to pre-injection levels about 40 s (0.67 min) after DHA injection (Fig. 2A). As shown in Figs. 2D

and 2E, DHA affected blood pressure and heart rate in a dose-dependent manner. In NA-infused rats, DHA did not produce any perceptible effects on both blood pressure and heart rate (Fig. 2B, 2D, and 2E). Similarly, DHA did not produce any significant effects on both blood pressure and heart rate in saline-infused rats (Fig. 2C, 2D, and 2E).

Fig. 3 shows the effects of EPA on blood pressure and heart rate of rats into which U46619 or NA was continuously infused. Similarly to DHA, EPA (30 mg/kg, i.v.) significantly lowered the blood pressure of U46619-infused rats (Fig. 3A), and its effects were dose-dependent (Fig. 3D). Although no prominent effects were detected on the heart rate of the rat shown in Fig. 3A, EPA decreased the heart rate in other rats in a dose-dependent manner (Fig. 3E). In NA- and saline-infused rats, EPA induced a small reduction in heart rate (Fig. 3B and 3C). The blood pressure lowering effects of EPA in NA- and saline-infused rats were dose-dependent but weaker than in U46619-infused rats (Fig. 3D). In both NA- and saline-infused rats, EPA did not show any perceptible effects on heart rate (Fig. 3E).

Fig. 4 shows the effects of LA on blood pressure and heart rate of rats into which U46619 or NA was continuously infused. LA (30 mg/kg) did not affect the blood pressure of rats receiving infusion of U46619, NA, or saline (Fig. 4A-D). In U46619-infused rats, LA induced a dose-dependent reduction of heart rate (Fig. 4E), although to a smaller extent than those of DHA or EPA.

3.3 Effects of SNP on Blood Pressure and Heart Rate of Rats with Continuous Infusion of U46619 or NA

The effects of SNP on blood pressure and heart rate of rats into which U46619, NA, or saline was continuously infused were determined (Fig. 5). SNP (100 µg/kg, i.v.) markedly lowered the blood pressure of rats receiving the injections of U46619, NA, or saline (Fig. 5A-C), with a pressure drop exceeding 60 mmHg (Fig. 5D). Blood pressure was reduced by SNP in a dose-dependent manner (Fig. 5D), and to a very similar degree in all rats. SNP also increased the heart rate in all rats (Fig. 5E), although we considered this increase a result from the marked reduction in blood pressure induced by SNP.

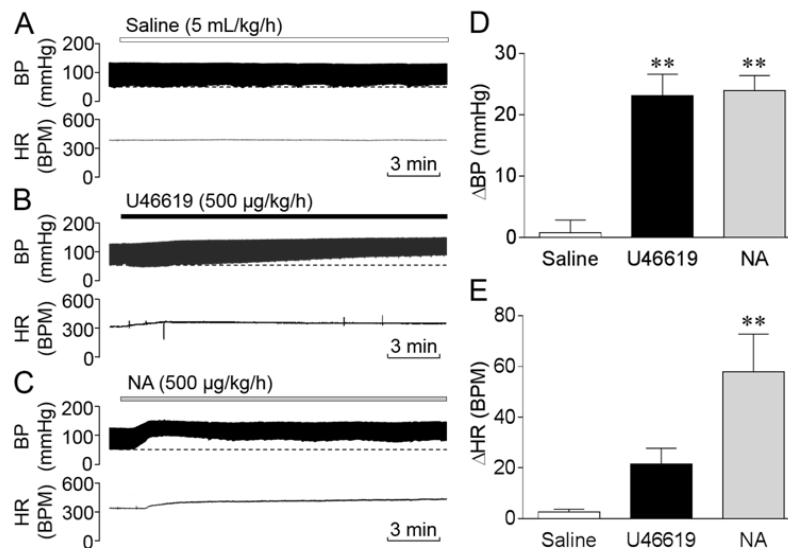


Fig. 1. Effects of continuous infusion of saline, U46619, and noradrenaline (NA) on blood pressure and heart rate of urethane anesthetized male Wistar rats

A-C: Representative traces showing the effects of continuous infusion of saline (5 mL/kg/h) (A), U46619 (500 µg/kg/h) (B), and noradrenaline (NA) (500 µg/kg/h) (C) on blood pressure and heart rate of rats anesthetized with urethane. Saline, U46619, and NA were infused into the jugular vein using an infusion pump. **D, E:** Summary of the results shown in A-C. The effects of saline, U46619, and NA on mean blood pressure (MBP) (D) and heart rate (HR) (E) are shown as differences in levels before and after infusion. Data are shown as mean values \pm SEM (n = 4). **P < 0.01: U46619, NA vs. saline

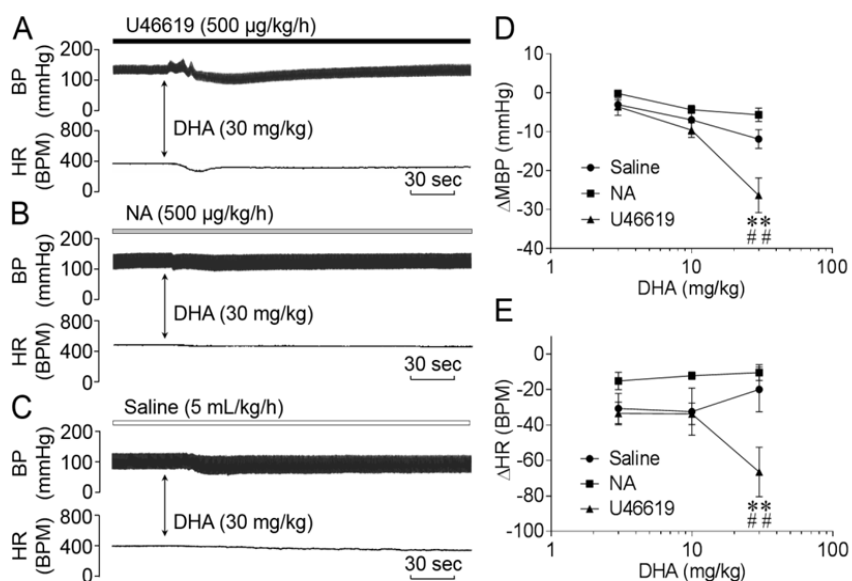


Fig. 2. Effects of docosahexaenoic acid (DHA) on blood pressure and heart rate of rats continuously infused with U46619, noradrenaline (NA), or saline

A-C: Representative traces showing the effects of bolus injection of DHA (30 mg/kg, i.v.) on the blood pressure and heart rate of rats infused with U46619 (500 µg/kg/h) (A), NA (500 µg/kg/h) (B), or saline (5 mL/kg/h) (C). DHA (30 mg/kg, i.v.) was administered via bolus through the femoral vein. **D, E:** Summary of the results shown in A-C. The effects of DHA (3–30 mg/kg, i.v.) on the mean blood pressure (MBP) (D) and heart rate (HR) (E) are shown as differences in levels before and after administration of cumulatively applied DHA (3–30 mg/kg, i.v.). Data are shown as mean values ± SEM (n = 4). **P < 0.01: U46619 vs. saline; ##P < 0.01: U46619 vs. NA.

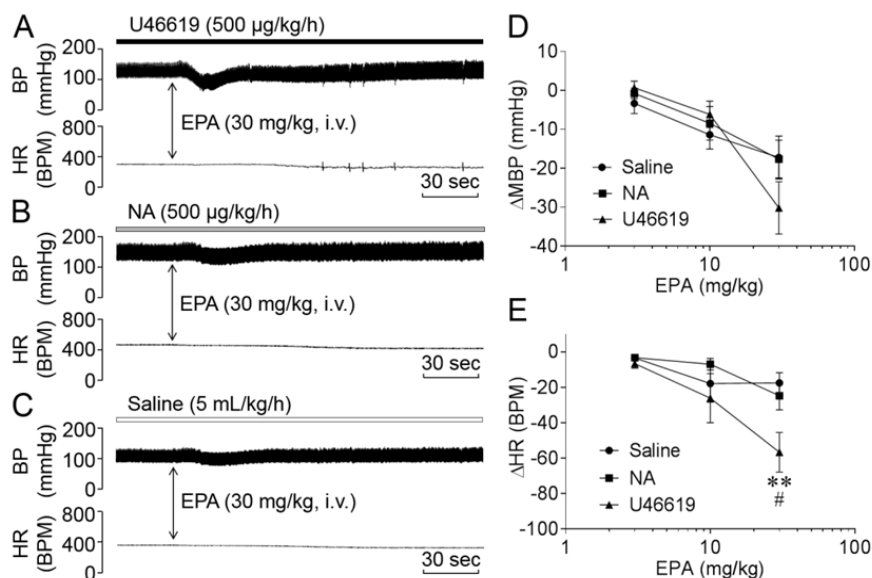


Fig. 3. Effects of eicosapentaenoic acid (EPA) on blood pressure and heart rate of rats continuously infused with U46619, noradrenaline (NA), or saline

A-C: Representative traces showing the effects of bolus injection of EPA (30 mg/kg, i.v.) on blood pressure and heart rate in rats infused with U46619 (500 µg/kg/h) (A), NA (500 µg/kg/h) (B), or saline (5 mL/kg/h) (C). EPA (30 mg/kg, i.v.) was administered via bolus through the femoral vein. **D, E:** Summary of the results shown in A-C. The effects of EPA (3–30 mg/kg, i.v.) on the mean blood pressure (MBP) (D) and heart rate (HR) (E) are shown as differences in levels before and after administration of cumulatively applied EPA (3–30 mg/kg, i.v.). Data are shown as mean values ± SEM (n = 6). **P < 0.01: U46619 vs. saline; #P < 0.05: U46619 vs. NA.

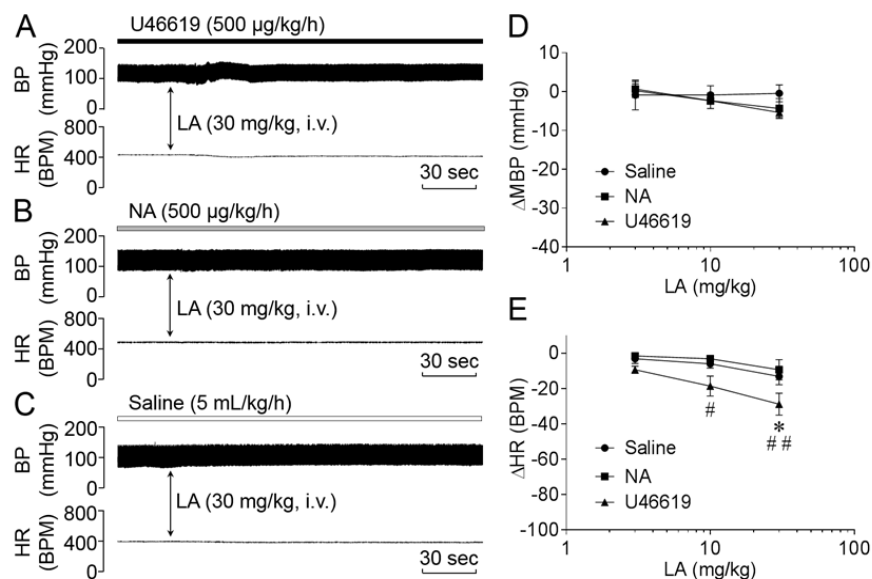


Fig. 4. Effects of linoleic acid (LA) on blood pressure and heart rate of rats continuously infused with U46619, noradrenaline (NA), or saline

A-C: Representative traces showing the effects of bolus injection of LA (30 mg/kg, i.v.) on blood pressure and heart rate of rats infused with U46619 (500 μg/kg/h) (A), NA (500 μg/kg/h) (B), or saline (5 mL/kg/h) (C). LA (30 mg/kg, i.v.) was administered via bolus through the femoral vein. **D, E:** Summary of the results shown in A-C. The effects of LA (3–30 mg/kg, i.v.) on the mean blood pressure (MBP) (D) and heart rate (HR) (E) are shown as differences in levels before and after administration of cumulatively applied LA (3–30 mg/kg, i.v.). Data are shown as mean values ± SEM (n = 3). *P < 0.05; U46619 vs. saline; #P < 0.05, ###P < 0.01: U46619 vs. NA.

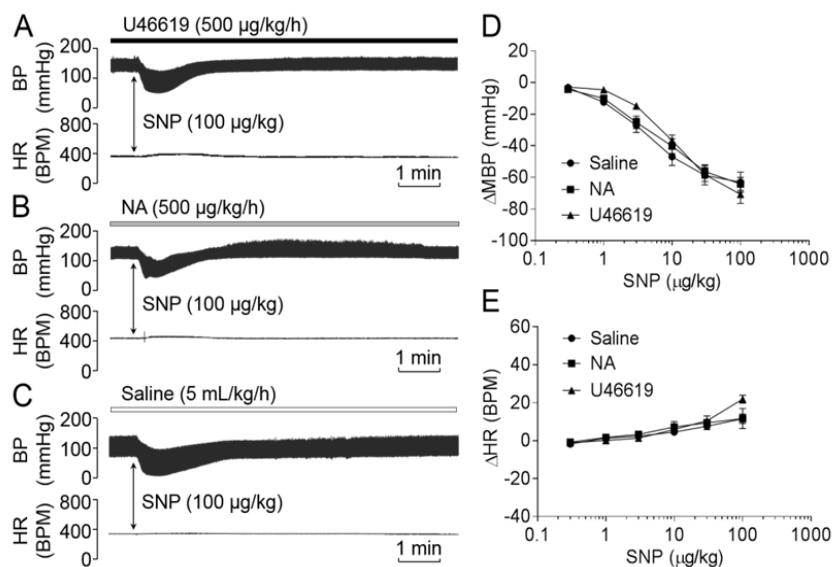


Fig. 5. Effects of sodium nitroprusside (SNP) on blood pressure and heart rate of rats continuously infused with U46619, noradrenaline (NA), or saline

A-C: Representative traces showing the effects of bolus injection of SNP (100 μg/kg, i.v.) on blood pressure and heart rate of rats infused with U46619 (500 μg/kg/h) (A), NA (500 μg/kg/h) (B), or saline (5 mL/kg/h) (C). SNP (100 μg/kg, i.v.) was administered via bolus through the femoral vein. **D, E:** Summary of the results shown in A-C. The effects of SNP (0.3–100 μg/kg, i.v.) on the mean blood pressure (MBP) (D) and heart rate (HR) (E) are shown as differences in levels before and after administration of cumulatively applied SNP (0.3–100 μg/kg, i.v.). Data are shown as mean values ± SEM (n = 3).

4. DISCUSSION

In this study, we investigated the acute effects of three intravenously administered PUFAs on blood pressure and heart rate of rats the blood pressure of which was elevated by continuous infusion of U46619 or NA. The results show that the n-3 type PUFAs DHA and EPA lowered the blood pressure and heart rate, and these effects were particularly evident in U46619-infused rats. In contrast, the n-6 PUFA LA did not significantly affect blood pressure or heart rate in both U46619- and NA-infused rats. The nitric oxide (NO) donor SNP lowered the blood pressure by almost the same degree in rats infused with U46619, NA, or saline. Therefore, we suggest that the acute blood pressure lowering effects of intravenously applied DHA and EPA are exerted selectively through TP receptor stimulation.

The intravenous administration of DHA, but not NA, induced a remarkable reduction in the blood pressure of U46619-infused rats. These findings suggest that DHA reduces blood pressure by interfering with a TP receptor-mediated mechanism of hypertension, not but inducing a general response to high blood pressure. This is consistent with the described effects of DHA on isolated rat aorta [4, 5] and mesenteric artery [6]. On the other hand, administration of DHA at 30 mg/kg results in blood levels of about 10^{-3} M if blood volume is assumed to be 1/13 of body weight. Although this concentration was 100 times higher than that required to inhibit U46619-induced contractions in isolated blood vessels [4, 6], the blood pressure reduction was transient, of about 40 s (0.67 min). This transient effect may be ascribed to the highly lipophilic characteristic of DHA, which may result in a prompt dissolving into adipose tissues and thus in a rapid drop in DHA blood levels. In fact, when DHA blood concentration was measured by liquid chromatography, it was found to increase to near 10^{-3} M immediately after intravenous administration, but decreased rapidly thereafter, and almost completely disappeared after 30 min (unpublished observation). Although EPA also reduced blood pressure in U46619-infused rats, the corresponding TP receptor-selective aspect of blood pressure reduction was not as evident as that of DHA. To further obtain evidence of a TP receptor-mediated blood pressure reduction by DHA, the influence of the NO donor SNP on the blood pressure of U46619-infused rats was examined and shown to result in a dose-dependent reduction similar to that of NA-infused rats (Fig. 5). Therefore, the blood pressure

reduction induced by NO is exerted against a non-stimulated hypertensive state.

DHA not only lowered blood pressure but also significantly reduced the heart rate of U46619-infused rats (Fig. 2). In contrast, SNP showed a reflexive heart rate increase in addition to a reduction in blood pressure. Therefore, the heart rate reducing effect of DHA is conceivably a result of direct action on atrial muscle. In this context, Takayama et al. reported that a TP receptor agonist increased the beating of isolated guinea pig sinoatrial nodal cells [26], which is consistent with a heart rate lowering action of DHA in U46619-infused rats resulting from an antagonistic effect of DHA vs. U46619 on the atrial muscle TP receptor.

Sato et al. [4, 6] reported that EPA exerts vasorelaxant actions similar to those of DHA in terms of potency and spectrum. Therefore, EPA was expected to induce the same level of blood pressure reduction as DHA, which was observed in U46619-infused rats in the present study. However, EPA is presumably inferior to DHA in terms of TP receptor selectivity, because a high dose (30 mg/kg, i.v.) of EPA induced a relatively strong blood pressure reduction in both NA- and saline-infused rats compared to U46619-infused rats. Although the reason for this observed difference is not clear at present, it may be related to differences in tissue transferability and metabolic pathway of DHA and EPA. EPA is more easily metabolized by cyclooxygenase and lipoxygenase than DHA, and more strongly affects blood vessel and antiplatelet action [27, 28]. These differences in pharmacodynamics and pharmacokinetics features between DHA and EPA might result in different blood pressure reduction effects.

In contrast to DHA and EPA, LA had no significant effect on the blood pressure of rats (Fig. 4). These results are consistent with the previously reported lack of LA-induced inhibitory effects on contractions of rat aorta [4] and mesenteric artery [6] in response to various vasoconstrictor agents, including a TP receptor agonist. In contrast, the chronic, sustained transdermal application of LA was reported to suppress angiotensin II-induced blood pressure elevation to a degree similar to that of chronic intake of oil fish [29]. In clinical studies, some groups reported a significant effect on blood pressure by LA [30–33], whereas other groups reported a lack of significant LA-mediated effects on blood pressure [34–40]. Therefore, although

LA does not induce acute blood pressure reduction when administered intravenously, it may lower the blood pressure after chronic oral administration. Further studies are needed to clarify this issue.

LA showed a tendency to decrease the heart rate in a dose-dependent manner, and this tendency was slightly more pronounced in U46619-infused as compared with NA- and saline-infused rats (Fig. 4). However, its effect was considerably weaker than those of DHA and EPA and the mechanism of action is unclear; we did not investigate this issue further in this study. Because intravenous injection of LA did not significantly affect the blood pressure of U46619-infused rats, we speculate that LA induces a decrease in heart rate through a mechanism distinct from TP receptor antagonism. It should be noted that spontaneously hypertensive rats (SHR) fed with perilla oil diets rich in the n-3 PUFA α -LA show a drop of approximately 10% in blood pressure compared to SHR fed with safflower oil diets rich in LA [41]. Therefore, the effects of α -LA on blood pressure and blood vessel tone should be explored more extensively in the future.

Although the beneficial effects of DHA, EPA, and the fish oil containing them on hypertension have been demonstrated in many epidemiological studies and animal experiments [42–47], the mechanism responsible for these effects has not been fully elucidated. In addition, most studies on the hypotensive action of DHA and EPA have examined the effects of long-term oral intake, whereas few reports on the acute effects of intravenous administration of these PUFAs are found in the literature [23]. In fact, the clinical hypotensive effects of DHA and EPA were described exclusively after chronic oral intake by hypertensive patients [7, 9]. A potential mechanism for the reduction in BP and HR produced by dietary PUFA intake is the action on vascular stiffness. DHA and EPA have been reported to improve arterial hemodynamics by reducing arterial stiffness, thus explaining some of their cardioprotective properties [48]. On the other hand, we showed that DHA and EPA selectively and strongly inhibit vasoconstriction mediated by prostanoid receptors (including TP receptor) in rat aorta without significantly affecting the contractions induced via α_1 -adrenoceptor stimulation or depolarization with a high-KCl solution [4]. These effects were also observed in rat mesenteric artery [6]. The amount of TXB₂, a main metabolite of TXA₂,

increases in the renal glomeruli [49, 50], and the mesenteric artery [51] of SHR. Furthermore, TXA₂ is suggested to play an important role in the early stages of the onset of hypertension [50]. Therefore, selective inhibition of TP receptor-mediated vasoconstriction may be a mechanism by which DHA and EPA exert their beneficial effects on hypertension.

Epidemiological studies have shown that continuous oral intake of DHA or fish oil significantly reduces the blood pressure of hypertensive patients without significantly affecting the blood pressure of healthy individuals [7, 9]. A plausible mechanism for this phenomenon could be the selective blood pressure-reducing actions of DHA and EPA, which inhibit TP receptor-mediated blood pressure elevation in the early stages of the onset of hypertensive patients while leaving the blood pressure of normotensive individuals unchanged.

To our knowledge, this is the first report showing that intravenous administration of EPA lowers blood pressure immediately. Because EPA is more abundant in fish oil than DHA, the contribution of EPA is considered significant in the blood pressure reduction effects of long-term oral intake of fish oil. This study shows that intravenous injection of EPA and DHA produced similar reductions in blood pressure, and therefore clinical application of n-3 PUFA is expected to increase.

According to the 2008 survey of the World Health Organization, more than 1 billion people over 25 years of age have been diagnosed as hypertensive in the world [52], and thus the importance of hypertension prevention is increasing. Our results provide a scientific basis for the recommended continuous ingestion of DHA, EPA, and the fish oil containing them to prevent high blood pressure.

5. CONCLUSION

Both DHA and EPA induce acute blood pressure reduction in anesthetized rats, and this blood pressure reduction is mediated via TP receptor.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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