

ORIGINAL**Propofol-induced relaxation of rat aorta is altered by aging**

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Abstract : Background : Propofol causes vasodilation via endothelium-dependent and -independent mechanisms. Because endothelial function is impaired with aging, the effects of propofol on endothelium-dependent vasodilation might be altered by aging. The aim of this study was thus to determine the effects of aging on vascular responses to propofol. **Methods :** Young (4-6 weeks old) or adult (16-25 weeks old) rats were anesthetized with sevoflurane. The thoracic aorta was dissected and cut into pieces 3-4 mm in length. In some rings, the endothelium was deliberately removed. The ring segment of the aorta was mounted for isometric force recording at a resting tension of 0.5-1.0 g in a 2 ml organ bath, containing Krebs-Ringer bicarbonate buffer. Arteries were precontracted with phenylephrine, and the function of endothelium was confirmed with acetylcholine. Then, we studied the concentration-dependent effects of propofol in endothelium-intact (control group) and -denuded aortic rings (denuded group), as well as those treated with N^o-nitro-L-arginine methylester (L-NAME group). **Results :** Relaxation due to propofol was observed in the control groups of both young and adult rats in a concentration-dependent manner, but the magnitude of relaxation was significantly greater in young rats. In addition, in young rats, relaxation due to propofol was significantly and equally reduced in both L-NAME and denuded groups at all propofol concentrations that we studied (10⁻⁶-10⁻³ M). In adult rats, relaxation due to propofol was quite similar between control and L-NAME groups at all propofol concentrations, whereas it was significantly reduced in the denuded group. **Conclusion :** These results suggest that endothelium-derived nitric oxide plays an important role in propofol-induced vasodilation in young rats, but not in adult rats. *J. Med. Invest.* 61 : 278-284, August, 2014

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INTRODUCTION

Propofol (2,6-diisopropyl-phenol) is an intravenous anesthetic agent widely used in the induction and maintenance of anesthesia. However, its cardiovascular effects have often caused a marked

decrease in blood pressure. The depressor effects of propofol have been ascribed to a decrease in systemic vascular resistance (1, 2) or cardiac output (3), or both (4, 5). The reduction in peripheral vascular resistance may be due to direct effects (6), possibly including endothelium-derived nitric oxide (NO) release (7), and to indirect effects via the sympathetic nervous system (8, 9).

Several studies have attempted to explain the complex actions of propofol. Propofol has been shown to relax vascular smooth muscle in both endothelium-dependent (7, 10-12) and -independent manners (13-15). Propofol modulates endothelium-dependent relaxation in some preparations. The direct actions of propofol on isolated venous and arterial tissues were investigated, with variable results. Petros *et al.* (7) suggested that propofol stimulates NO production from cultured endothelial cells in a concentration-dependent manner. Park *et al.* (10) demonstrated that propofol produces concentration-dependent vasodilation of distal coronary arteries; this effect is endothelium-dependent and appears to be mediated by multiple substances, including NO and a vasodilatory prostanoid. On the other hand, others suggested that propofol produces endothelium-independent relaxation, even at clinically meaningful low concentrations, and acts as a calcium-channel blocker (13, 14).

Aging exerts functional changes on endothelial cells. Several studies have indicated that endothelial function is impaired with age and endothelium-dependent relaxation might decline with age (16-18). Therefore, the effect of drugs on endothelial-dependent relaxation is expected to be greater in young animals. Previously, our group reported the effects of propofol on adenosine triphosphate-sensitive potassium channels in rat ventricular myocytes (19) and in Cos-7 cells transfected with various types of K_{ATP} channel subunit (20) on volume-sensitive chloride channels (21) and on hypotonic swelling-induced membrane depolarization (22) in human coronary artery smooth muscle cells. The aim of this study was to determine the effects of age and endothelial function on vascular responses to propofol using thoracic aortic rings in young and adult rats.

METHODS

This study was approved by the Animal Investigation Committee of Tokushima University (Tokushima, Japan) and was conducted according

to the animal use guidelines of the American Physiological Society (Bethesda, MD).

Young (4-6 weeks old) and adult (16-25 weeks old) Wistar rats were anesthetized by inhalation of sevoflurane and then killed by opening the abdominal aortic artery. The thoracic aorta was dissected and adherent perivascular tissues were carefully removed and cut into 3 to 4 mm in length. In some rings, the endothelium was removed by gentle rubbing of its surface with cotton. The ring segment of the aorta was suspended between two stainless steel hooks in a 2 ml organ bath (Micro Easy Magnus; Medical Kishimoto, Kyoto, Japan), containing Krebs-Ringer bicarbonate buffer (in mmol/l: NaCl, 118.4; KCl, 4.9; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4 , 1.2; and glucose, 11.1). It was bubbled with a 95% O_2 /5% CO_2 gas mixture. These preparations were equilibrated for 2 hours under a resting tension of 0.5 g for young rats and 1.0 g for adult rats.

The vessels were submaximally precontracted with phenylephrine (3×10^{-7} M) and supplemented with acetylcholine (3×10^{-7} M) to assess the integrity of the endothelium. No relaxation in response to acetylcholine in the denuded preparation indicated effective functional removal of the endothelium. After acetylcholine testing, the rings were re-equilibrated for 60 min and then contracted with phenylephrine. Propofol was added at exponentially increasing concentrations (10^{-6} - 10^{-3} M) to individual chambers of endothelium-intact and -denuded groups. To test whether propofol-induced vasodilation is involved in nitric oxide release, 3×10^{-4} M N^{ω} -nitro-L-arginine methyl ester (L-NAME; a specific inhibitor of endothelium-derived relaxing factor-nitric oxide synthase) was added to endothelium-intact rings 20 min before contraction using phenylephrine. Then, we checked concentration-response curves to propofol. We studied the concentration-dependent effects of propofol on aortic rings for endothelium-intact (control group) and -denuded rings (denuded group), as well as those treated with L-NAME (L-NAME group). Relaxation responses to propofol are expressed as the percentage of the precontracted tension induced by 3×10^{-7} M phenylephrine.

Drugs used

A commercially available preparation of propofol (Zeneca Pharmaceutical Co., Osaka, Japan) was used. Phenylephrine, acetylcholine, and L-NAME were purchased from Sigma (Sigma-Aldrich Japan,

Tokyo, Japan).

Statistical analysis

Data are shown as mean and standard error of the mean (mean \pm SEM). To compare the data obtained from the different groups of animals, statistical evaluation was performed by two-factor factorial ANOVA. When a significant interaction was observed, complementary analysis was performed by Bonferroni/Dunn post hoc test to identify differences among groups. A value of $P < 0.05$ was considered to be significant.

RESULTS

In young rats, the relaxation response to propofol

was significantly greater in endothelium-intact rings (control group) than in L-NAME-treated rings (L-NAME group) and endothelium-denuded rings (denuded group) at all propofol concentrations (Figure 1). In adult rats, vasodilation by propofol was not significantly different between the control group and the L-NAME group at all propofol concentrations, whereas it was significantly reduced in the denuded group (Figure 2). In the denuded group, propofol produced a little contraction at low concentrations (10^{-6} - 10^{-5} M) and relaxation at high concentrations (10^{-4} - 10^{-3} M).

Figure 3 shows a comparison between young and adult rats in each group. Young and adult rats in the control group exhibited relaxation due to propofol in a concentration-dependent manner, but young rats showed significantly greater relaxation to propofol

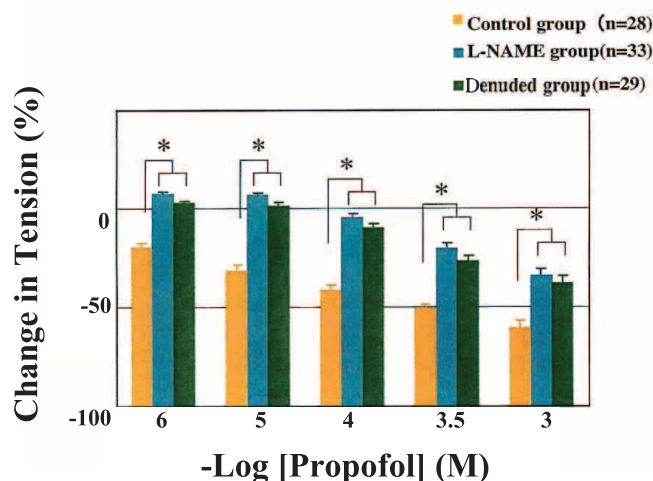


Figure 1. Concentration-dependent effects of propofol in young rats. Relaxation responses due to propofol are expressed as the percentage of the precontracted tension induced by phenylephrine. * Significant difference from the control group ($P < 0.05$). Data are presented as mean \pm SEM.

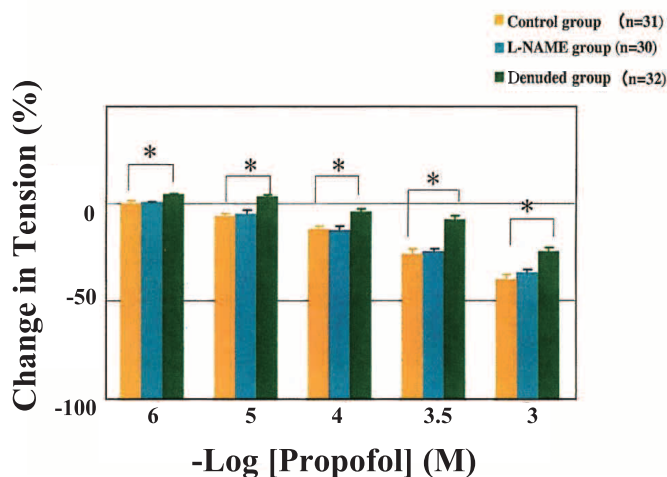


Figure 2. Concentration-dependent effects of propofol in adult rats. Relaxation responses due to propofol are expressed as the percentage of the precontracted tension induced by phenylephrine. * Significant difference from the control group ($P < 0.05$). Data are presented as mean \pm SEM.

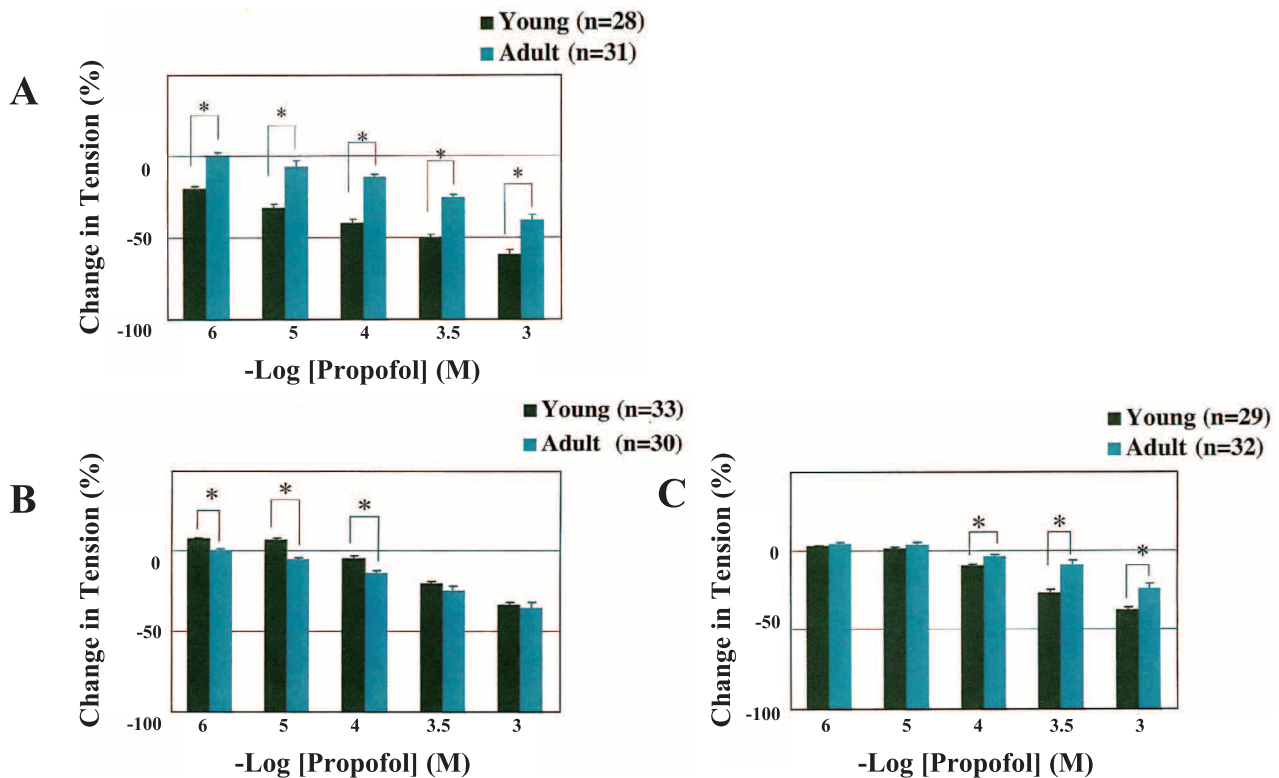


Figure 3. Comparison between young and adult rats. (A) Comparison between control groups in young and adult rats. (B) Comparison between L-NAME groups in young and adult rats. (C) Comparison between denuded groups in young and adult rats. * $P < 0.05$ between groups. Data are presented as mean \pm SEM.

than adult rats at all propofol concentrations (Figure 3A). In young rats, propofol-induced relaxation at a low concentration (10^{-6} - 10^{-4} M) was suppressed by the addition of L-NAME (Figure 3B). In adult rats, propofol-induced relaxation at a high concentration (10^{-4} - 10^{-3} M) was suppressed by removal of the endothelium (Figure 3C). Therefore, the relaxation due to high concentrations (10^{-4} - 10^{-3} M) of propofol may have been endothelium-independent.

DISCUSSION

This study showed that, when endothelium function was well preserved, as in young rats, endothelium-dependent vasodilation occurred even at a low concentration of propofol. In young rats, there was concentration-dependent vasorelaxation in all groups. However, propofol-induced relaxation was suppressed at a low concentration (clinical concentration) by removal of the endothelium or the addition of L-NAME in young rats. This difference suggests the involvement of the L-arginine-nitric oxide pathway in the vasodilatory effect of propofol in young rats. In adult rats, the vasodilatory mechanism seemed to be mainly dependent on the vascular

smooth muscle cells because vasodilation by propofol was not significantly different between endothelium-intact (control) and L-NAME-treated rings.

Vascular aging is associated with structural and functional changes (16-18). The major proposed mechanism for the impairment of endothelium-dependent vasodilation by aging is a decrease in the production of endothelium-derived relaxing factor (EDRF), now identified as nitric oxide (NO) (23-25). In addition, Hongo *et al.* suggested findings of a decrease in the number of receptors to vasoactive agents on the endothelial cells, a decrease in the affinity of receptors on the endothelial membrane, namely, vasoactive agents, a decrease in the responsiveness of the smooth muscle to EDRF, exaggeration of endothelium-dependent contraction in old and/or hypertensive rats, a decrease in the production of an endothelium-dependent component of prostaglandins, or decreased sensitivity of those prostaglandins to smooth muscles (26). These factors suggested that endothelium dysfunction by aging could affect propofol-induced vasodilation. Therefore, we can suppose that this difference of relaxation at a low concentration of propofol between young and adult rats may be due to good endothelium function.

The direct action of propofol on arteries and veins has been investigated, with variable results. Propofol is known to relax arteries in both endothelium-dependent and -independent manners. In other words, propofol causes vasodilation by two mechanisms: The first mechanism is dependent on the presence of functionally intact endothelium. NO is formed from L-arginine by NO synthase (NOS) in the vascular endothelium, and regulates vascular tone by increasing the level of 3',5'-cyclic guanosine monophosphate (cGMP) in the vascular smooth muscle. Petros *et al.* suggested that propofol stimulates NO production in a concentration-dependent manner from cultured endothelial cells (7). Park *et al.* (10) also demonstrated that there appears to be an endothelium-independent effect of propofol in the high normal to supraclinical range, since endothelial denudation did not totally abolish propofol-induced dilation.

The second mechanism is a direct effect on vascular smooth muscle that is independent of the endothelium. Specifically, propofol produces vasodilation by an endothelium-independent mechanism that is probably mediated by the inhibition of extracellular Ca influx through voltage-gated Ca channels. This response is similar to that of Ca channel blockers (13, 14). In addition, it is acknowledged that mediators other than NO could modulate the endothelium-dependent vasodilation and could passively interact with NO. For example, Tanabe *et al.* (27) have shown, using A10 cells, that propofol suppresses the stimulation of the arachidonate cascade by vasopressin, at least partly by inhibiting phosphoinositide-hydrolyzing phospholipase C and phosphatidylcholine-hydrolyzing phospholipase D, resulting in the inhibition of PGI₂. Additionally, anesthetics modulate the activity of K_{ATP} channels of vascular smooth muscle. It has been reported that propofol impaired vasodilation mediated by K_{ATP} channels in vascular smooth muscle cells (28, 29).

In this way, many reports suggest that the effects of propofol on relaxation are complex and that relaxant responses to propofol may be at least in part dependent on the action of endothelium, which involves interactions with vascular smooth muscle calcium channels (11, 13). However, this study showed that, when endothelium function was well preserved, as in young rats, endothelium-dependent vasodilation occurred even at a low concentration of propofol. In addition, there was little difference between control and L-NAME groups of adult rats. These results suggest that, in adult rats, the

vasodilatory mechanism is mainly dependent on vascular smooth muscle cells because endothelium function is altered by aging.

In rings without endothelium, a low concentration of propofol produced a little contraction in adult rats. Coughlan *et al.* (30) reported a modest vasoconstrictive effect of propofol at low concentrations, whereas dilation occurred at higher concentrations. Nakamura *et al.* (31) also observed a constrictive effect of propofol at low concentrations (10^{-6} - 10^{-5} M) and relaxation at a high concentration (10^{-4} M) in canine coronary arterial strips contracted previously with PGF₂ α or potassium chloride (KCl). There may be other substances that induce contraction, but these are currently poorly understood.

The peak plasma concentrations of propofol have been reported to be 4-10 μ g/ml (approximately $2-5 \times 10^{-5}$ M) in patients in whom general anesthesia was induced by bolus infusion of propofol (32), and on the order of 2-5 μ g/ml (approximately $1-3 \times 10^{-5}$ M) when patients were anesthetized with an infusion of propofol supplemented with inhalation of nitrous oxide (33). However, as it has been estimated that 97-99% of propofol is bound to plasma proteins *in vivo* (32, 34), the concentration of free propofol that is available to affect vascular smooth muscle has been estimated to be 10^{-6} M or less, providing that propofol is not injected very rapidly. Consequently, the vasodilatory effect of propofol, as shown by us, in rat thoracic aortas may be observed within clinically detectable levels.

Our study had several limitations. First, we did not study the effect of the intralipid, which is the vehicle of propofol. Nakamura *et al.* (31) observed that intrafat, containing soya bean oil, egg phosphatide and glycerol, at a similar concentration to the emulsion formation of propofol had a slight relaxant effect at a concentration corresponding to propofol at 10^{-4} M. They suggested that the vascular effect of soya bean oil with egg phosphatide may contribute to the vasodilation induced by the emulsion formation of propofol at high concentrations. Second, EDRF includes PGI₂ and endothelium-derived hyperpolarizing factor (EDHF), besides NO. Additionally, endothelium-derived contracting factor (EDCF) may also have an influence on the results in this experiment system. However, we did not examine these effects. Third, we studied the effects of propofol on rat thoracic aorta, but these effects may differ from those on human artery. Therefore, we should be careful in extrapolating

the current results to humans.

In conclusion, propofol at lower concentration relaxes arteries via endothelial NO only in young rats, but not in adult rats. These results suggest that NO plays an important role in propofol-induced vasodilation in young rats, but not in adult rats. In adult rats, the vasodilatory mechanism is mainly dependent on the vascular smooth muscle cells because endothelium function is altered by aging. These findings may indicate one of the factors explaining the effects of aging on vascular responses to propofol.

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CONFLICT OF INTEREST

None

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