

Beneficial Effect of Propofol on Arterial Adenosine Triphosphate-sensitive K^+ Channel Function Impaired by Thromboxane

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Background: It is not known whether thromboxane A_2 impairs adenosine triphosphate (ATP)-sensitive K^+ channel function *via* increased production of superoxide in blood vessels and whether propofol as a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor restores this modification.

Methods: Rat aortas without endothelium were used for isometric force recording, measurements of membrane potential, and superoxide production and Western immunoblotting. Vasorelaxation to an ATP-sensitive K^+ channel opener levcromakalim was obtained during contraction to phenylephrine (3×10^{-7} M) or a thromboxane A_2 analogue U46619 (3×10^{-8} M). In some experiments, aortas were incubated with an ATP-sensitive K^+ channel antagonist glibenclamide, a superoxide inhibitor Tiron, a nonselective NADPH oxidase inhibitor apocynin, a hydrogen peroxide scavenger catalase, a xanthine oxidase inhibitor allopurinol, a thromboxane receptor antagonist SQ29548 or propofol (3×10^{-7} to 3×10^{-6} M).

Results: Levcromakalim-induced vasorelaxation was abolished by glibenclamide in rings contracted with either vasoconstrictor agent. Tiron, apocynin, and propofol, but not catalase, augmented the vasodilator response as well as the hyperpolarization by levcromakalim in aortas contracted with U46619. Tiron, apocynin, SQ29548, and propofol, but not allopurinol, similarly reduced *in situ* levels of superoxide within aortic vascular smooth muscle exposed to U46619. Protein expression of a NADPH oxidase subunit p47phox increased in these arteries, and this augmentation was abolished by propofol.

Conclusions: Thromboxane receptor activation induces vascular oxidative stress *via* NADPH oxidase, resulting in the impairment of ATP-sensitive K^+ channel function. Propofol reduces this stress *via* inhibition of a NADPH oxidase subunit p47phox and, therefore, restores ATP-sensitive K^+ channel function.

VASCULAR smooth muscle cells contain several sources of reactive oxygen species, among which nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are

predominant.^{1,2} Studies in cultured cells from pulmonary arterial endothelium and corpus cavernosum demonstrated that thromboxane A_2 , which is known as an eicosanoid derived from cyclooxygenase, increases superoxide production *via* the activation of NADPH oxidase.^{3–6} However, it is still unclear whether thromboxane A_2 augments this enzymatic activity in the intact artery. Accumulating findings demonstrated that adenosine triphosphate (ATP)-sensitive K^+ channels make significant contributions in the regulation of vascular smooth muscle tone under normal physiologic as well as pathophysiological conditions,⁷ whereas increased oxidative stress reportedly induces malfunction of these channels.^{8,9} However, it has been unknown whether thromboxane A_2 impairs the K^+ channel function *via* increased production of superoxide in blood vessels.

Propofol potentially possesses antioxidant properties caused by its chemical structure similar to that of phenol-based free-radical scavengers such as vitamin E.¹⁰ Previous studies found that clinically relevant concentrations of this intravenous anesthetic reduce levels of superoxide, resulting in the recovery of vascular function related to nitric oxide derived from endothelial as well as neuronal synthase.^{11,12} Clinically relevant concentrations of propofol may restore vascular ATP-sensitive K^+ channel function exposed to oxidative stress, although it has not been determined. Also, it has not been proven whether a clinically used anesthetic plays a role as a NADPH oxidase inhibitor by evaluating the protein expression of this enzyme.

Therefore, the current study was designed to examine the role of vascular oxidative stress derived from NADPH oxidase on thromboxane receptor activation in vascular ATP-sensitive K^+ channel function and the mechanism as well as the effect of propofol within clinically relevant concentrations acting on this stress.

Materials and Methods

The animal care and use committee in Wakayama Medical University (Wakayama, Japan) approved this study. Forty Male Wistar rats (16 to 20 weeks) were obtained from Charles River Japan Inc. (Yokohama, Japan). Rats were anesthetized with inhalation of 3% halothane. Under this anesthetic condition, the rats were killed by exsanguination, and thoracic aortas were harvested. Thoracic aortic rings of 2.5 mm in length were studied in modified Krebs-Ringer's bicarbonate solution (control

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solution) of the following composition (in mM): NaCl 119, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.17, KH₂PO₄ 1.18, NaHCO₃ 25, and glucose 5.5. Endothelial cells were removed mechanically by gentle rubbing of the lumen using a small forceps to avoid the modification mediated by endothelium-derived vasodilator substances as well as endothelial ATP-sensitive K⁺ channels.¹³ The removal of endothelial cells was verified by the absence of relaxation in response to acetylcholine (10⁻⁶ M).

Organ Chamber Experiments

Eight rings cut from the same artery were studied in parallel. Each ring was connected to an isometric force transducer and suspended in an organ chamber filled with 10 ml of control solution (37°C, pH 7.4) bubbled with 95% O₂ and 5% CO₂. The ring was gradually stretched to the optimal point of its length-tension curve, as determined by the contraction to phenylephrine (3 × 10⁻⁷ M). In most of studied arteries, optimal resting tension achieved approximately at 1.5 g. Preparations were equilibrated for 90 min. Some rings were treated with an ATP-sensitive K⁺ channel antagonist glibenclamide (10⁻⁶ M), a superoxide inhibitor Tiron (10 mM), a nonselective NADPH oxidase inhibitor apocynin (1 mM), a hydrogen peroxide scavenger catalase (1200 U/ml), a xanthine oxidase inhibitor allopurinol (10⁻⁴ M), propofol (3 × 10⁻⁷ or 10⁻⁶ M), or dimethyl sulfoxide (2 × 10⁻⁶ M). These agents were applied 20 min before the contraction induced by phenylephrine or U46619. During submaximal contraction to phenylephrine (3 × 10⁻⁷ M) or a thromboxane A₂ analogue U46619 (3 × 10⁻⁸ M), the concentration-response curves to an ATP-sensitive K⁺ channel opener levcromakalim or a voltage-dependent Ca²⁺ channel antagonist diltiazem were simultaneously obtained in a cumulative fashion (from 10⁻⁸ to 3 × 10⁻⁶ M or from 10⁻⁸ to 10⁻⁴ M, respectively) with 4- to 6-min interval. After the completion of above protocols, all data were calculated. The vasorelaxation was expressed as a percentage of the maximal relaxation in response to papaverine (3 × 10⁻⁴ M), which was added at the end of experiments to produce the maximal relaxation (100%) of arteries.

Electrophysiological Experiments

Aortic rings were longitudinally cut and fixed on the bottom of an experimental chamber. The arteries were continuously perfused with control solution (37°C) bubbled with 95% O₂-5% CO₂ gas mixture. A glass micro-electrode (tip resistance 40 to 80 MΩ) filled with 3 M KCl and held by a micromanipulator (Narishige, Tokyo, Japan), was inserted into a smooth muscle cell from the intimal side of the vessel.¹⁴ The electrical signal was amplified using a recording amplifier (Electro 705; World Precision Instruments Inc., Sarasota, FL). The membrane potential was continuously monitored and recorded on a chart recorder (SS-250F-1; SENKONIC Inc., Tokyo, Japan). Membrane potentials in the pres-

ence of levcromakalim (10⁻⁷ M) in combination with phenylephrine (3 × 10⁻⁷ M) or U46619 (3 × 10⁻⁸ M) were recorded in arteries treated with or without glibenclamide (10⁻⁶ M), Tiron (10 mM), apocynin (1 mM), or propofol (10⁻⁶ M). These agents were applied 20 min before membrane potential recordings.

Measurements of *in Situ* Superoxide Production

An oxidative fluorescent dye hydroethidine was used to evaluate levels of superoxide *in situ* as described previously.¹² Cells are permeable to hydroethidine; in the presence of superoxide, hydroethidine is oxidized to fluorescent ethidium bromide, in which form it is trapped by intercalation with DNA. This method provides sensitive detection of superoxide levels *in situ*. Unfixed rat aortas with 20-μm-thick were placed on glass slides. Hydroethidine (2 × 10⁻⁶ M) in phosphate-buffered saline (pH = 7.4) was applied to each slice. Slides were incubated in a light-protected chamber at 37°C for 20 min. Images were obtained with a FLUOVIEW FV300 laser scanning confocal microscope (OLYMPUS Inc., Tokyo, Japan) equipped with a krypton/argon laser. Fluorescence was detected with a 585-nm long-pass filter. Laser settings were identical for acquisition of images from all of arteries. Before the application of hydroethidine, arteries were incubated with the normal solution for 20 min in which phenylephrine (3 × 10⁻⁷ M) or U46619 (3 × 10⁻¹⁰, 3 × 10⁻⁹, 3 × 10⁻⁸, or 3 × 10⁻⁷ M) was added. In some experiments, Tiron (10 mM), apocynin (1 mM), a thromboxane receptor antagonist SQ29548 (10⁻⁶ M), allopurinol (10⁻⁴ M), or propofol (3 × 10⁻⁷, 10⁻⁶, or 3 × 10⁻⁶ M) was also applied. Aortas exposed to hydroethidine in the sole presence of Tiron (10 mM) served as the control, and the fluorescence in the artery was expressed as a ratio.¹²

Western Immunoblotting Analysis

Cytosolic and membranous fractions were prepared and used for Western immunoblotting analysis.¹⁵ Arteries were incubated in the modified Krebs-Ringer's bicarbonate solution (37°C, pH = 7.4, control solution) bubbled with 95% O₂-5% CO₂ gas mixture and thereafter quickly frozen (-80°C). Some aortas were incubated for 20 min in the control solution, the solution with the addition of phenylephrine (3 × 10⁻⁷ M), U46619 (3 × 10⁻⁸ M), or U46619 (3 × 10⁻⁸ M) in combination with propofol (10⁻⁶ M). Blood vessels were powdered under liquid nitrogen and solubilized in ice-cold sterile water (1 ml) containing 0.1% Triton X-100. The lysate was centrifuged at 600g for 15 min at 4°C, and the supernatant fluid was used for the measurement of total protein levels.¹⁶ A portion of the supernatant fluid was centrifuged at 100,000g for 30 min at 4°C, and the pellet was used as a membrane fraction.

Samples (10 μg for membrane protein) were run on 12.5% sodium dodecyl sulfate (SDS) polyacrylamide gels.

Blotted membranes were probed for 120 min at 4°C with anti-Nox1, anti-Nox2, anti-Nox4, anti-p47phox (Upstate Cell Signaling, Lake Placid, NY), anti-p22phox (Santa Cruz Biotechnology, Santa Cruz, CA), and anti-adaptin- α (Thermo Fisher Scientific, Rockford, IL) (1 μ g/ml each). After washing with phosphate-buffered saline containing 0.05% Tween 20 for 30 min, the membrane was incubated with horseradish peroxidase-conjugated antiimmunoglobulin G antibody (eBioscience, San Diego, CA) diluted at 1:2000 in phosphate-buffered saline-Tween 20 buffer at room temperature for 60 min. The blots were washed three times for 10 min in phosphate-buffered saline-Tween 20 buffer and subsequently visualized with an enhanced chemiluminescence detection system (Amersham GE Healthcare UK Ltd., Little Chalfont, Buckinghamshire, England), exposed to x-ray film, and analyzed by NIH image software produced by Wayne Rasband (National Institutes of Health, Bethesda, MD). To determine loading/transfer variations of protein, all blots were stained with Ponceau Red (washable, before incubation with antibodies) as well as Coomassie Brilliant Blue (permanent, after the enhanced chemiluminescence detection system). Intensity of total protein bands per lane was evaluated by densitometry. Negligible loading/transfer variation was observed between samples.

Drugs

The following pharmacological agents were used: allopurinol, apocynin, diltiazem, dimethyl sulfoxide, glibenclamide, levcromakalim, phenylephrine, propofol, SQ29548, Tiron, and U46619 (Sigma Aldrich Inc., St. Louis, MO) and hydroethidine (Polysciences Inc., Warrington, PA). Drugs were dissolved in distilled water such that volumes of less than 60 μ l are added to the perfusion system. The stock solutions of apocynin, glibenclamide, levcromakalim, propofol, and Tiron were prepared in dimethyl sulfoxide, and the highest concentration of dimethyl sulfoxide in our experimental condition was 1.74×10^{-6} M. The solution containing hydroethidine (7 mg) was produced using N,N-dimethylacetamide (1 ml). The concentrations of drugs are expressed as final molar concentration.

Statistical Analysis

The data were expressed as means \pm SD; n refers to the number of rats from which the aorta was taken. Power calculations were done with responses to the reduction of superoxide levels by propofol (3×10^{-7} M) in the aortas treated with U46619 (3×10^{-8} M) as the primary endpoint, and we calculated that a sample size of 5 gave 99% power to detect a change of 13.3% in levels of superoxide at a significance level of 0.05 (SD = 4.3%). Statistical analyses were performed by StatView[®] version 5.0 (SAS Institute Inc., Cary, NC) and Sample Power 2.0 (SPSS Japan Inc., Tokyo, Japan). Data were

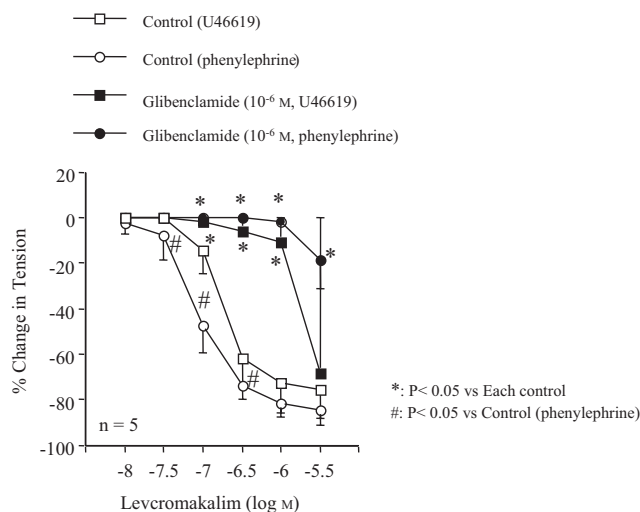


Fig. 1. Concentration-response curves to levcromakalim (10^{-8} to 3×10^{-6} M) in the presence or in the absence of glibenclamide (10^{-6} M), obtained in the rat aorta without endothelium contracted with phenylephrine (3×10^{-7} M) or U46619 (3×10^{-8} M). The data were expressed as means \pm SD; n refers to the number of rats from which the aorta was taken, and they were expressed as percent of maximal relaxation induced by papaverine (3×10^{-4} M). Difference between the control aorta and the aorta treated with glibenclamide (* $P < 0.05$) and that between the control aorta contracted with phenylephrine and the control contracted with U46619 (# $P < 0.05$) are statistically significant.

evaluated by using repeated-measures of analysis of variance (for comparison among groups in the length-tension study) or a factorial analysis of variance (for comparison among groups in other studies) as appropriate, followed by Student-Newman-Keuls test as a *post hoc* analysis. Differences were considered to be statistically significant when $P < 0.05$.

Results

Organ Chamber and Electrophysiological Experiments

Levcromakalim concentration-dependently induced relaxation in aortas contracted with phenylephrine (3×10^{-7} M) or U46619 (3×10^{-8} M), whereas the relaxation in those treated with U46619 was attenuated (fig. 1). Glibenclamide (10^{-6} M) inhibited the relaxation in aortas contracted with either vasoconstrictor agent (fig. 1). Constrictor responses in control aortas (950 ± 100 mg [$n = 5$] or 933 ± 184 mg [$n = 5$] for phenylephrine and U46619, respectively) and those in aortas treated with glibenclamide (10^{-6} M) (920 ± 85 mg [$n = 5$] and 897 ± 152 mg [$n = 5$] for phenylephrine and U46619, respectively) did not differ between phenylephrine (3×10^{-7} M) and U46619 (3×10^{-8} M). In addition, glibenclamide did not alter contraction in response to either agent.

Tiron (10 mM) augmented vasodilation in response to levcromakalim in aortas contracted with U46619

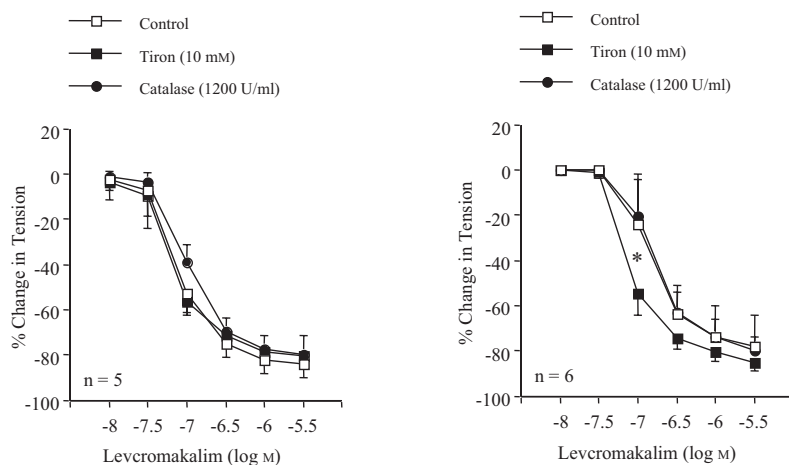


Fig. 2. Concentration-response curves to levcromakalim (10^{-8} to 3×10^{-6} M) in the presence or in the absence of Tiron (10 mM) or catalase (1200 U/ml), obtained in the rat aorta without endothelium contracted with phenylephrine (3×10^{-7} M) or U46619 (3×10^{-8} M). The data were expressed as means \pm SD; n refers to the number of rats from which the aorta was taken, and they were expressed as percent of maximal relaxation induced by papaverine (3×10^{-4} M). Difference between the control aorta and the aorta treated with Tiron is statistically significant (* $P < 0.05$).

Contraction with Phenylephrine (3×10^{-7} M)

Contraction with U46619 (3×10^{-8} M)

(3×10^{-8} M), whereas it did not alter the dilation in those treated with phenylephrine (3×10^{-7} M) (fig. 2). Catalase (1200 U/ml) failed to alter dilation in both aortas contracted with U46619 and those with phenylephrine (fig. 2).

Propofol (3×10^{-7} , 10^{-6} M) augmented the vasodilator responses to levcromakalim in the aortas contracted with U46619 (3×10^{-8} M) but not those with phenylephrine (3×10^{-7} M) (fig. 3).

In the aortas contracted with U46619 (3×10^{-8} M), apocynin (1 mM), but not allopurinol (10^{-4} M), augmented the vasodilator responses to levcromakalim, whereas apocynin (1 mM) and Tiron (10 mM) did not alter relaxation in response to diltiazem (fig. 4). Dimethyl sulfoxide (2×10^{-6} M) did not produce any effects on the vasodilation in aortas contracted with U46619 (3×10^{-8} M) (data not shown).

Membrane potential in the presence of levcromakalim (10^{-7} M) in combination with U46619 (3×10^{-8} M)

increased compared with that induced by levcromakalim (10^{-7} M) in combination with phenylephrine (3×10^{-7} M) (-40.8 ± 1.9 mV and -48.8 ± 2.0 mV, respectively; $P < 0.05$ compared with phenylephrine). In the presence of levcromakalim (10^{-7} M) in combination with U46619 (3×10^{-8} M) or phenylephrine (3×10^{-7} M), glibenclamide (10^{-6} M) significantly produced depolarization, compared with that in aortas without this ATP-sensitive K^+ channel antagonist (-36.2 ± 1.1 mV and -34.8 ± 1.3 mV for condition in the presence of phenylephrine or U46619, respectively; $P < 0.05$ compared with each control). Tiron (10 mM), apocynin (1 mM), and propofol (10^{-6} M) similarly induced hyperpolarization in response to levcromakalim (10^{-7} M) in the presence of U46619 (3×10^{-8} M) (fig. 5), whereas these agents did not affect the resting membrane potential (-44.4 ± 2.7 , -42.8 ± 1.9 , -44.8 ± 3.6 , or -45.2 ± 3.0 mV for control, Tiron, apocynin and propofol, respectively [$n = 5$ each]; not statistically significant).

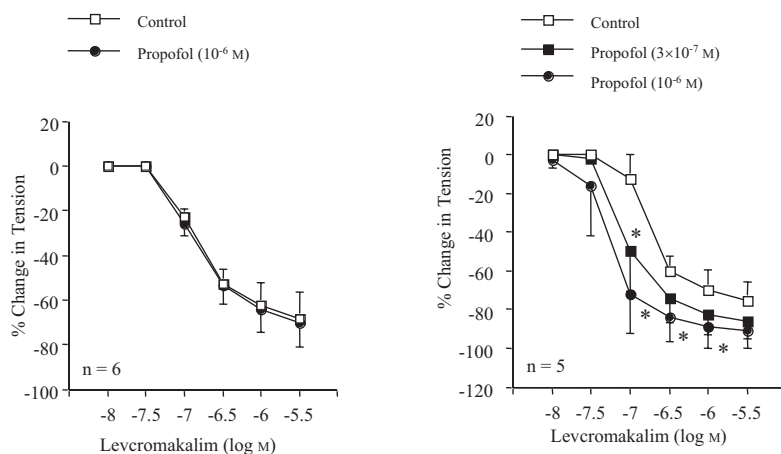
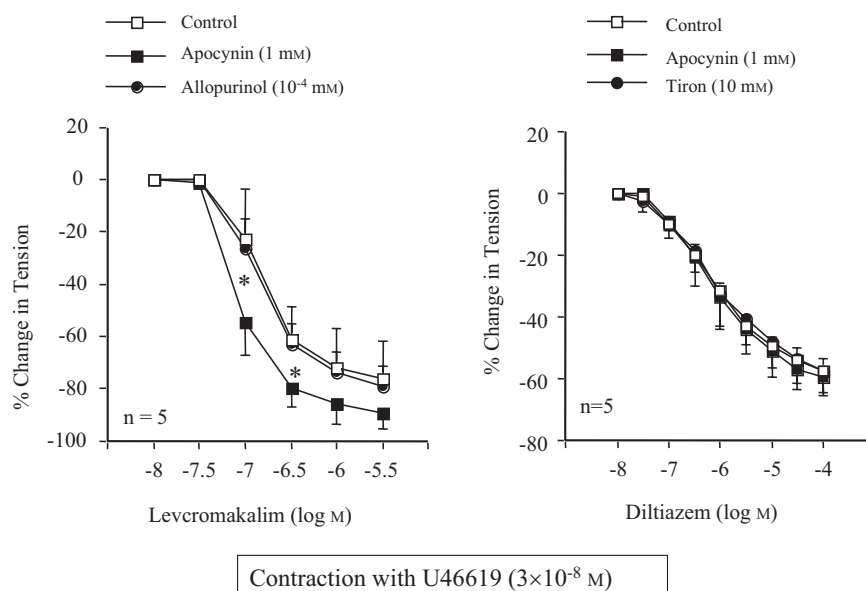


Fig. 3. Concentration-response curves to levcromakalim (10^{-8} to 3×10^{-6} M) in the presence or in the absence of propofol (3×10^{-7} or 10^{-6} M), obtained in the rat aorta without endothelium contracted with phenylephrine (3×10^{-7} M) or U46619 (3×10^{-8} M). The data are expressed as percent of maximal relaxation induced by papaverine (3×10^{-4} M). The data are expressed as means \pm SD; n refers to the number of rats from which the aorta was taken. Difference between the control aorta and the aorta treated with propofol is statistically significant (* $P < 0.05$).

Contraction with Phenylephrine (3×10^{-7} M)

Contraction with U46619 (3×10^{-8} M)

Fig. 4. Concentration-response curves to levromakalim (10^{-8} to 3×10^{-6} M) or diltiazem (10^{-8} to 10^{-4} M) in the presence or in the absence of apocynin (1 mM), allopurinol (10^{-4} M), or Tiron (10 mM), obtained in the rat aorta without endothelium contracted with U46619 (3×10^{-8} M). The data are expressed as percent of maximal relaxation induced by papaverine (3×10^{-4} M). The data are expressed as means \pm SD; n refers to the number of rats from which the aorta was taken. Difference of relaxation in response to levromakalim between the control aorta and the aorta treated with apocynin is statistically significant (* $P < 0.05$).



Measurements of *in Situ* Superoxide Production

U46619 (3×10^{-9} to 3×10^{-7} M) concentration-dependently increased *in situ* levels of superoxide within vascular smooth muscle compared with those incubated with U46619 (3×10^{-10} M) or phenylephrine (3×10^{-7} M) (fig. 6). Tiron (10 mM), apocynin (1 mM), and SQ29548 (10^{-6} M), but not allopurinol (10^{-4} M), similarly reduced levels of superoxide in the aortas exposed to U46619 (3×10^{-8} M) (fig. 6). Propofol (3×10^{-7} to 3×10^{-6} M) concentration-dependently reduced levels of superoxide in these arteries (fig. 6).

Western Immunoblotting Analysis

Protein expression of NADPH oxidase subunits (p47phox, but not p22phox, Nox2, Nox1, and Nox4) increased in membrane fractions of aortas exposed to U46619 (3×10^{-8} M) but not in those to phenylephrine (3×10^{-7} M) (fig. 7), whereas total protein fractions did

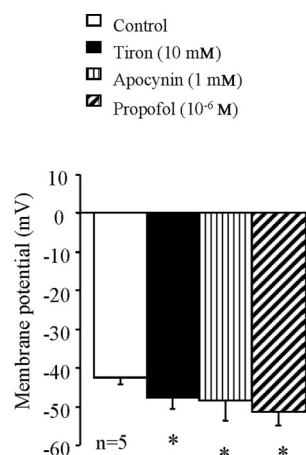
not differ among groups (data not shown). The augmentation of expression in arteries treated with U46619 was abolished by propofol (10^{-6} M) (fig. 7).

Discussion

The Effect of Thromboxane A₂ on Vascular ATP-sensitive K⁺ Channel Function and the Role of Oxidative Stress

In the current study, phenylephrine (3×10^{-7} M) and U46619 (3×10^{-8} M) similarly contracted aortas, whereas the latter solely inhibited the dilation as well as hyperpolarization in response to levromakalim. In addition, a selective ATP-sensitive K⁺ channel antagonist glibenclamide almost abolished dilation and significantly reduced hyperpolarization irrespective of the agent used for contraction.^{7,17} Therefore, these results suggest that a thromboxane A₂ analogue, but not an α_1 -adrenoceptor

Fig. 5. Membrane potential of the rat aorta without endothelium in the presence of levromakalim (10^{-7} M) in combination with U46619 (3×10^{-8} M) treated with or without Tiron (10 mM), apocynin (1 mM), or propofol (10^{-6} M). The data were expressed as means \pm SD; n refers to the number of rats from which the aorta was taken. Difference between the control aorta and the aorta treated with apocynin, propofol, or Tiron is statistically significant (* $P < 0.05$).



In the presence of levromakalim (10^{-7} M) in combination with U46619 (3×10^{-8} M)

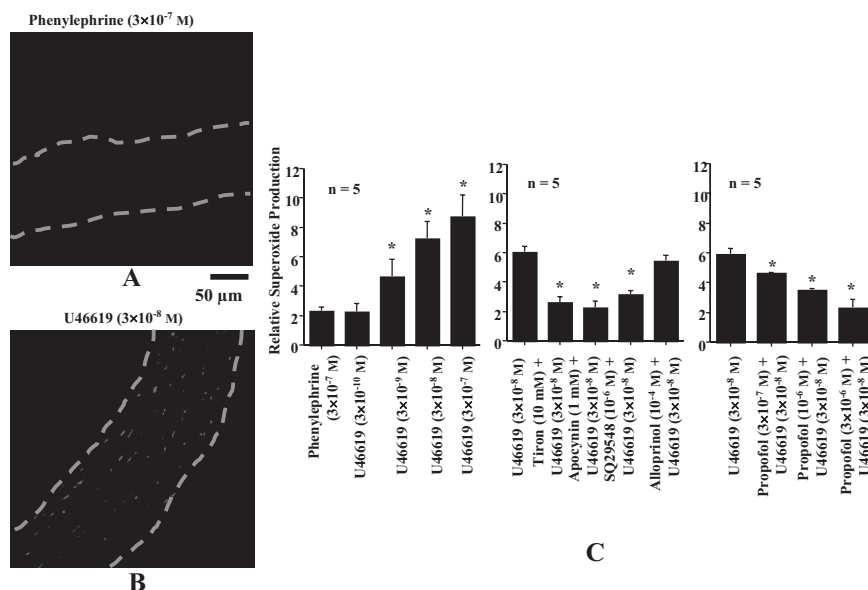


Fig. 6. (A and B) Representative images of *in situ* superoxide production. Gray dots indicate external margins of aortas. Note increased intensity of white fluorescence in the aorta treated with U46619 (3×10^{-8} M). (C) Relative superoxide production in the aortas treated with phenylephrine (3×10^{-7} M), U46619 (3×10^{-10} to 3×10^{-7} M), U46619 (3×10^{-8} M) in combination with Tiron (10 mM), apocynin (1 mM), SQ29548 (10^{-6} M) or allopurinol (10^{-4} M), or U46619 (3×10^{-8} M) in combination with propofol (3×10^{-7} to 3×10^{-6} M). The data were expressed as means \pm SD; n refers to the number of rats from which the aorta was taken. Difference between the aorta treated with phenylephrine and the aortas treated with U46619, that between the aorta treated with U46619 and the aorta treated with U46619 in combination with Tiron, apocynin, or SQ29548, and that between the aorta treated with U46619 and the aorta treated with U46619 in combination with propofol are statistically significant (* $P < 0.05$).

agonist, impairs the vascular ATP-sensitive K^+ channel function and that this difference between vasoconstrictor agents is not dependent on the degree of contraction each produced. It is of importance to note that increased oxidative stress seen in some pathophysiological conditions induces malfunction of ATP-sensitive K^+ channels in vascular smooth muscle cells.^{8,9} A superoxide inhibitor Tiron and a nonselective NADPH oxidase inhibitor apocynin (which also acts as a superoxide inhibitor), but not a hydrogen peroxide scavenger catalase, recovered ATP-sensitive K^+ channel function in the current study, indicating that superoxide *via* NADPH oxidase is a most likely source of the K^+ channel inhibition in blood vessels induced by thromboxane A_2 .^{8,18}

Vascular smooth muscle cells contain several superoxide-producing pathways, among which NADPH oxidase

is predominant.² Findings in cultured cells from pulmonary arterial endothelium or corpus cavernosum documented that acute exposure to a thromboxane A_2 mimetic predisposes to superoxide production in these cells *via* NADPH oxidase activation, including the membrane translocation of gp91phox (Nox2) or p47phox.⁴⁻⁶ Considering these previous results, we have conducted the current study to simultaneously evaluate roles of major NADPH oxidase subunits related to Nox2 activation, including Nox2, p22phox, and p47phox, in the oxidative stress produced by thromboxane A_2 in the intact artery.^{1,2} As we expected, U46619, but not phenylephrine, increased levels of superoxide within the vascular smooth muscle, and this augmentation was completely inhibited by a superoxide scavenger Tiron, a nonselective NADPH oxidase inhibitor apocynin, or a

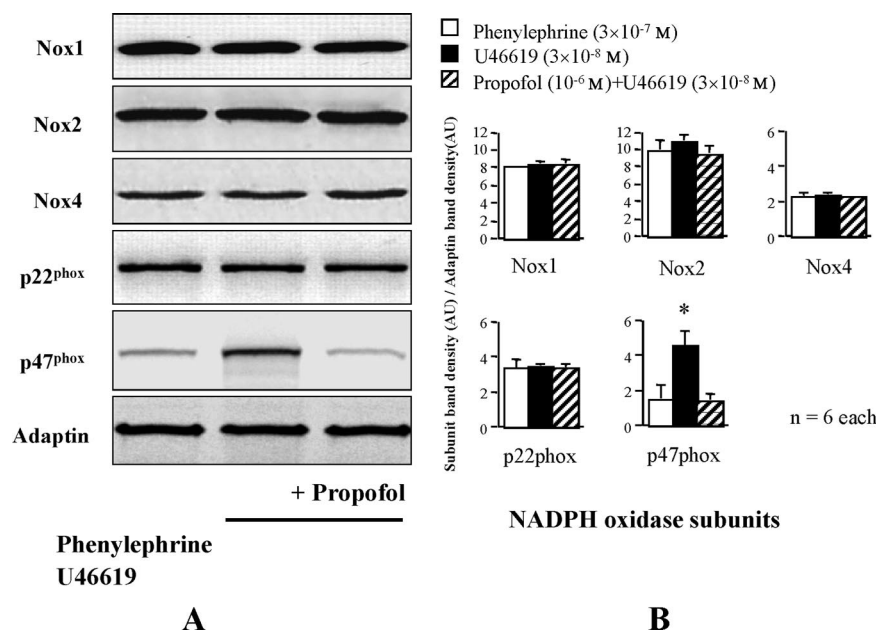


Fig. 7. (A) Protein expression of nicotinamide adenine dinucleotide phosphate oxidase (NADPH) subunits, including Nox1, Nox2, Nox4, p22phox, p47phox, and adaptin in the membrane from the rat aorta without endothelium, after 20-min incubation in the control solution with the addition of phenylephrine (3×10^{-7} M), U46619 (3×10^{-8} M), or U46619 (3×10^{-8} M) in combination with propofol (10^{-6} M) are shown. The adaptin band shows equal loading of protein. (B) In the bar graph, the subunit band density (assay unit; AU) per adaptin band density (AU) is expressed as means \pm SD; n refers to the number of rats from which the aorta was taken. Difference between the control aorta and the aorta treated with U46619 is statistically significant (* $P < 0.05$).

thromboxane receptor antagonist SQ29548.^{8,18,19} In the current study, protein expression of NADPH oxidase subunits was also examined because recent studies have questioned the selectivity of apocynin as a NADPH oxidase inhibitor.¹⁸ We have confirmed that the expression of p47phox is solely augmented by the exposure toward U46619. It is well known that interactions between membrane-bound subunits such as Nox2 or p22phox and cytosolic subunits, including p47phox are essential upon activation of Nox2 and that assembly of p47phox is most important among these to support the action of Nox2.¹ Indeed, our results that membrane translocation of p47phox was predominant upon the application of thromboxane A₂ are consistent with a previous study on cultured cells from corpus cavernosum.⁴ Therefore, it can be concluded that thromboxane receptor activation in the intact arterial smooth muscle increases levels of superoxide resulting from activation of a Nox2-related NADPH oxidase subunit p47phox. However, the signal transduction pathway to produce p47phox translocation coupling the activation of thromboxane receptor remains to be determined.

The Role of Propofol as an NADPH Oxidase Inhibitor in Oxidative Stress of the Vascular Smooth Muscle Cells

Propofol reportedly possesses potential antioxidant properties caused by its chemical structure similar to that of phenol-based free-radical scavengers such as vitamin E.¹⁰ Previous *in vivo* or *in vitro* studies documented that this intravenous anesthetic reduces oxidative stress toward blood vessels.^{11,12} These results suggest that this anesthetic may be protective against the vascular dysfunction caused by increased oxidative stress. Indeed, propofol (3×10^{-7} to 10^{-6} M) recovered vascular ATP-sensitive K⁺ channel function *via* reduction of superoxide levels within arterial walls. In addition, 10^{-6} M of this agent completely inhibited protein expression of a Nox2-related NADPH oxidase subunit p47phox. The plasma concentration of propofol during induction of anesthesia in humans has been reported as up to 3×10^{-5} M, and burst suppression doses of propofol for cerebral protection are up to 6×10^{-5} M.²⁰⁻²² Effective concentrations of propofol (3×10^{-7} to 10^{-6} M) to inhibit NADPH oxidase activity, resulting in restoration of vascular ATP-sensitive K⁺ channel function are probably within clinical range, even if one considers plasma-free concentrations calculated from both above clinical plasma concentrations and the substantial binding of this compound to plasma proteins (from 97 to 98%).²¹ Therefore, our results strongly indicate that clinically relevant concentrations of propofol can play a role as a NADPH oxidase inhibitor. It is crucial to note the difference between results in the current study and our previous study showing the inhibition of propofol on vasorelaxation in response to ATP-sensitive K⁺ channel openers.²³ Upon the comparison

of these studies, it has become evident that higher plasma-free concentration of propofol (above 10^{-5} M) is probably needed to inhibit the K⁺ channel function, indicating the benefit of this intravenous anesthetic in the clinical practice considering its protective effect toward oxidative stress in blood vessels. A recent study showing that propofol enhances plasma γ -tocopherol levels in surgical patients, also suggests a multi-mode of action of this agent regarding the organ protection²⁴; further studies are required to clarify the whole scenario why this anesthetic is protective toward oxidative stress.

Study Limitations

The current study was conducted in aortic rings as well as the vascular smooth muscle. It is still unclear whether the beneficial effects of propofol seen in our study have any relevance to pathogenesis in such a conduit artery, although recent studies have demonstrated that increased oxidative stress induced by NADPH oxidase plays a crucial role in the aortic atherosclerosis.²⁵ It is also true that smaller resistance arteries greatly contribute to the regulation of hemodynamics *in vivo*; therefore, further studies are warranted to clarify that propofol similarly reduces oxidative stress in these peripheral vasculatures. In addition, it should be noted that throughout the current study, higher oxygen (hyperoxia) was used to incubate aortic rings because we intended to make a comparison of the current data with our previous study.²³ Such a higher concentration of oxygen may modify the propofol effects toward oxidative stress. Indeed, a previous study demonstrated that lower oxygen tension is capable of inducing augmentation of a superoxide-inactivating system in the blood vessels.²⁶ However, it remains unclear whether changes of oxygen tension influence the results obtained in the current study.

Conclusions

The current study has demonstrated the following new findings. First, thromboxane A₂ induces overproduction of superoxide *via* a Nox2-related NADPH oxidase subunit p47phox in vascular smooth muscle cells of the intact artery. Second, increased levels of superoxide produced by thromboxane A₂ impair ATP-sensitive K⁺ channel function in the vascular smooth muscle. Third, clinically relevant concentrations of propofol restore the channel function acting as a NADPH oxidase inhibitor. Thromboxane A₂ reportedly plays a role in a variety of cardiovascular diseases, including myocardial infarction, cerebral vasospasm, hypertension, preeclampsia, and thrombotic disorders.^{3,27,28} Accumulating findings demonstrated that ATP-sensitive K⁺ channels contribute to pathophysiological vasodilation during hypoxia, acidosis, and inflammation.^{7,29-31} These findings together

with our results suggest possibilities that thromboxane receptor activation modifies ATP-sensitive K^+ channel function in above pathophysiological conditions by modulating levels of superoxide *via* NADPH oxidase, and that clinically used propofol may be protective against vascular malfunction related to such pathogenesis.

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