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Inhibitory Effects of Propofol on Acetylcholine-induced, Endothelium-dependent Relaxation and Prostacyclin Synthesis in Rabbit Mesenteric Resistance Arteries

Akiko Yamashita, M.D., Junko Kajikuri, B.S.,† Masuo Ohashi, M.D.,‡ Yuichi Kanmura, M.D., Ph.D.,§ Takeo Itoh, Ph.D.‖*

Background: **Propofol (2,6-diisopropylphenol) modulates endothelium-dependent relaxation in some arterial preparations. The effect of propofol on endothelium-dependent, prostacyclin-mediated responses in mesenteric resistance arteries has not yet been clarified.**

Methods: **The effect of propofol was examined on acetylcholine-induced membrane potential changes in the presence of N^G-nitro-L-arginine (L-NOARG) in endothelium-intact rabbit mesenteric resistance arteries *in vitro*. The effects of propofol were also examined on the endothelium-dependent relaxation and prostacyclin synthesis that was induced by acetylcholine in the presence of L-NOARG and nicardipine. The effect of propofol on the relaxation induced by a prostacyclin analogue was examined in strips treated with L-NOARG and diclofenac.**

Results: **Acetylcholine produced an initial and a slow membrane hyperpolarization. Propofol, 10 μM, and diclofenac each inhibited the acetylcholine-induced slow hyperpolarization, but not the initial hyperpolarization. Acetylcholine produced an endothelium-dependent relaxation that was significantly inhibited by propofol, 10 μM, and diclofenac. Propofol, 10 μM, greatly inhibited the acetylcholine-induced synthesis of prostacyclin, as did diclofenac. Propofol, 10 μM, had no effect on the relaxation induced by a prostacyclin analog.**

Conclusions: **In rabbit mesenteric resistance arteries, propofol inhibits the synthesis of prostacyclin and thus attenuates acetylcholine-induced, endothelium-dependent responses. Our results may help to explain why some actions seen with propofol in some preparations (*e.g.*, vasoconstriction) are not seen after the endothelium is removed. (Key words: Endothelium-derived hyperpolarizing factor; membrane hyperpolarization; prostaglandin; vascular endothelium.)**

PROPOFOL (2, 6-diisopropylphenol) is a widely used intravenous anesthetic agent with an action that is rapid in onset and of short duration, and its elimination is rapid.[1] Induction of anesthesia with propofol is often accompanied by a mild-to-severe hypotension[1,2] caused by decreases in cardiac output and peripheral vascular resistance.[2–8] Propofol modulates the function of the endothelium in some arterial preparations, thus causing a modulation of vascular tone. For example, propofol produces an endothelium-dependent vasodilation that is partly inhibited by indomethacin (an inhibitor of cyclooxygenase) in rat aorta and pulmonary artery[9] and by N^G-nitro-L-arginine (L-NOARG; an inhibitor of nitric oxide synthase) or methylene blue (an inhibitor of guanylyl cyclase) in bovine coronary artery,[10] suggesting that propofol may stimulate the release of nitric oxide (NO) and vasodilator prostanoids from endothelial cells. In contrast, in rat thoracic aorta, propofol inhibits the endothelium-dependent relaxation and cyclic guanosine 3′,5′-monophosphate (cGMP) synthesis induced by acetylcholine,[11] suggesting that propofol may interfere with the actions of endothelium-derived NO. Thus, there is apparently contradictory evidence concerning the effects of propofol on endothelial function, and its action may depend on species, region, and experimental conditions.

In many types of experimental vessel preparations,

* Research Fellow, Department of Anesthesiology and Critical Care Medicine, Kagoshima University Faculty of Medicine.

† Research Fellow, Department of Pharmacology, Nagoya City University Medical School.

‡ Graduate Student, Department of Pharmacology, Nagoya City University Medical School.

§ Associate Professor, Operating Rooms, Kyushu University Hospital.

‖ Chairman and Professor, Department of Pharmacology, Nagoya City University Medical School.

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Address reprint requests to Dr. Itoh: Department of Pharmacology, Nagoya City University Medical School, Mizuho-ku, Nagoya 467-8601, Japan. Address electronic mail to: titoh@med.nagoya-cu.ac.jp

exogenously administered acetylcholine causes the release of a variety of endothelium-derived substances, including endothelium-derived relaxing factor (EDRF; which has been identified as nitric oxide or a nitric oxide–containing agent),[12–16] endothelium-derived hyperpolarizing factor (EDHF; which may be a metabolite of arachidonic acid derived from cytochrome P450 monooxygenase[17,18] or an endogenous cannabinoid[19] or potassium ion[20]), and prostacyclin.[14,15,21,22] Each of these substances has been shown to produce hyperpolarization,[12–15] and a corresponding relaxation,[12–23] in the adjacent vascular smooth muscle. It has also been found that barbiturates inhibit cytochrome P450 monooxygenase and possibly the synthesis of EDHF.[24] However, the effect of propofol on the actions of EDHF and prostanoids has not been clarified in small splanchnic resistance arteries, which are important in the control of total peripheral vascular resistance and in the maintenance of splanchnic blood flow, *e.g.,* in patients undergoing surgery.[25,26]

The present study was performed to clarify the actions of propofol on the effects produced by endothelium-derived prostacyclin in rabbit mesenteric resistance arteries. We first observed the effect of propofol on acetylcholine-induced membrane potential changes in endothelium-intact strips in the presence of L-NOARG. We then examined the effect of propofol (1) on the acetylcholine-induced relaxation seen during the contraction evoked by norepinephrine, and (2) on the acetylcholine-stimulated synthesis of prostacyclin (by measuring 6-keto-prostaglandin $F_{1\alpha}$, a stable metabolite of prostacyclin) in the presence of L-NOARG and nicardipine (an L-type Ca^{2+} channel inhibitor). All the effects of propofol were compared with those of diclofenac (a cyclooxygenase inhibitor). Lastly, we examined the effect of propofol on the relaxation induced by beraprost, a stable analog of prostacyclin,[27] in strips treated with L-NOARG and diclofenac.

Materials and Methods

Twelve male Japan White albino rabbits (supplied by Kitayama Labes Co., Nagano, Japan), weighing 1.9–2.5 kg, were anesthetized by intravenous injection of pentobarbitone sodium (40 mg/kg) and then killed by exsanguination. The protocols used conformed with guidelines on the conduct of animal experiments issued by Nagoya City University Medical School and by the Japanese government (law no. 105, notification no. 6) and were approved by the Committee on the Ethics of Animal Experiments of Nagoya City University Medical School. The third and fourth branches of the mesenteric artery distributing to the region of the ileum (diameter, approximately 70–100 μm) were excised immediately, then cleaned by removal of connective tissue in Krebs solution under a binocular microscope at room temperature. After each artery had been cut open along its long axis using a small scissors, circularly cut strips were carefully prepared so as not to damage the endothelium, as described previously.[28] Seven to 13 strips were obtained from each rabbit, and these were used for measurements of membrane potential (21 strips obtained from 12 rabbits), isometric force (21 strips from 11 rabbits), and 6-keto-prostaglandin $F_{1\alpha}$ (24 strips from 4 rabbits).

In 17 strips, the endothelium was carefully removed by gentle rubbing of the internal surface of the vessel using small pieces of razor blade, and satisfactory ablation of the endothelium was pharmacologically verified by the absence of a relaxing effect when 3 μM acetylcholine was applied during a contraction induced by norepinephrine (using 9 of the 17 strips), as described previously.[29–31]

Recording of Membrane Potential Changes

An artery strip was placed in a chamber of 0.5 ml volume. Both ends of the strip were pinned down to the bottom of the chamber, and it was superfused with Krebs solution at a flow rate of about 3 ml/min. Glass microelectrodes were made from borosilicate glass tubing (OD = 1.2 mm with a glass filament inside; Hilgenberg, Malsfeld, Germany), then filled with 1 M KCl. The resistance of the electrodes was 80–100 MΩ. The electrode was inserted into smooth muscle cells from the luminal side. Membrane potentials recorded using a microelectrode amplifier (MEZ-8301; Nihon Kohden, Tokyo, Japan) were displayed on a cathode-ray oscilloscope (V-252; Hitachi Denshi Co., Tokyo, Japan). The data were stored at an acquisition rate of 200 Hz using an AxoScope 1.1.1/Digidata 1200 data-acquisition system (Axon Instruments, Foster, CA) on an IBM/AT-compatible personal computer.

The effects were examined by comparing the responses to administration of 1 μM acetylcholine before and in the presence (after 15 min pretreatment) of 10 μM propofol in the same cells (n = 8). All tests were carried out in a solution containing 0.1 mM L-NOARG, 5 μM guanethidine (to prevent norepinephrine-outflow from sympathetic nerves), and 0.3 μM nicardipine when en-

dothelium-intact preparations were used. The concentrations of these agents were selected so as to produce their maximal effects. In four preparations, the effect of diclofenac was examined by comparing the responses to 1 μM acetylcholine before and in the presence (after 15 min pretreatment) of 3 μM diclofenac in a solution containing 0.1 mM L-NOARG, 5 μM guanethidine, and 0.3 μM nicardipine, and the effect of propofol on the acetylcholine-induced response was then examined in the presence of 3 μM diclofenac in the same cells (n = 4 cells from four rabbits).

When endothelium-denuded preparations were used, the solution contained 5 μM guanethidine with 0.3 μM nicardipine. After 2 h incubation with the solution, acetylcholine (1 μM) was applied for 2 min two times with a 20-min interval (nine cells from nine rabbits). In five of these nine cells, beraprost, 10 nM, was then applied for 3 min while recordings were made from the same cells.

Recording of Mechanical Activity

Circularly cut strips (0.2–0.3 mm long, 0.07–0.10 mm wide, 0.02–0.03 mm thick) were prepared for force recording. A fine silk thread tied to each end of the strip was fixed to a small piece (about 1 mm × 1 mm) of Scotch double-sided adhesive tape (3M, St. Paul, MN). One piece of tape was fixed to the chamber and the other to the strain gauge (UL-2; Minebea, Tokyo, Japan), allowing us to record isometric tension as described previously.[29,30] The chamber had a volume of 0.9 ml, and the solutions were gently introduced using a syringe from one end of the chamber and simultaneously aspirated by a pump from the other end. The resting force (1.2 ± 0.3 mg) was adjusted to obtain a maximum contraction in Krebs solution containing 128 mM K^+.

In smooth muscle of the rabbit mesenteric resistance artery, we had previously found that propofol inhibits the norepinephrine-induced contraction as a result of an inhibition of Ca^{2+} release and of Ca^{2+}-influx through L-type Ca^{2+} channels.[31] The former mechanism is apparent at concentrations of propofol more than 30 μM, whereas the latter comes into operation at more than 10 μM. To avoid the complexity of the action of propofol on the acetylcholine-induced relaxation, 0.3 μM nicardipine (an L-type Ca^{2+} channel inhibitor) was added to the bath, and the concentration of propofol was limited to 10 μM. The concentration of nicardipine chosen for these experiments completely blocks the tonic contraction induced by 128 mM K^+ in this preparation.[31] Furthermore, 0.1 mM L-NOARG together with 5 μM guanethidine and 3 μM propranolol (to prevent β-adrenoceptor stimulation by exogenously applied norepinephrine) was also added to the bath.

After application of the solution containing 0.1 mM L-NOARG together with 0.3 μM nicardipine, 5 μM guanethidine plus 3 μM propranolol for 45 min, the strips were contracted by 10 μM norepinephrine for 8 min at 25-min intervals to obtain reproducible responses. Acetylcholine (1 μM) was then applied once for 2 min in strips precontracted with 10 μM norepinephrine, which was applied using the time schedule described previously. This protocol was repeated so as to obtain a reproducible acetylcholine (1 μM)-induced relaxation (control response). Propofol, 10 μM, was then applied to the same strips for 15 min before and was present during the application of 10 μM norepinephrine (n = 6). A similar protocol was also performed using 3 μM diclofenac instead of, or together with, propofol. In this case, the effect of diclofenac, 3 μM, on the acetylcholine (1 μM)-induced response was first observed, and the effect of propofol, 10 μM, was then observed in the presence of 3 μM diclofenac in the same strips (n = 7). The amplitude of the norepinephrine-induced tonic contraction recorded before the application of acetylcholine in the absence of propofol or diclofenac was normalized as 100%.

The concentration–response relationship for the effect of propofol, 0.1–10 μM, on the acetylcholine (1 μM)-induced relaxation in strips precontracted with 10 μM norepinephrine was obtained in a solution containing 0.1 mM L-NOARG, 5 μM guanethidine, 3 μM propranolol, and 0.3 μM nicardipine. A given concentration of propofol was pretreated for 15 min and was present during the application of 10 μM norepinephrine (n = 4). The various concentrations of propofol were cumulatively applied from low to high.

The effect of 10 μM propofol on the relaxation induced by beraprost was examined during the contraction induced by 10 μM norepinephrine in a solution containing 0.1 mM L-NOARG, 5 μM guanethidine, 0.3 μM nicardipine, 3 μM propranolol, and 3 μM diclofenac (n = 4). After a 2-h incubation in this solution, 10 μM norepinephrine was applied for 12 min at 30-min intervals to obtain reproducible responses. Various concentrations of beraprost, 1–100 nM, were then cumulatively applied from low to high during the norepinephrine-induced tonic contraction. After application of 10 μM propofol for 15 min (as a pretreatment), this protocol was repeated in the presence of 10 μM propofol (n = 4).

Assay for 6-Keto-Prostaglandin $F_{1\alpha}$

Six preparations (four strips with endothelium and two strips without endothelium) were obtained from each of four rabbits. After equilibration for 2 h in Krebs solution, strips with or without endothelium were transferred to tubes containing 0.4 ml Krebs solution and equilibrated for 1 h at 36°C. The Krebs solution contained guanethidine (5 μM), L-NOARG (0.1 mM), and nicardipine (0.3 μM). Acetylcholine (final concentration, 1 μM) was then added to the tube for 3 min. For the assay, a 50-μl sample of solution was taken from the tube, and the concentration of 6-keto-prostaglandin $F_{1\alpha}$-like immunoreactivity was measured using an enzyme immunoassay kit purchased from Amersham Pharmacia Biotech (Tokyo, Japan). This kit combines the use of peroxidase labeled for 6-keto-prostaglandin $F_{1\alpha}$ conjugate, a specific antiserum that can be immobilized onto precoated microtitre plates, and a one-pot stabilized substrate solution. The assay protocol for this kit followed a manual supplied by Amersham Pharmacia Biotech. To normalize the concentrations, the area of each strip was measured, and the content of 6-keto-prostaglandin $F_{1\alpha}$-like immunoreactivity was expressed per centimeter^2 for measurements made after a 3-min period with or without acetylcholine. When used, propofol, 10 μM, or diclofenac, 3 μM, was pretreated for 15 min and was present throughout the application of 1 μM acetylcholine.

Solutions

The Krebs solution contained 137.4 mM Na^+, 5.9 mM K^+, 1.2 mM Mg^{2+}, 2.6 mM Ca^{2+}, 15.5 mM HCO_3^-, 1.2 mM $H_2PO_4^-$, 134 mM Cl^-, and 11.5 mM glucose. The concentration of K^+ was modified by the isotonic replacement of NaCl with KCl. The solutions were bubbled with 95% oxygen and 5% carbon dioxide, and their *p*H was adjusted to 7.3 or 7.4 (using NaOH or HCl) at 34–36°C.

Drugs

Drugs used were norepinephrine, diclofenac sodium, and nicardipine (Sigma Chemical Co., St. Louis, MO), guanethidine (Tokyo Kasei, Tokyo, Japan), acetylcholine hydrochloride (Daiichi Pharmaceutical Co., Tokyo, Japan), N^G-nitro-L-arginine (L-NOARG; Peptide Institute Inc., Osaka, Japan), and propranolol (Nacalai, Kyoto, Japan). Pure propofol, 99.9%, was kindly provided by Zeneca Pharmaceuticals (Mereside, Macclesfield, United Kingdom), and it was diluted as required in Krebs solution with the aid of a sonicator (Sine Sonic 100; Kokusai Denki Electric Co., Tokyo, Japan). L-NOARG was directly diluted in Krebs solution (0.1 mM). Nicardipine, 10 mM, was initially dissolved in dimetyl sulfoxide and further diluted in Krebs solution. The other agents were dissolved in ultra-pure Milli-Q water (Milli-Q SP/Milli RX system; Japan Millipore Corp., Tokyo, Japan). Beraprost sodium was kindly provided by Yamanouchi Pharmaceutical Co. (Tokyo, Japan).

Table 1. Effects of Propofol and Diclofenac on Resting Membrane Potential and Acetylcholine-induced Hyperpolarization in Smooth Muscle Cells of Endothelium-intact Rabbit Mesenteric Resistance Arteries

	RMP (mV)	Initial Hyperpolarization (mV)	Slow Hyperpolarization (mV)
Control-1	−59.4 ± 2.3	−7.0 ± 1.6	−5.3 ± 1.7
Propofol	−57.2 ± 1.0	−6.3 ± 1.2	−1.0 ± 1.9*
Control-2	−56.3 ± 3.4	−10.2 ± 2.4	−8.7 ± 3.3
Diclofenac	−55.0 ± 1.1	−8.3 ± 1.5	−0.4 ± 0.6*
Diclofenac + propofol	−55.1 ± 1.0	−8.9 ± 1.5	−0.5 ± 0.5*

Acetylcholine (1 μM) was applied for 2 min before (Control-1) and after application of 10 μM propofol in the same cells (n = 8). In another set of experiments, acetylcholine (1 μM) was applied before (Control-2) and after application of 3 μM diclofenac in the same cells (n = 8). Propofol (10 μM) was then applied with 3 μM diclofenac (n = 4). The peak amplitude of the initial and slow components of the hyperpolarization were measured with respect to the potential before the application of acetylcholine. Values are mean ± SD.
*$P < 0.05$ *vs.* the corresponding control.

Statistical Analysis

In the present experiments, the results obtained in the absence (control) and presence of propofol were obtained in the same strip unless otherwise noted, with *n* indicating the number of strips, which equals the number of animals. The values recorded are expressed as mean ± SD. Statistical analysis was performed using a one-way repeated-measures analysis of variance (Stat View 4.02; Abacus Concepts, Berkeley, CA) followed by the Scheffé F test for *post hoc* analysis (Super ANOVA; Abacus Concepts). The significance of the concentration-dependent effects of propofol or beraprost was examined by use of this analysis. The statistical significance of the effects of 10 μM propofol or 3 μM diclofenac were examined by the use of paired (table 1) or unpaired Student *t* tests. *P* values < 0.05 were considered significant.

Results

Effects of Propofol on Acetylcholine-induced Membrane Potential Changes

In endothelium-intact strips, the resting membrane potential in smooth muscle cells of the rabbit mesenteric

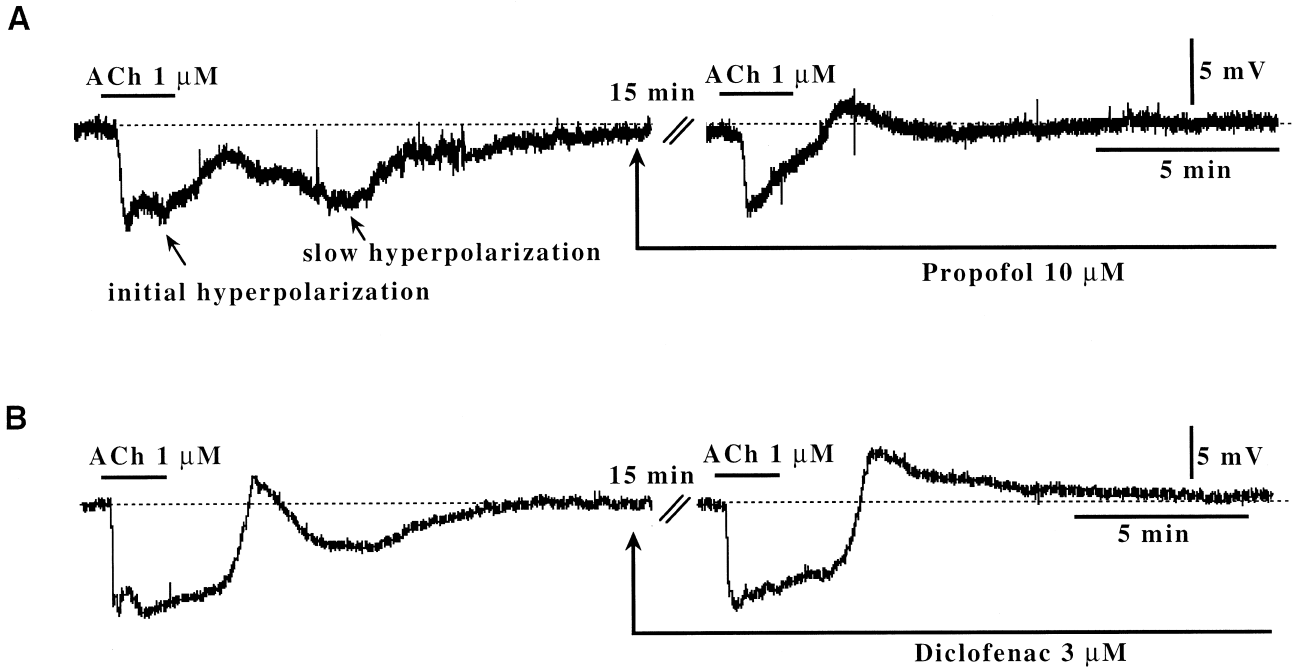

Fig. 1. Original recordings of the effects of propofol and diclofenac on acetylcholine-induced membrane potential changes in endothelium-intact rabbit mesenteric arteries. Acetylcholine (ACh, 1 μM) was applied for 2 min before (control) and after application of 10 μM propofol (*A*) or 3 μM diclofenac (*B*). The latter two drugs were pretreated for 15 min, as indicated. All tests were carried out in a solution containing 0.1 mM L-NOARG, 5 μM guanethidine, and 0.3 μM nicardipine. The two sets of traces were obtained from different vessels. Dotted line indicates the resting membrane potential level.

artery was −59.4 ± 2.3 mV (n = 8), and no spontaneous membrane activity was observed. Application of acetylcholine, 1 μM, for 2 min produced a membrane hyperpolarization with two components: an initial hyperpolarization and a subsequent slow hyperpolarization that appeared after the withdrawal of acetylcholine (fig. 1 and table 1).

In endothelium-intact strips, propofol, 10 μM, modified neither the resting membrane potential nor the acetylcholine-induced initial hyperpolarization, but this agent greatly attenuated the acetylcholine-induced slow hyperpolarization (fig. 1A and table 1). Similarly, diclofenac, 3 μM, had no effect on the resting membrane potential, but it blocked the acetylcholine-induced slow hyperpolarization with no change in the acetylcholine-induced initial hyperpolarization (fig. 1B and table 1). In the presence of diclofenac, the acetylcholine-induced slow hyperpolarization was already attenuated, and addition of propofol, 10 μM, had no further effect on it and no effect on either the resting membrane potential or the acetylcholine-induced initial membrane hyperpolarization (table 1).

In the smooth muscle cells of endothelium-denuded strips, the resting membrane potential was −55.8 ± 1.9 mV (n = 9), and acetylcholine, 1 μM, produced no significant change in membrane potential (hyperpolarization by 0.1 ± 0.8 mV, n = 9). Under these conditions, beraprost, 10 nM, induced a slowly developed membrane hyperpolarization (by 12.4 ± 2.9 mV, $P < 0.01$, n = 5).

Effect of Propofol on Acetylcholine-induced Relaxation

Acetylcholine, 1 μM, produced a relaxation during the contraction induced by 10 μM norepinephrine in endothelium-intact strips in a solution containing 0.1 mM L-NOARG with 5 μM guanethidine, 3 μM propranolol, and 0.3 μM nicardipine (figs. 2A and 2B). In contrast, acetylcholine, 1 μM, did not significantly modify the norepinephrine-induced tonic contraction in endothelium-denuded strips (−5.8 ± 7.6% relaxation; n = 6).

Propofol, 10 μM, attenuated the acetylcholine-induced, endothelium-dependent relaxation (from 86% to 48%; n = 7; figs. 2A, 2C). Diclofenac, 3 μM, also attenuated the acetylcholine-induced relaxation (from 76% to 43%; n = 6; figs. 2B, 2D). In the presence of diclofenac, propofol had no effect on the residual acetylcholine-induced relaxation (figs. 2B, 2D). The inhibitory action of propofol

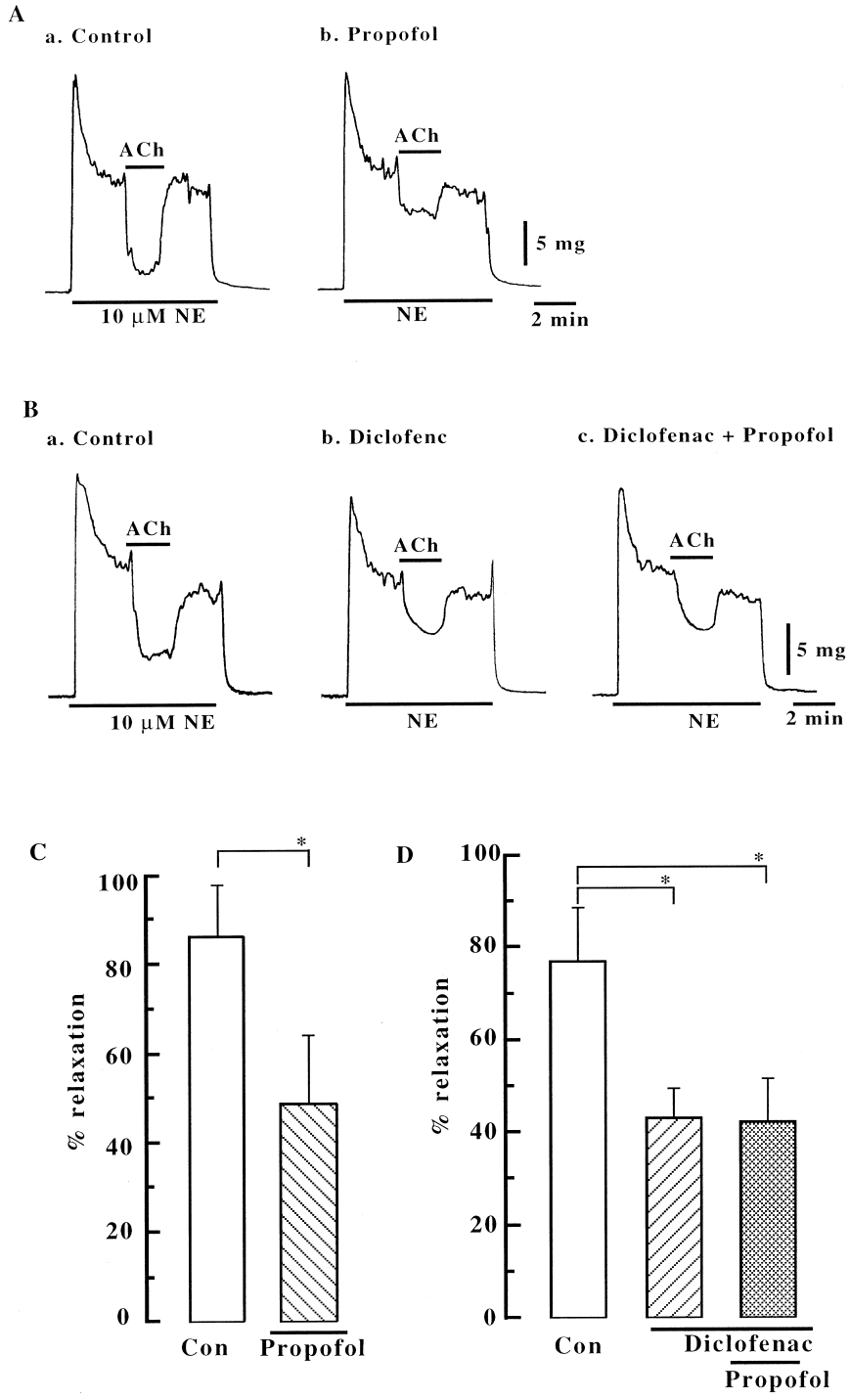

Fig. 2. Effects of propofol and diclofenac on acetylcholine-induced relaxation in endothelium-intact strips of rabbit mesenteric artery. (*A*) Acetylcholine (ACh, 1 μM) was applied for 2 min (indicated by the horizontal bars) during the tonic contraction induced by 10 μM norepinephrine (NE) before (control) and after a 15 min pretreatment with 10 μM propofol (Ab) or 3 μM diclofenac (Bb). (Bc), after the recording of the response shown in (Bb), acetylcholine was again applied 15 min after the addition of 10 μM propofol to the existing 3 μM diclofenac. Norepinephrine was applied for 8 min at 25-min intervals. The responses shown in (*A*) and (*B*) were obtained from different strips. (*C* and *D*) Summary of the effects of propofol (10 μM, n = 6) or diclofenac (3 μM) without and with propofol (10 μM; n = 7) on the acetylcholine-induced maximum relaxation. Mean of data with SD shown by vertical lines. All tests were carried out in a solution containing 0.1 mM L-NOARG, 5 μM guanethidine, 0.3 μM nicardipine, and 3 μM propranolol. *Significant difference from the corresponding control ($P < 0.001$).

on the acetylcholine-induced relaxation was concentration-dependent and obtained at concentrations of 0.3 μM or more (fig. 3).

Effect of Propofol on Beraprost-induced Relaxation

Beraprost, 1–100 nM, produced a relaxation during the contraction induced by 10 μM norepinephrine in a con-

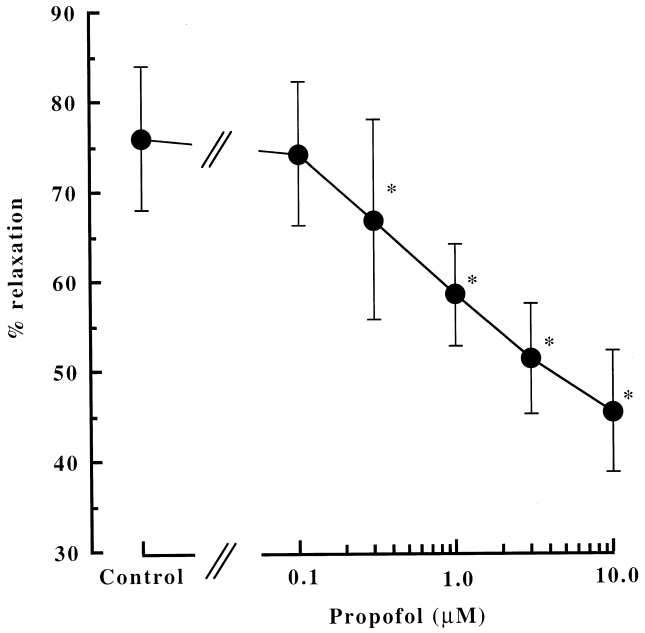

Fig. 3. Concentration-dependence of effect of propofol on acetylcholine-induced maximum relaxation in endothelium-intact strips of rabbit mesenteric artery. Acetylcholine (1 μM) was applied for 2 min during a 10 μM norepinephrine (NE)-induced tonic contraction before (control) and after application of various concentrations of propofol (0.1–10 μM). Propofol at each concentration was pretreated for 15 min and was then present throughout the experiment. Each symbol shows the mean of data from four strips, with SD shown by vertical lines. All tests were carried out in a solution containing 0.1 mM L-NOARG, 5 μM guanethidine, 0.3 μM nicardipine, and 3 μM propranolol. *Significant difference from control ($P < 0.05$).

centration-dependent manner (figs. 4A and 4B; $P < 0.05$). Propofol, 10 μM, had no effect on the relaxation induced by beraprost at any given concentration (1–100 nM; figs. 4A and 4B; $P > 0.1$).

Effect of Propofol on Acetylcholine-induced Production of 6-Keto-Prostaglandin $F_{1\alpha}$

Under resting conditions, the concentration of 6-keto-prostaglandin $F_{1\alpha}$ was 0.695 ± 0.336 pmol/cm^2 and 0.060 ± 0.037 pmol/cm^2 in endothelium-intact and -denuded strips, respectively (n = 4), each measurement being made for a 3-min period in the absence of acetylcholine (no propofol, no diclofenac). A 3-min application of acetylcholine, 1 μM, significantly increased this metabolite only in endothelium-intact strips (fig. 5). Diclofenac, 3 μM, blocked and propofol, 10 μM, greatly inhibited this acetylcholine-induced synthesis of 6-keto-prostaglandin $F_{1\alpha}$.

Discussion

Propofol Inhibits the Synthesis of Prostacyclin by the Endothelium

In the smooth muscle of the guinea pig coronary artery, acetylcholine produces an initial, followed by a sustained, membrane hyperpolarization when the endothelium is intact.[14] These hyperpolarizations are mediated by three factors derived from the endothelium: the initial hyperpolarization is generated by EDHF, and the slow hyperpolarization is generated by NO and prostanoids.[14] Similarly, in the smooth muscle of the rabbit mesenteric artery, acetylcholine produces an endothelium-dependent membrane hyperpolarization mediated

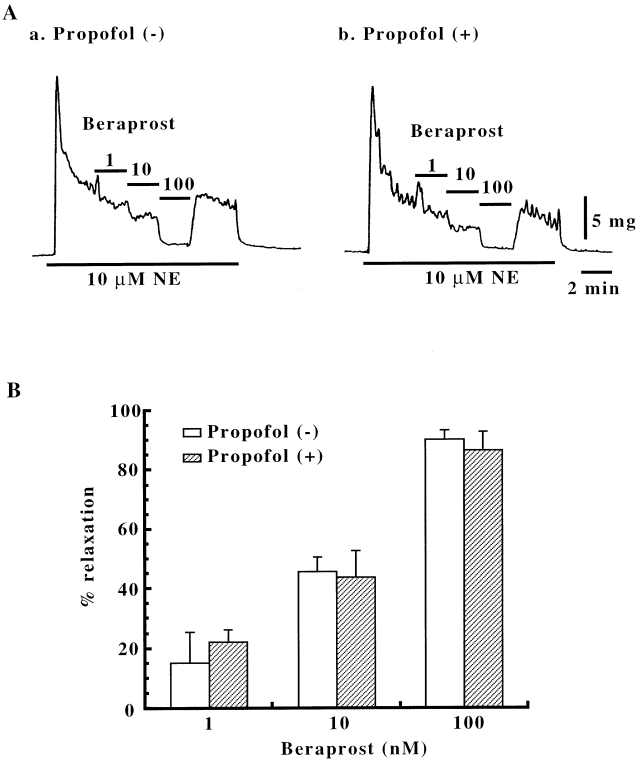

Fig. 4. Effect of propofol on beraprost-induced relaxation in endothelium-intact strips treated with both L-NOARG (0.1 mM) and diclofenac (3 μM). (*A*) Beraprost (1–100 nM) was cumulatively applied for 2 min from low to high concentration (indicated by the horizontal bars) during the tonic contraction induced by 10 μM norepinephrine (NE) before (Aa) and after a 15-min pretreatment with 10 μM propofol (Ab) (the two traces were obtained from the same strip). Norepinephrine was applied for 12 min at 30-min intervals. (*B*) Summary of the effects of propofol (10 μM) on the beraprost-induced maximum relaxation. Mean of data from four strips, with SD shown by vertical lines. All tests were carried out in a solution containing 0.1 mM L-NOARG, 5 μM guanethidine, 0.3 μM nicardipine, 3 μM propranolol, and 3 μM diclofenac.

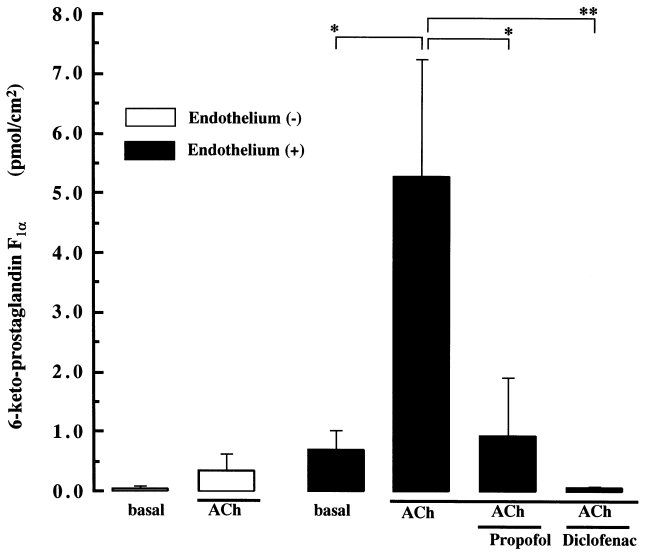

Fig. 5. Effects of propofol (10 μM) and diclofenac (3 μM) on acetylcholine-induced synthesis of prostacyclin, as estimated from the amount of 6-keto-prostaglandin $F_{1\alpha}$–like immunoreactivity in endothelium-intact (*closed columns*) and endothelium-denuded (*open columns*) strips of rabbit mesenteric artery. Each column represents the mean of data from four strips, with SD shown by vertical lines. All tests were carried out in a solution containing 0.1 mM L-NOARG, 5 μM guanethidine, and 0.3 μM nicardipine. *$P < 0.05$ compared with the level induced by acetylcholine in the absence of agents under endothelium-intact conditions. **$P < 0.001$.

by EDHF, NO, and prostanoids.[15] Nishiyama *et al.*[32] recently reported that in the smooth muscle of the guinea pig coronary artery, acetylcholine produced an endothelium-dependent hyperpolarization with two components: an initial component mediated by EDHF and a slow component mediated by prostanoids. Similarly, in the present experiments, acetylcholine produced an endothelium-dependent hyperpolarization with two components. Because the solution used in the present experiments contained L-NOARG (an inhibitor of NO synthase), these hyperpolarizations could be mediated by EDHF or prostanoids, or both.

It is well known that prostacyclin is the major prostanoid derived from the endothelium in various types of vascular beds and that it causes a vasorelaxation.[21,22] In the present experiments, (1) diclofenac, a cyclooxygenase inhibitor, blocked the acetylcholine-induced slow hyperpolarization, but had no effect on the initial one. Furthermore, (2) beraprost (10 nM), a stable analog of prostacyclin,[27] hyperpolarized the smooth muscle cell membrane in endothelium-denuded strips, as previously found using iloprost (another stable analog of prostacyclin).[14,15] Moreover, (3) in endothelium-intact (but not endothelium-denuded) strips, acetylcholine increased the production of 6-keto-prostaglandin $F_{1\alpha}$, a stable metabolite of prostacyclin, an effect that was blocked by diclofenac. On the basis of these findings, we conclude that in the rabbit mesenteric artery, acetylcholine induces a muscle relaxation and stimulates the release of endothelium-derived prostacyclin, which then induces a slow membrane hyperpolarization of the smooth muscle cells.

In the present experiments, acetylcholine relaxed the strips immediately after its application, and the relaxation in response to acetylcholine was terminated as soon as the acetylcholine was removed. On the other hand, the slow component of the hyperpolarization (apparently as result of prostanoid release triggered by acetylcholine) appeared approximately 1.5–2 min after the removal of the acetylcholine, by which time the relaxation response had disappeared. These results suggest that the slow prostanoid-induced membrane hyperpolarization is not the mechanism responsible for the prostacyclin-induced relaxation in this artery. However, in the present experiments, the acetylcholine-induced hyperpolarization was observed in the absence of norepinephrine, whereas the acetylcholine-induced relaxation was obtained in the presence of norepinephrine. Thus, there is a possibility that the presence or absence of this vasospasmogenic agent be responsible for the lack of an apparent time relationship between the onset of the slow hyperpolarization and that of the relaxation evoked by acetylcholine. This remains to be clarified.

In the present experiments, (1) propofol inhibited the slow, but not the initial hyperpolarization induced by acetylcholine, and its effect could not be seen in the presence of diclofenac because diclofenac had much the same effect. Furthermore, (2) propofol attenuated the acetylcholine-induced relaxation during the norepinephrine contraction, just as diclofenac did, so this effect was not seen in the presence of diclofenac. Moreover, (3) although propofol greatly inhibited the production of 6-keto-prostaglandin $F_{1\alpha}$, it had no effect on the relaxation induced by a prostacyclin analog. On the basis of these findings, we conclude that by inhibiting the acetylcholine-stimulated synthesis of prostacyclin in endothelial cells, propofol attenuates certain acetylcholine-induced, endothelium-dependent responses (*i.e.*, those that are mediated by prostacyclin).

Effect of Propofol on EDHF-mediated Responses

In the presence of L-NOARG, acetylcholine produced an endothelium-dependent relaxation during the norepi-

nephrine-contraction, and diclofenac attenuated this relaxation by about one half. In our preliminary experiments, we found that the remaining acetylcholine-induced relaxation was abolished in strips pretreated with 0.1 μM charybdotoxin (an inhibitor of large and intermediate conductance Ca^{2+}-activated K^+ channels) together with 0.1 μM apamin (an inhibitor of small conductance Ca^{2+}-activated K^+ channels). Furthermore, acetylcholine still produced a large initial hyperpolarization in the presence of L-NOARG together with diclofenac (fig. 1B). These results suggest that EDHF plays a role in the acetylcholine-induced relaxation seen in the presence of L-NOARG with diclofenac.

In the present experiments, propofol had no effect on the acetylcholine-induced initial hyperpolarization in endothelium-intact strips treated with L-NOARG. Furthermore, in the presence of L-NOARG together with diclofenac, propofol had no effect on the acetylcholine-induced membrane response and relaxation. On the basis of these findings, it is suggested that propofol has no effect on those functions that are mediated by EDHF in the rabbit mesenteric artery.

On the assumption that the plasma protein binding for propofol is 97–98%, the concentrations of propofol (0.3–10 μM) used in the present study may be supratherapeutic. However, the situation is not straightforward because the microkinetic behavior of propofol within the vascular space has not yet been properly characterized.[9] Furthermore, it is not known whether only unbound propofol possesses vasomotor activity or whether the bound fraction (or any part of it) may also have a vasomotor effect.[9] It should also be pointed out that the propofol-induced impairment of endothelial prostacyclin production described here does not explain the clinically encountered propofol-induced decrease in blood pressure.

In conclusion, propofol inhibits the synthesis of prostacyclin in the endothelial cells of small mesenteric resistance arteries in the rabbit and attenuates the acetylcholine-induced, endothelium-dependent relaxation. However, in this tissue propofol may not affect the actions of EDHF, another endothelium-derived vasorelaxing factor. Because the present study was conducted in the presence of an NO synthase inhibitor, we cannot draw any conclusions about the possible influence of propofol on the actions of the NO derived from the endothelium in small mesenteric resistance arteries. However, when taken together with previous data, our finding that propofol has an inhibitory action on the synthesis of prostacyclin in endothelial cells in such arteries may help to explain why some of the variety of effects seen with propofol in some preparations (*e.g.*, vasoconstriction) are not seen in endothelium-denuded preparations.

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