Relaxant effects of propofol on human omental arteries and veins

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Summary
We have investigated the relaxant effects of propofol on smooth muscle tension in human omental arteries and veins to determine if endothelium-related mechanisms are involved. Isolated vessel segments were precontracted with endothelin-1 and propofol was added cumulatively (10⁻⁷–10⁻⁴ mol litre⁻¹). In both artery and vein segments, propofol induced relaxation, which was not dependent on an intact endothelium. Relaxation was reduced when the extracellular K⁺ concentration was increased and in the presence of tetraethylammonium chloride (TEA). In intact segments, N-nitro-L-arginine methyl ester (L-NAME), indomethacin, glibenclamide, 4-aminopyridine, clotrimazole and atropine did not affect the concentration–response curve of propofol. This indicates that propofol relaxes human omental arteries and veins in an endothelium independent manner, and that hyperpolarization caused by activation of the K⁺ channel, BKCa, may be involved. (Br. J. Anaesth. 1998; 80: 655–659)

Keywords: anaesthetic i.v.; propofol; arteries; omental; veins; omental

Propofol anaesthesia often causes undesirable hypotension. The vascular effects of propofol are poorly understood, but it seems to affect both the arterial and venous circulation, resulting in a reduction in systemic vascular resistance¹–⁵ and an increase in capacitance,⁶⁷ respectively.

Propofol reduces vascular smooth muscle tone both directly⁸⁻¹⁰; and indirectly,¹¹–¹⁴ for example via effects on sympathetic activity. Relaxation of vascular smooth muscle may be achieved by several mechanisms. Endothelium-dependent relaxation includes nitric oxide formation,¹² prostanoid synthesis,¹³ and release of endothelium-derived hyperpolarizing factors activating smooth muscle potassium (K⁺) channels.¹⁴ Endothelium-independent relaxation includes altering the availability of free cytosolic calcium, for example via effects on voltage-gated Ca²⁺ channel activity.¹⁵

Several studies have attempted to explain the complex actions of propofol. Propofol has been shown to relax vascular smooth muscle in both an endothelium-dependent¹⁶–²⁰ and independent manner.²¹–²⁶ Various mediators for the action of propofol have been suggested. Formation of nitric oxide or prostanoids, or both,¹⁶–²⁵ have been shown to be involved. Propofol has also been suggested to alter free cytosolic calcium concentrations in vascu-

lar smooth muscle.¹²¹²⁻²⁶ It seems that propofol may interact at several cellular targets, thereby causing relaxation of vascular smooth muscle.

The aim of this study was to investigate the direct relaxant effects of propofol on human vascular smooth muscle and to determine if endothelium-related mechanisms are involved. Experiments were performed on intact and endothelium-denuded vessel segments and in the presence or absence of inhibitors of the synthesis or action, or both, of nitric oxide, prostanoids and endothelium-derived hyperpolarizing factors.

Materials and methods
The following compounds were used: propofol (Diprivan, Zeneca); 10% Intralipid (Pharmacia Upjohn); 9,11-dideoxy-11α,9α-epoxy-methano-prostaglandin F₂α (U46619, Sigma, thromboxane A₂ analogue); substance P acetate (SP, Sigma); endothelin-1 (ET-1, Sigma); N-nitro-L-arginine methyl ester hydrochloride (L-NAME, Sigma, inhibitor of nitric oxide synthase); sodium-indomethacin (Confortid, Dunex, inhibitor of cyclo-oxygenase); tetraethylammonium chloride (TEA, Sigma, non-selective inhibitor of K⁺ channel, BKCa, may be involved). (Br. J. Anaesth. 1998; 80: 655–659)

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The study was approved by the Ethics Committee, University of Lund. Macroscopically normal segments of human omental arteries and veins were obtained from 22 patients aged 36–88 (median 69) yr undergoing abdominal surgery. Patients with endocrine tumours, abdominal infections and previous radiotherapy were excluded. The male/female ratio was 0.7. Experiments were performed within 1–24 h. Vessel segments, after being dissected free from fat and connective tissue, were stored in aerated KRS at 4°C. There was no correlation between responses and storage time.

MEASUREMENT OF CHANGES IN ISOMETRIC TENSION

Vessels with an outer diameter of 0.5–1.0 mm were cut into 2–4-mm long ring segments. The segments were placed in 2-ml tissue baths on two L-shaped hooks, one of which was attached to a Grass FTO3C force-displacement transducer for isometric measurement of tension. Vessel tension was recorded on a Grass polygraph model 7b. The baths were thermostatically maintained at 37°C and contained lipid KRS (see composition above), which was aerated continuously with 88.5% oxygen and 11.5% carbon dioxide, to give approximate values of pH 7.4, PCO₂ 5.0 kPa and PO₂ 40 kPa. With the use of lipid KRS, it was possible to maintain a constant lipid concentration in the baths, even when different concentrations of propofol were added.

Vessel segments were stretched gradually to a resting tension of 6 mN during an equilibration period of 60–90 min to obtain the optimal tension level. After the equilibration period, KCl 90 mmol litre⁻¹ was added to the baths. When the resulting contraction had reached a plateau, the lipid KRS was changed three times within 10 min, during which tension returned to baseline. KCl 90 mmol litre⁻¹ was then added 1–2 times, each time followed by wash-out until consistent contractions were elicited. With this procedure, endothelium stays functionally and anatomically intact.

Experimental procedure

In the first series of experiments, we investigated the endothelium dependency of the action of propofol. The endothelium of one artery and one vein segment from six patients was removed by gentle injection of the oxygen–carbon dioxide gas mixture through the vessel lumen for 5 min. To confirm that the endothelium had been removed successfully, substance P (SP), an agent which is totally dependent on a functional endothelium for its action in these preparations, was added to vessel segments precontracted with the thromboxane A₂ analogue U46619 (10⁻⁸–3 × 10⁻⁷ mol litre⁻¹). SP caused no response in endothelium-denuded vessel segments but caused relaxation in control vessel segments. Vessel segments were rinsed several times after addition of SP. Then ET-1 1 – 3 × 10⁻⁹ mol litre⁻¹ was added. This
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Concentration corresponds to the EC$_{50}$ value in these preparations.$^{32}$ ET-1 was first added at $1 \times 10^{-9}$ mol litre$^{-1}$. If no contraction was elicited, the concentration was increased stepwise until the segments contracted. Within 10 min, ET-1-induced contraction reached a plateau. Pilot experiments had shown that this contraction remained constant without fade for at least 30 min. When a stable level of tension had been reached, propofol was added cumulatively in 0.5 log$_{10}$ units ($10^{-7}$–$10^{-4}$ mol litre$^{-1}$) and the resulting relaxation measured. Propofol was added in a cumulative manner to assess any potential concentration dependency of the responses and to construct concentration–response curves. Each concentration of propofol present in the bath for 2 min or until a stable tension was reached after relaxation. The cumulative concentration–response experiments with propofol took 15–20 min and were therefore performed on vessels with a stable ET-1 contraction.

In the second series of experiments, we determined by which mechanisms propofol-induced relaxation was mediated. The effect of propofol on segments precontracted with ET-1 was investigated in the presence or absence of indomethacin $10^{-5}$ mol litre$^{-1}$, L-NAME $3 \times 10^{-4}$ mol litre$^{-1}$, KCl 30 mmol litre$^{-1}$, TEA $10^{-2}$ or $10^{-3}$ mol litre$^{-1}$, glibenclamide $10^{-3}$ mol litre$^{-1}$, 4-AP 10$^{-3}$ mol litre$^{-1}$, clotrimazole $10^{-5}$ mol litre$^{-1}$ or atropine $10^{-6}$ or $10^{-5}$ mol litre$^{-1}$. Control experiments were performed simultaneously on separate segments from the same patient, generating paired data with or without treatment. To exclude an effect of the solvent DMSO used in the glibenclamide and clotrimazole experiments, control experiments were also performed in the presence of DMSO at the equivalent concentration (141 mmol litre$^{-1}$) alone. The vasoactive compounds were added to the organ baths 10 min before addition of ET-1.

**Statistical Analysis**

When similar experiments were performed on more than one segment from the same patient, the mean for each patient was calculated before presentation and statistical analysis. The number of patients is indicated by “n”. Values are expressed as mean $\pm$ SEM. The Wilcoxon signed rank test was used for each within-patient variation. Removal of the endothelium did not affect the concentration–response curve of propofol (fig. 2A).

Precontraction induced by ET-1 or the response to propofol was not affected by indomethacin (artery $n = 6$, vein $n = 5$), L-NAME (artery $n = 7$, vein $n = 8$), glibenclamide (artery $n = 5$, vein $n = 5$), 4-AP (artery $n = 7$, vein $n = 7$), clotrimazole (artery $n = 6$, vein $n = 5$), atropine (artery $n = 5$, vein $n = 5$) or the solvent DMSO (artery $n = 4$, vein $n = 4$) (data not shown).

An increase in extracellular KCl concentration did not affect precontraction induced by ET-1, but reduced the relaxation induced by propofol in both artery and vein segments (fig. 3A, B).

TEA $10^{-3}$–$10^{-2}$ mol litre$^{-1}$ did not affect precontraction induced by ET-1. TEA $10^{-2}$ mol litre$^{-1}$ reduced propofol-induced relaxation in both artery and vein segments. At TEA $10^{-3}$ mol litre$^{-1}$, propofol-induced relaxation was inhibited only at the highest concentration of propofol and only in veins (fig. 4A, B).

**Discussion**

We have shown that propofol produced concentration-dependent relaxant effects on human omental arteries and veins. The propofol concentrations used were high and exceeded those seen clinically. Clinically relevant plasma concentrations of propofol have been estimated to be $1–5 \times 10^{-5}$ mol litre$^{-1}$. However, in vivo and in vitro concentrations are not easy to compare, as the in vivo situation cannot be mimicked completely. Several factors may interfere, such as plasma protein binding and lipid solubility, two factors which are both reported to be important for propofol. Hence the clinical relevance of our findings remains to be determined. The poor water solubility of propofol requires the use of a lipid solvent for its administration. In this study, we chose 10% Intralipid as it is the vehicle used clinically. The observed effects of propofol cannot be a result of 10% Intralipid as the equivalent concentration of Intralipid was always present in the control organ baths.
The dependency of a functional endothelium for the action of propofol has been investigated in several species and vascular beds. Our results indicate that propofol relaxes human omental arteries and veins in an endothelium-independent manner. This also occurs in the rat aorta, canine coronary artery, pig coronary artery, and rat pulmonary artery. Propofol has also been found to have endothelium-dependent effects. In the bovine coronary artery, propofol is more potent in endothelium-intact vessel segments than in endothelium-denuded vessel segments. In rat coronary artery, the relaxant effect of propofol is endothelium-dependent. Propofol-induced relaxation was high and selectivity for BKCa might be questioned. In addition, TEA has also been found in the pig coronary artery, where propofol caused less relaxation in vessel segments precontracted with KCl than with norepinephrine, 5-hydroxytryptamine or carbachol. Moreover, in human omental arteries and veins, propofol is less potent in inhibiting KCl-induced contractions than contractions induced by U46619. The reverse of these findings seems to be true for the rat thoracic aorta, where propofol produced greater relaxation in vessel segments precontracted with KCl than with phenylephrine. The evidence for propofol-induced hyperpolarization in our study is indirect. Electro-physiological measurements of actual membrane potential are needed to verify the findings.

Hyperpolarization can be achieved by activating K+ channels, resulting in K+ efflux and reduction of smooth muscle tone. To investigate if K+ channels are involved in propofol-induced relaxation, the effect of three inhibitors of K+ channels (TEA, glibenclamide and 4-AP) was investigated. Glibenclamide and 4-AP did not affect the responses to propofol, indicating that neither KATP nor Kv is activated by propofol. In contrast, TEA inhibited propofol-induced relaxation. The effect of TEA indicates that propofol in human omental arteries and veins may activate BKCa, which results in hyperpolarization and relaxation. In canine coronary arteries, TEA at these concentrations did not affect the response to propofol, again confirming the complex and variable actions of propofol.

The TEA concentration required for inhibition of propofol-induced relaxation was high and selectivity for BKCa might be questioned. In addition, TEA has been proposed to have muscarinic antagonist properties. In our study, the muscarinic antagonist atropine had no effect on propofol-induced relaxation, indicating that relaxation was not mediated via muscarinic receptors. This also implies that the effect of TEA on propofol-induced relaxation is unlikely to be non-specific inhibition of muscarinic receptors.

Clofazimazole, an inhibitor of the cytochrome P450 system, has been shown previously to inhibit nitric oxide–prostanoid-independent relaxation. Clofazimazole is also an inhibitor of the intermediate conductance Ca2+-activated K+ channel (I_{NCX}). In our study, clofazimazole had no effect on propofol-induced relaxation, indicating that propofol may not relax human omental arteries and veins via a cytochrome P450-derived product, and it does not activate I_{NCX}.

Figure 4 Concentration–response curves for propofol in human omental artery (A) and vein (B) segments precontracted with endothelin-1 (ET-1) in the presence or absence (control) of TEA 10^{-6} and 10^{-7} mol litre^{-1}. Propofol-induced relaxation was reduced by TEA. The Wilcoxon signed rank test was used (*P<0.05). Values are mean (SEM), n=6–7.
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In summary, the relaxant effect of propofol on human omental arteries and veins was endothelium-independent and probably involved hyperpolarization, possibly resulting from activation of BK_{cal}.

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References


