Inhibition of ATP-sensitive potassium channels attenuates propofol-induced vasorelaxation

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ABSTRACT

Background: Infusion of propofol often causes significant vasodilation, which is followed by a profound drop in blood pressure. However, the exact underlying molecular mechanisms of this clinically important phenomenon remain unclear.

Objective: To determine the biological role of endothelium in propofol-induced vasorelaxation and the underlying molecular mechanisms of this response in the rat aorta.

Design, setting and subjects: Ex vivo assessment of vasomotor function in rat aortic rings, with or without endothelium, after addition of propofol or etomidate. In vivo randomised study of haemodynamic changes in Sprague Dawley rats after administration of propofol, with or without prior infusion of a K_{ATP} antagonist. In vitro measurement of intracellular calcium in cultured vascular smooth muscle cells (VSMC) treated with propofol. The experiments were conducted in a research laboratory at the National Cheng Kung University, Taiwan, from August 2008 to July 2009.

Interventions: Changes in isometric tension of pre-contracted rat aortic rings were recorded after cumulative addition of propofol (3–300 μM). An ATP-sensitive potassium (K_{ATP}) channel blocker, glibenclamide (10 μM), was incubated in the organ bath before the addition of propofol. Haemodynamic changes after intravenous administration of propofol in the presence or absence of PNU-37883A (a vascular-specific K_{ATP} channel blocker) were recorded in anaesthetised rats. Alterations in intracellular calcium and ATP levels in cultured VSMC treated with propofol were measured.

Results: Compared with etomidate, propofol induced a significant concentration-dependent vascular relaxation response that was independent of the presence of endothelium. The relaxation response was almost completely abolished by K_{ATP} channel antagonism. Levels of intracellular calcium were significantly attenuated in cultured VSMC treated with propofol (10 mM). Pre-treatment with PNU-37883A significantly attenuated propofol-induced hypotension in anaesthetised rats.

Conclusions: Development of hypotension after systemic administration of propofol is mainly caused by its direct relaxation effect on vascular smooth muscle. This response is mainly mediated by activation of K_{ATP} channels.

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Propofol (2,6-diisopropylphenol) is an alkyl phenol derivative that induces sedation and hypnosis within 1 minute after intravenous administration. Short duration of clinical effect, ease of titration, rapid cessation and limited accumulation of the parent compound or metabolites make propofol one of the most commonly used intravenous anaesthetics for surgical procedures and in patients requiring sedation in the intensive care unit.[1] However, administration of propofol is associated with profound hypotension, which is primarily a result of reduced systemic vascular resistance.[2] The direct effect of propofol on vasomotor function has been extensively investigated. However, several controversies have arisen with regard to the direct vasoactive effect of propofol. One of the unresolved questions is whether propofol mediates its vasoactive responses via activation of vascular endothelium or independently of the endothelium.[3] Furthermore, previous studies have demonstrated that propofol hyperpolarises vascular smooth muscle cells (VSMC) through activation of high-conductance Ca^{2+}-sensitive K^+ (BK_{Ca}) channels,[4] ATP-sensitive potassium (K_{ATP}) channels[5] and endothelium-derived relaxing factors[5,6] on the small conductance arteries. However, the molecular mechanisms of propofol-induced vasorelaxation in large conductance arteries, and thereby its effect on haemodynamics, have not been elucidated. The aim of our study was to determine the biological role of endothelium in propofol-induced vasorelaxation, and the underlying molecular mechanisms of this clinically important response to propofol in the rat aorta.

Methods

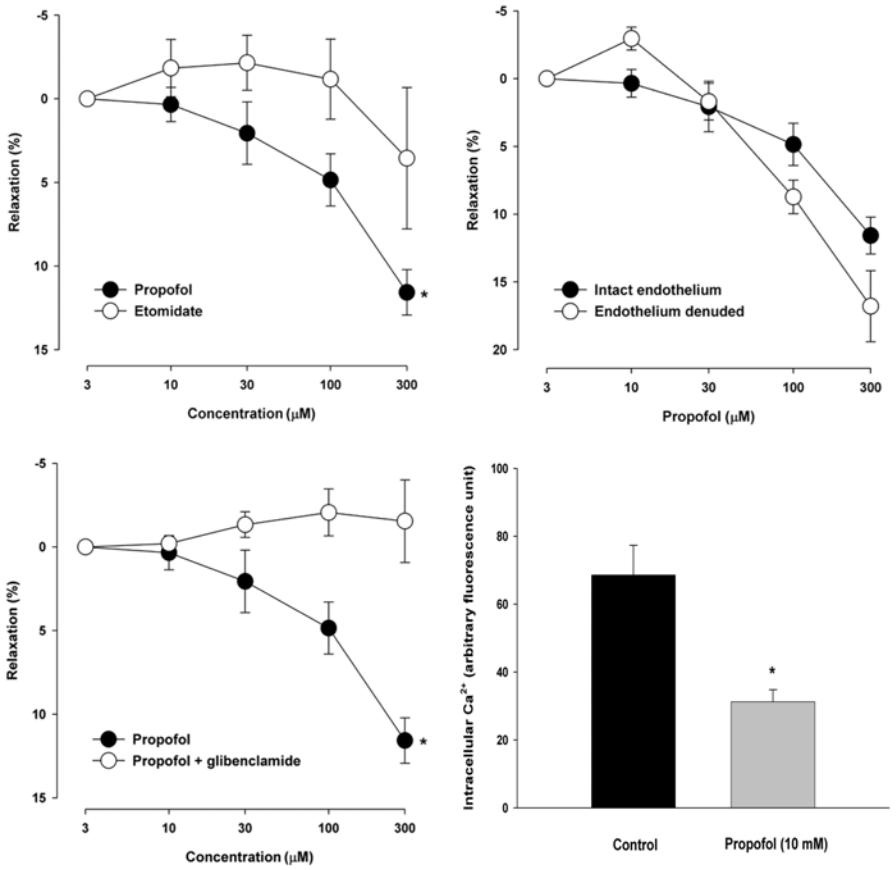
Animals

Sprague Dawley rats (approximately 300 g in weight) were obtained from the animal centre of the National Cheng Kung University, and all experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee. Rats were sacrificed by injection of pentobarbital (250 mg/kg, IP).

Ex vivo assessment of vasomotor function

Aortic rings (2 mm long) were isolated and mounted in organ chambers containing 25 mL Krebs solution. The chambers were maintained at 37° C and aerated continuously with 94% O_2/6% CO_2. Changes in isometric force

Figure 1. Results of ex vivo experiments



1A: *Measurements of isometric force of rat aortic segments in response to propofol or etomidate. Changes in isometric force were recorded after cumulative addition of propofol and etomidate (final concentrations of 0–300 μM). * P < 0.05 propofol vs etomidate analysed by analysis of variance; n = 6–8 different animals in each group.* ***1B:*** *Vasoreactivity of isolated rat aorta in response to cumulative addition of propofol in the presence (●) or absence (○) of vascular endothelium. The results showed that the two concentration response curves are superimposed.* ***1C:*** *The relaxation response induced by propofol was completely abolished by pre-incubating the preparation with a specific ATP-sensitive potassium (K_{ATP}) channel blocker, glibenclamide (10 μM). * P < 0.05 analysed by ANOVA; n = 5–6 different animals in each group.* ***1D:*** *Concentrations of intracellular calcium measured in cultured pulmonary artery vascular smooth muscle cells after 30 minutes' treatment with or without propofol (10 mM). The intracellular Ca^{2+}-dependent fluorescence intensity was measured by flow cytometry. * P < 0.001 analysed by unpaired t-test; n = 4–8 different experiments.*

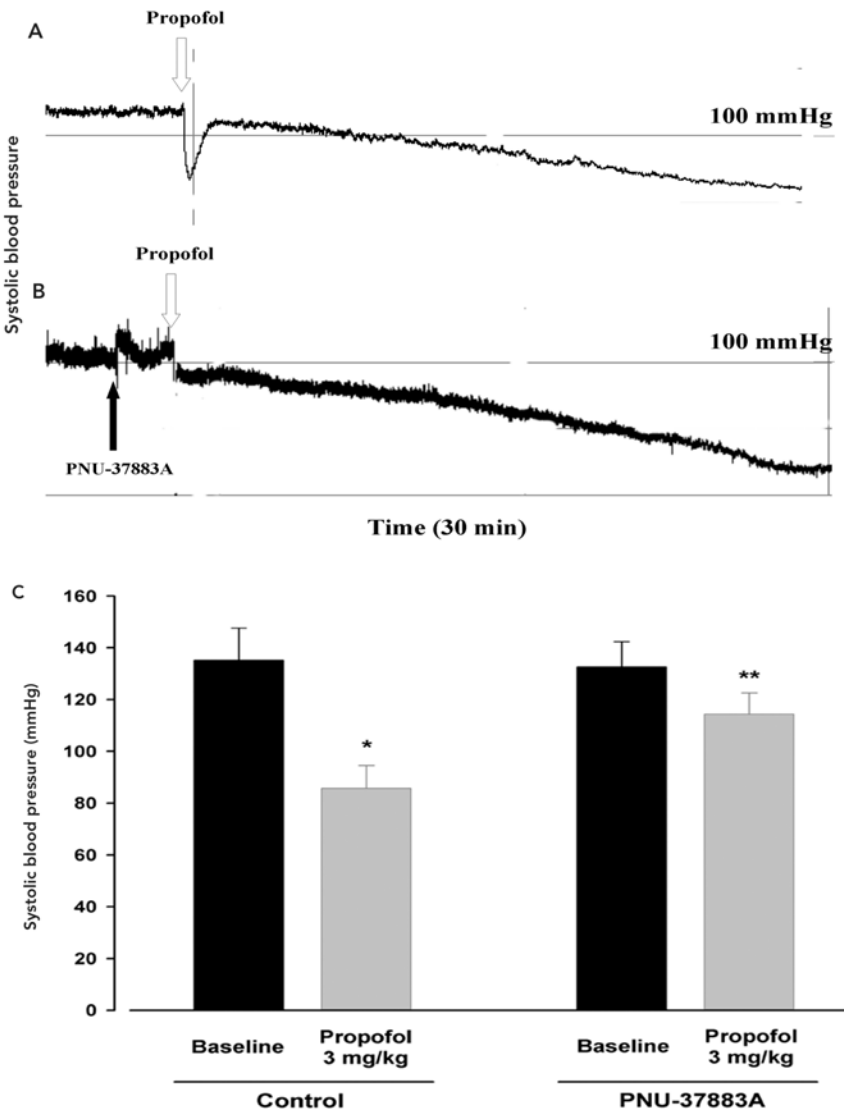
were recorded continuously using an isometric force-displacement transducer (model FT03; Grass Instruments, West Warwick, RI, USA). In some preparations, the endothelium was removed by gentle rubbing of the lumen using a wire. Each ring was gradually stretched to 2.5 g. After a 45-minute equilibration period, the rings were contracted by cumulative addition of phenylephrine (10^{-9} to 10^{-5} M; Sigma-Aldrich, St Louis, Mo, USA), and the EC_{50} of phenylephrine (concentration required to induce 50% of maximum contraction) was determined from the contraction response. Endothelial removal was confirmed by the absence of relaxation response to 10^{-7} M acetylcholine (Sigma-Aldrich, St Louis, Mo, USA).[7]

Concentration–response curves were constructed after cumulative addition of propofol (3–300 μM; AstraZeneca, Macclesfield, UK) or etomidate (3–300 μM; B Braun Melsungen, Melsungen, Germany) during pre-contraction of the vessel rings with an EC_{50} of phenylephrine. To determine the activity of K_{ATP} channels, glibenclamide (final concentration 10 μM; Sigma-Aldrich, St Louis, Mo, USA) was incubated with the isolated blood vessels before each contraction. Glibenclamide was dissolved in dimethyl sulfoxide before being added to the organ bath, and the final concentration of dimethyl sulfoxide was 0.1% (v/v).[8] Papaverine (3×10^{-4} M; Sigma-Aldrich, St Louis, Mo, USA) was used to induce complete relaxation of the vessels.

In vivo haemodynamic measurement

Rats were anaesthetised by intraperitoneal injection of ketamine (6 mg/kg). The trachea of the animals was cannulated and supported by mechanical ventilation at a fixed ventilation rate of 40 cycles/min and a tidal volume of 40 mL/kg. Blood pressure was monitored through a fluid-filled pressure-transducing system by placing a catheter in the femoral artery. The contralateral femoral vein was also cannulated with a silicone catheter for drug and fluid administration. After intravascular cannulation was set up and haemodynamics had been stabilised for at least 20 minutes, a loading dose of propofol (3 mg/kg) was administered via the femoral vein, followed by continuous infusion of propofol (1 mg/kg/h) through a syringe pump. In some animals, a water-soluble vascular-specific K_{ATP} antagonist, PNU-37883A (3 mg/kg), was infused 2 minutes before the administration of propofol. Changes in blood pressure were recorded until 30 minutes after the bolus dose of intravenous propofol.

Figure 2. Results of in vivo experiments



Representative original systolic blood pressure tracing measured in the rat femoral artery after bolus intravenous administration of propofol (3 mg/kg). ***2A:*** *A profound drop in blood pressure was detected after the administration of propofol in control animals.* ***2B:*** *In rats pretreated with intravenous infusion of PNU-37883A (3 mg/kg, 2 minutes before an intravenous bolus of propofol), there was a slight elevation in blood pressure, followed by a less significant drop in blood pressure. * $P < 0.001$ compared with baseline in the control group. ** $P = 0.01$ compared with post-propofol infusion in the control group. $P = 0.78$ for comparison of the two baseline systolic blood pressures; $P = 0.09$ for comparison of systolic blood pressures before and after propofol treatment in the PNU-37883A group. Data were analysed by repeated-measure analysis of variance; n = 3–4 different animals in each group.*

In vitro detection of intracellular calcium in cultured vascular smooth muscle cells

Cultured human pulmonary artery VSMC were then loaded with Fluo-2/AM (3 μM; Molecular Probes, Life Technologies, Carlsbad, Calif, USA) and treated with propofol (10 mM) for 30 minutes. Intracellular Ca^{2+} measurements were performed as previously described.[9] Briefly, cells were loaded with 2 μM Fluo-3/AM (Invitrogen, Life Technologies, Carlsbad, Calif, USA) in Tyrode solution containing 2 mM $CaCl_2$. Fluo-3/AM-loaded cells were re-suspended in 1 mL Tyrode solution and the Ca^{2+}-dependent fluorescence intensity was measured by flow cytometry in fluorescence channel FL-1 with an excitation wavelength of 488 nm and an emission wavelength of 530 nm.

Statistical analysis

Results are presented as mean ± SEM. Data were compared by an unpaired *t*-test or ANOVA, as appropriate. Statistical significance was accepted at a level of $P < 0.05$.

Results

Ex vivo experiment

Compared with etomidate (serving as a positive control), propofol induced a significantly greater relaxation response in the aortic segment isolated from the control rats, in a concentration-dependent manner (Figure 1A). This response was not affected by the removal of endothelium (Figure 1B). The propofol-induced vasorelaxation response was almost completely abolished by incubation with glibenclamide (Figure 1C). The intracellular calcium concentration was significantly reduced in cultured pulmonary artery VSMC treated with propofol (Figure 1D).

In vivo experiment

In the in vivo experiment, the bolus dose of propofol (3 mg/kg) caused a significant drop in blood pressure (up to 37% of the baseline measurement) in the control group ($P < 0.001$) (Figure 2A). Inhibition of vascular K_{ATP} channels by pretreatment with PNU-37883A significantly reduced this hypotensive response secondary to infusion of propofol (Figures 2A, 2B and 2C).

Discussion

It has been suggested that propofol-induced hypotension arises from inhibition of sympathetic responses, inhibition

of catecholamine secretion, suppression of myocardial contractility, and dilation of vascular smooth muscle.[4,10-12] Some studies have shown that the direct relaxation effect of propofol on vascular tone is endothelium-dependent,[6] while others have suggested that this response is endothelium-independent.[11-14] Using a pressurised micropipette vessel chamber system, Park and colleagues measured the relaxation responses of rat coronary arteries after the administration of different concentrations of propofol.[15] Their results showed that the direct vasodilatory effect of propofol on these small-resistance arteries was endothelium-dependent and was mediated by endothelium-derived nitric oxide and prostanoids. However, in a study by Morena et al, the vasodilatory effect of propofol was similar in endothelium-intact and endothelium-denuded vessel rings isolated from human mesenteric arteries.[13] Morena et al also showed that neither nitric oxide synthase inhibitor or cyclooxygenase inhibitor (indomethacin) affected the propofol-induced relaxation response in human resistance arteries.[13] Similar studies on human and porcine coronary arteries have reported that propofol relaxes isolated coronary arteries in an endothelium-independent manner.[11] Studies of large conduit arteries have also found that the vasodilation response produced by propofol was not endothelium-dependent.[4,14]

Following the concepts of these earlier studies, we performed ex vivo experiments comparing the vasodilatory effects of propofol and a less-vasoactive anaesthestic agent, etomidate, in isolated rat aorta with intact endothelium or mechanically denuded endothelium. In addition, following the findings of Wallerstedt et al,[4] we determined the upstream molecular mechanism of propofol-operated, voltage-gated calcium channels on vasculature. It is well recognised that the activity of K^+ channels present at the plasma membrane governs the membrane potential and affects the voltage-gated calcium channel activity and calcium entry in vascular smooth muscle.[16] Among these K^+ channels, the K_{ATP} channel is one of the most important ion channels in the regulation of vascular tone.[17,18] Opening of K_{ATP} channels hyperpolarises the membrane and leads to the closure of voltage-gated calcium channels.[18] Reduced entry of extracellular calcium into the cytosol thus relaxes the vascular smooth muscle.[18]

Consistent with most previous reports, our results demonstrated that propofol induced relaxation of pre-contracted rat aorta in a dose-dependent manner, and that the response was independent of the presence of endothelium. Furthermore, the relaxant response was significantly attenuated by pre-incubation with a specific K_{ATP} channel blocker, glibenclamide, indicating that propofol-induced vasorelaxation is mainly mediated by activation of K_{ATP} channels. We measured the concentration of intracellular calcium in cultured VSMC after incubation with 10 mM propofol. The results showed that propofol attenuated intracellular calcium levels in VSMC, confirming the involvement of reduced calcium influx and the closure of voltage-gated calcium channels in propofol-induced vascular smooth muscle relaxation. Clinically, plasma levels of propofol range from 11 to 85 μM after intravenous administration (2 mg/kg).[19] However, concentrations of propofol tested in our ex vivo experiments (3–300 μM) fell beyond the therapeutic range for human subjects. The difference in this response is mainly due to the fact that rats can tolerate significantly higher doses of propofol per kilogram of body weight than humans.[20] Thus, the therapeutic plasma concentration of propofol in rats needs to be higher than the plasma concentration for human subjects.

We performed in vivo haemodynamic measurements in anaesthetised rats to determine the regulation of K_{ATP} channels in propofol-induced vasodilation. Intravenous administration of a bolus dose of 3 mg/kg propofol caused a significant drop in systemic blood pressure. PNU-37883A, a water-soluble, vascular-specific K_{ATP} channel antagonist,[21] was used to examine the effect of K_{ATP} channel blockade on propofol-induced hypotension. Intravenous infusion of PNU-37883A before the administration of propofol slightly elevated systemic blood pressure, indicating the pressor effect of K_{ATP} channel blockade on baseline blood pressure. Interestingly, propofol-induced hypotension was significantly attenuated by pretreatment with PNU-37883A. This in vivo finding further supports the results of our ex vivo experiments showing that propofol induces smooth muscle relaxation and that the subsequent vasodilation is mainly mediated through activation of vascular K_{ATP} channels.

Conclusion

Our ex vivo and in vivo experiments showed that propofol relaxes vascular smooth muscle via activation of K_{ATP} channels in vascular smooth muscle cells. The vasorelaxation effect of propofol is independent of the presence of vascular endothelium. The fall in blood pressure after systemic administration of propofol is almost completely abolished by in vivo inhibition of K_{ATP} channels. This observation further supports our ex vivo finding that the vasoactive property of propofol is most likely mediated through the activation of K_{ATP} channels. However, our results do not suggest that inhibition of K_{ATP} channels would have any clinically beneficial effect on propofol-induced hypotension, as K_{ATP} channels are an important biological protective coupler during metabolic distress.[22]

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