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SPECIAL REPORT

Propofol stimulates nitric oxide release from cultured porcine aortic endothelial cells

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Propofol, an intravenous anaesthetic agent, causes marked vasodilatation *in vivo*. In the present study the effects of propofol on the release of nitric oxide (NO) from vascular endothelial cells was determined *in vitro*. Application of propofol to co-cultures of porcine aortic endothelial and smooth muscle cells resulted in a rapid increase in cyclic GMP formation. This increase was significantly inhibited following pretreatment of the cells with either N^G-nitro-L-arginine (L-NOARG) or in the presence of haemoglobin. When applied to smooth muscle cells alone, propofol did not result in an increase in cyclic GMP levels. These results demonstrate that propofol stimulates the production and release of NO from cultured endothelial cells and suggest that the vasodilatation and hypotension observed when propofol is given *in vivo* may be due to NO release.

Keywords: Endothelial cells; nitric oxide; N^G-nitro-L-arginine (L-NOARG); propofol

Introduction The synthesis of nitric oxide (NO) from L-arginine is a widespread pathway modulating both the cardiovascular and the immune system. Vascular endothelial cells generate NO from L-arginine via a Ca^{2+}/calmodulin-dependent constitutive NO synthase enzyme. This results in elevation of guanosine 3':5'-cyclic monophosphate (cyclic GMP) levels in smooth muscle cells and subsequent vascular relaxation (Moncada *et al.*, 1991).

Propofol (2,6 diisopropyl-phenol) is a rapidly acting intravenous anaesthetic agent, which upon administration results in a marked reduction in systemic vascular resistance (Grounds *et al.*, 1985). In the present study we have examined whether propofol stimulates the release of NO from cultured vascular endothelial cells.

Methods Porcine aortic endothelial cells (PAEC) were isolated and cultured in Dulbecco's modified Eagle's medium supplemented with penicillin (100 units ml^{-1}), streptomycin (100 $\mu g\ ml^{-1}$), glutamine (4 mM), foetal calf serum (10% v/v) and newborn calf-serum (10% v/v). Endothelial cells were used at either passage one or two (Bogle *et al.*, 1991). Porcine aortic smooth muscle cells (PASMC) were isolated by a modified explant technique (Crossman *et al.*, 1990). When confluent, endothelial cells were mixed with smooth muscle cells (1:1) and plated into 24 well plates for use 24 h later.

Co-cultures were rinsed twice with HEPES-buffered Krebs solution of the following composition (mM): NaCl 131, KCl 5.5, $CaCl_2$ 2.5, $MgCl_2$ 1, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 5.5, HEPES 20, pH 7.4, 37°C. Following this, cells were exposed to either propofol alone or propofol in the presence of inhibitors of NO formation for 5 min. Cells were pretreated with inhibitors for 10 min. Subsequently, 0.5 ml ice-cold hydrochloric acid (0.1 N) was added and plates placed on ice for 20–30 min to allow extraction of cyclic nucleotides. Cyclic GMP levels were determined by specific radioimmunoassay following acetylation (Crossman *et al.*, 1990). Cell number was determined after trypsinization by counting the number of cells in representative wells using a Coulter counter.

Propofol (2,6 diisopropyl-phenol) was obtained from ICI. Intralipid, is an oil in water emulsion containing fractionated soybean oil, egg phospholipids and glycerol, pH 7.0 obtained from KabiVitrum Ltd., Bucks.

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Concentration-dependence data were log transformed and a pooled estimate of the standard deviation calculated. A Dunnett's t test was performed on this pooled estimate. Other data were analysed by an unpaired t test. $P<0.05$ was considered as significant.

Results In co-cultures of endothelial and smooth muscle cells, application of bradykinin (100 nM, 1 min) resulted in a significant increase in cyclic GMP production (control: 8.0 ± 2.8 fmol/10^6 cells, bradykinin 31.2 ± 11.4 fmol/10^6 cells, $P<0.01$, $n=3$). Similarly, application of sodium nitroprusside (SNP, 0.1 mM, 1 min) resulted in a large increase in cyclic GMP production (control: 8.0 ± 2.8, SNP: 91 ± 31, $P<0.01$, $n=3$).

Figure 1 shows the concentration-dependence of propofol-induced cyclic GMP formation in co-cultures of porcine aortic endothelial and smooth muscle cells. Application of propofol (0.03–1 mM) resulted in a rapid increase in cyclic GMP formation. Propofol vehicle (dimethyl sulphoxide, DMSO 0.1%) or clinical vehicle (intralipid 10%) had no significant

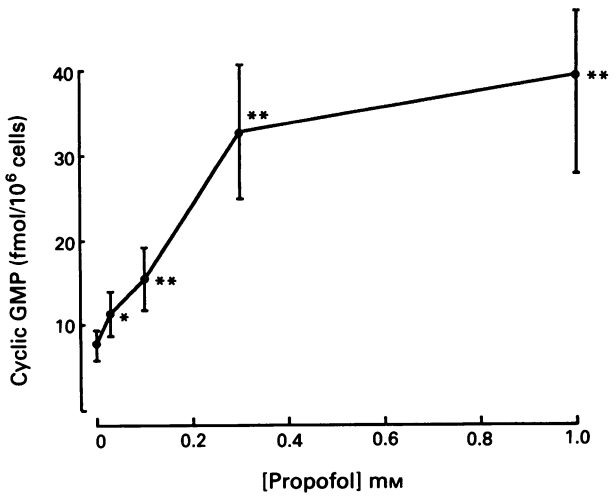

Figure 1 Concentration-dependence of propofol-induced cyclic GMP production. Porcine aortic endothelial and smooth muscle cell co-cultures were incubated with propofol (0.03–1.0 mM; 5 min). Results are mean ± s.d. obtained from 4 experiments on different cell batches. *$P<0.05$; **$P<0.01$ vs control.

effect on co-culture cyclic GMP levels (control cyclic GMP: 8.0 ± 2.8 fmol/10^6 cells, DMSO 6.9 ± 4.4 fmol/10^6 cells, intralipid 6.6 ± 3.9 fmol/10^6 cells, $n = 3$). When propofol (0.3 mM, 5 min) was added to cultures of smooth muscle cells no significant increase in cyclic GMP production was observed (8.5 ± 3.9 fmol/10^6 cells).

Figure 2 shows the effects of propofol on cyclic GMP production following pretreatment of co-cultures with inhibitors of NO production or action. Pretreatment of cells with either N^G-nitro-L-arginine (L-NOARG, 0.1 mM, 10 min) or haemoglobin (10 μM, 10 min) resulted in a significant inhibition of the effects of propofol on cyclic GMP production.

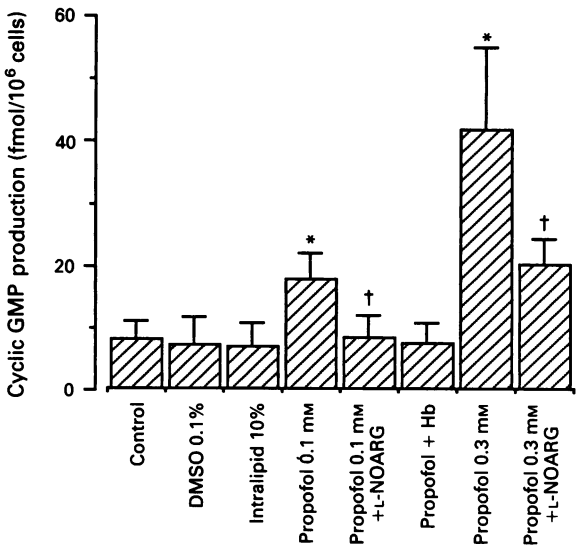

Figure 2 Role of nitric oxide in propofol-induced cyclic GMP production. Co-cultures of porcine aortic endothelial cells and porcine aortic smooth muscle cells were incubated with propofol (0.1 mM or 0.3 mM; 5 min) in the presence of either N^G-nitro-L-arginine (L-NOARG) 0.1 mM or haemoglobin (Hb) 0.01 mM. Results are mean ± s.d. obtained from 3 experiments on different cell batches. *$P < 0.05$ vs control; †$P < 0.05$ vs propofol alone.

Discussion The results of this study indicate that propofol stimulates NO production in a concentration-dependent manner from cultured endothelial cells. This increase in NO synthesis and release results in a subsequent cyclic GMP formation in vascular smooth muscle cells *in vitro*. Intralipid, the vehicle in which propofol is suspended for clinical use, did not increase NO production. This observation suggests that the *in vivo* effects of propofol on systemic vascular resistance are unlikely to be mediated by its vehicle.

To confirm that the increase in cyclic GMP observed in these experiments were due to NO, we demonstrated that an inhibitor of NO synthesis, L-NOARG, blocked the effects of propofol. Furthermore, haemoglobin an agent which binds and inactivates NO also inhibited propofol-induced cyclic GMP formation.

A recent study examined the effects of propofol on rat aortic and pulmonary artery rings and demonstrated a marked relaxation, which was endothelium-independent (Park *et al.*, 1992). This report suggested that in the rat, vascular smooth muscle may be more sensitive to the actions of propofol than the endothelium. In contrast our results would suggest that the predominant action of propofol on NO production is endothelium-dependent since there was no significant increase in NO production when propofol was applied to smooth muscle cells alone. These differences could be accounted for either by the different experimental techniques employed (isolated vascular rings versus cultured cells) or species differences.

Intracellular cyclic GMP levels may increase as a result of either increased production or inhibition of breakdown by phosphodiesterase enzymes (Waldman & Murad, 1987). This latter effect is unlikely to contribute to the changes observed in our experiments since the effects of propofol were abolished following inhibition of NO generation.

Although it is evident from this study that propofol stimulates NO production from endothelial cells it is possible that it may in addition reduce peripheral vascular resistance and blood pressure by depressing sympathetic drive, as do other general anaesthetic agents. Whether NO release is the predominant mechanism responsible for the vasodilatation requires further study.

In conclusion, we suggest that stimulated release of NO is a possible mechanism responsible for the reduction in systemic vascular resistance and hypotension observed when propofol is used *in vivo*. Further animal studies are needed to investigate the usefulness of NO synthase inhibitors in preventing hypotension associated with intravenous injection of propofol.

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