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Propofol prevents endothelial dysfunction induced by glucose overload

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1 Surgical operations often induce acute hyperglycemia, which is known to affect endothelial functions. In this study, we examined the effects of propofol, a commonly used general anaesthetic, on bovine aortic endothelial cell (BAEC) dysfunction induced by glucose overload.

2 D-glucose overload (23 mM) induced an accumulation of superoxide anion (O_2^-), assessed by MCLA chemiluminescence, to a similar extent as that generated by 233 μU ml^{-1} xanthine oxidase (XO) and 100 μM xanthine. Propofol inhibited this accumulation with an IC_{50} of 0.21 μM, whereas much higher concentrations of propofol were required to scavenge O_2^- generated by 250 μU ml^{-1} XO and 100 μM xanthine (IC_{50}: 13.5 μM).

3 D-glucose overload attenuated ATP-induced NO production which was detected using diaminofluorescence-2 (DAF-2). The inhibition was reversed by propofol with an EC_{50} of 0.60 μM. In contrast, inhibitions caused by xanthine/XO were not altered by propofol (1 μM).

4 D-glucose overload suppressed ATP-induced Ca^{2+} oscillations and capacitative Ca^{2+} entry (CCE), which were both restored by superoxide dismutase, indicating that O_2^- was responsible. Propofol restored these attenuated Ca^{2+} oscillations and CCE with EC_{50} of 0.31 and 1.0 μM, respectively.

5 D-glucose overload (23 mM) increased the intracellular glucose concentration 4 fold, compared with cells exposed to 5.75 mM glucose, and 1 μM propofol reduced this increase to 2.8 fold.

6 We conclude from these results that anaesthetic concentrations of propofol prevent the impairment of Ca^{2+}-dependent NO production in BAEC induced by glucose overload. This effect is mainly due to the reduction of O_2^- accumulation, and involves, at least in part, the inhibition of cellular glucose uptake.

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Keywords: Calcium; endothelium; glucose overload; nitric oxide; propofol; superoxide anion

Abbreviations: BAEC, bovine aortic endothelial cells; CCE, capacitative Ca^{2+} entry; DAF-2, diaminofluorescence-2; MCLA, 2-methyl-6(p-methoxyphenyl)-3,7-dihydroimidazo [1,2-a] pyrazin-3-one; XO, xanthine oxidase

Introduction

Acute hyperglycemia is a commonly observed intra-operative metabolic alteration, not only in diabetics but also in non-diabetics, which is due to physiological stress reaction and high glucose transfusion. For instance, it was reported that 8% of diabetic patients showed peri-operative plasma glucose concentrations of more than 400 mg dl^{-1} (22.2 mM) (Walts *et al*., 1981), and non-diabetic patients receiving cardioplegic solution showed concentrations of 31.8±4.8 mM (Werb *et al*., 1989). Cardiovascular surgical complications such as coronary diseases, brain ischemia, and thrombotic diseases are the most common cause of peri-operative mortality (Sieber, 1997). Considering that a hyperglycemic environment affects endothelial functions (Kimura *et al*., 2001b; Tesfamariam, 1994), it could be speculated that these peri-operative cardiovascular complications might be partially due to hyperglycemia-induced endothelial dysfunction.

Superoxide anion (O_2^-) is one of the suggested candidates for the pathogenesis of acute endothelial damage in a hyperglycemic environment (Tesfamariam, 1994; Tesfamariam & Cohen, 1992). For instance, endothelium-derived vasodilatation is impaired in rat aortic rings treated with elevated glucose (44 mM) due to oxidative stress (Tesfamariam & Cohen, 1992). Furthermore, acute D-glucose overload attenuates NO production due to the impairment of Ca^{2+} mobilization by O_2^- in bovine aortic endothelial cells (BAEC) (Kimura *et al*., 2001b). Endothelium-derived NO plays various physiological roles, such as the regulation of vascular tone and anti-coagulative action (for a review, see Moncada *et al*., 1991). Therefore, peri-operative hyperglycemia-induced O_2^- accumulation and the concomitant impaired NO production in endothelium might be involved in the pathogenesis of operative cardiovascular complications.

Propofol (2,6-diisopropylphenol) has been reported to have anti-oxidative properties, probably due to a phenolic hydroxyl group in its chemical structure (Aarts *et al*., 1995; Bao *et al*., 1998; Demiryurek *et al*., 1998). Therefore, anaesthesia using propofol may be beneficial in preventing peri-operative hyperglycemia-induced endothelial dysfunctions. Furthermore, propofol has also been reported to inhibit the production of reactive oxygen species in

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neutrophils (Mikawa *et al*., 1998). Thus it would be intriguing to investigate whether propofol inhibits the generation of and/or scavenges O_2^- in endothelium in a hyperglycemic environment.

In this study, we examined the effects of propofol on D-glucose overload-induced O_2^- accumulation and subsequent endothelial dysfunction. The results obtained suggest that propofol may prevent glucose overload-induced O_2^--mediated endothelial damage by mechanisms independent of its anti-oxidative actions.

Methods

Culture of bovine aortic endothelial cells (BAEC)

Thoracic aorta from one-year-old calves were obtained from a local slaughterhouse. Endothelial cells were cultured in Dulbecco's Modified Eagle Medium with 10% foetal bovine serum as previously described (Oike *et al*., 2000). Passage 2 cells were used in all subsequent experiments and each cell batch stained for Dil-Acl-LDL (Kimura *et al*., 2001a) with no evidence of other contaminating cells being present. Cells used were isolated from seven aortae, and there was virtually no difference in results between cell batches.

Measurement of superoxide anion by MCLA

We quantified O_2^- generated from BAEC by using an O_2^--sensitive luciferin derivative, 2-methyl-6-(p-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (Shimmura *et al*., 1992) (MCLA; Tokyo Kasei Kogyo, Tokyo, Japan) as previously described (Kimura *et al*., 2001b). Since it is difficult to convert MCLA chemiluminescence into absolute values of O_2^- (Shimmura *et al*., 1992), we expressed the amount of O_2^- as the equivalent concentration of xanthine oxidase (XO) that reacts with xanthine to generate O_2^- (Kimura *et al*., 2001b). The MCLA chemiluminescence induced by xanthine/XO depends linearly on the XO concentration.

Measurement of the intracellular production of NO

NO was measured by using diaminofluorescein-2 (DAF-2), an NO-sensitive fluorescent dye (Kojima *et al*., 1998). Cells were loaded with the diacetylated form of DAF-2 (10 μM; Daiichi Pure Chemicals, Co. Ltd., Tokyo, Japan) for 30 min at 37°C. The chamber with loaded cells was mounted on an inverted-microscope (Axiovert 135; Carl Zeiss, Jena, Germany). Cells were excited at 490 nm every 60 s, and the emitted fluorescence at 515 nm wavelength was measured by using an Attofluor digital fluorescence microscopy system (Atto Instruments, Rockville, MD, U.S.A.). Since DAF-2 fluorescence increases almost linearly with NO concentration (Kojima *et al*., 1998), we expressed intracellular NO production as the net increase in the intensity of DAF-2 fluorescence in 15 min relative to its basal value.

Measurement of intracellular Ca^{2+} concentration

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured in the same set-up as for NO measurements, but with a different filter set, i.e., excitation at 340 and 380 nm and emission at 510 nm. Cells grown on coverslip were loaded with fura-2 (fura-2/AM; Dojindo, Kumamoto, Japan) as previously described (Oike *et al*., 2000). The fura-2 fluorescence images were recorded at a rate of approximately 1 Hz, and converted to apparent Ca^{2+} concentration as previously described (Oike *et al*., 2000). All experiments were performed at room temperature (20–25°C).

Measurement of intracellular glucose concentration

For the measurement of the intracellular glucose concentration, BAEC grown to confluence on 35 mm culture dishes were incubated with a 5.75 or 23 mM M-glucose solution for 4 h. After washing three times with glucose-free phosphate-buffered saline, cells were lysed with 30 μl of 0.1% Triton-X in the presence of 1% NaF, an inhibitor of glycolysis. We confirmed in preliminary experiments that this lysis buffer does not contain glucose. Glucose concentrations in the lysates were measured by the glucose oxidase method (Okuda & Miwa, 1971), using a commercial kit (Glu Neo, Shino Test CO., Tokyo, Japan). Values obtained were converted to intracellular glucose concentrations using a total cell volume of 0.29 μl per dish. This was calculated using 1 pl as the intracellular volume of an endothelium (Baydoun *et al*., 1990) and taking the total cell number on a confluent 35 mm culture dish as 291 000.

Exposure of the cells to high glucose environment

Cells were pretreated for 4 h with each solution (see below). We did not use culture medium with elevated glucose concentration, since it contains an excess amount of antioxidative amino acids and vitamins, and this would artificially reduce the impairing effects of high glucose-induced O_2^- accumulation.

Solutions and drugs

The standard solution used in the present study was a modified Krebs solution (11.5 mM glucose solution) containing (in mM): NaCl 132, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 1.5, glucose 11.5, HEPES 11.5; pH adjusted to 7.3 with NaOH. We also used Krebs solutions containing 5.75 or 23 mM D-glucose, and 11.5 mM D-glucose plus 11.5 mM L-glucose. The osmolarity of the solutions containing different amounts of glucose was kept constant by changing the NaCl concentration, i.e., 135 mM and 126 mM NaCl in the presence of 5.75 mM and 23 mM glucose, respectively.

Propofol (Aldrich, Milwaukee, WI, U.S.A.) was dissolved in dimethylsulfoxide (DMSO). The final concentration of DMSO was adjusted to 0.01% for each solution to avoid its possible non-specific action. All other drugs were purchased from Sigma (St. Louis, MO, U.S.A.).

Statistical analysis

Pooled data are given as mean ± standard error of the mean, and statistical significance between two groups was determined by using Student's unpaired *t*-test. Probabilities less than 5% ($P<0.05$) were regarded as significant.

Results

Propofol inhibits glucose overload-induced superoxide anion (O_2^-) generation

Firstly we examined the effects of propofol on the accumulation of O_2^- induced by glucose overload in BAEC. Cells were pretreated with solutions containing various concentrations of glucose for 4 h. MCLA chemiluminescence was measured for 10 min at the end of this period. Photon counts obtained after treatment with 5.75, 11.5 and 23 mM D-glucose show a concentration-dependent increase in O_2^- generation which was equivalent to amounts induced by the catalytic reaction between 100 μM xanthine and 37 ± 12, 45 ± 10 and 233 ± 23 μU ml^{-1} XO respectively ($n=7$). The generation of O_2^- was only evident in the presence of BAEC and was not enhanced by the non-metabolizable L-glucose. Moreover, elevated MCLA chemiluminescence was completely abolished by 150 U ml^{-1} superoxide dismutase (SOD, Table 1). Thus, although MCLA chemiluminescence is highly specific for O_2^- and singlet molecular oxygen (Shimmura *et al*., 1992), the fact that the latter is not scavenged by SOD (Goda *et al*., 1974) suggests that the signal detected in our study is due to O_2^- generated following the metabolism of D-glucose by BAEC.

The addition of propofol during incubations with 23 mM D-glucose solution reduced O_2^- production in a concentration dependent manner ($IC_{50}=0.21$ μM; Figure 1A). However, propofol (10 μM) did not significantly affect, whereas SOD (150 U ml^{-1}) completely abolished O_2^- generated in 5.75 and 11.5 mM D-glucose solutions (Table 1). In contrast to its marked effect on the actions of D-glucose, propofol was comparatively less effective in inhibiting O_2^- generation by xanthine (100 μM) and XO (250 μU ml^{-1}) which, at the concentrations used, produce equivalent amounts of O_2^- to 23 mM D-glucose. As shown in Figure 1B, propofol did scavenge xanthine/XO-induced O_2^- but its IC_{50} value (13.5 μM) was much higher than that for inhibiting D-glucose overload-induced O_2^- accumulation.

Activation of protein kinase C is one of the putative candidates causing O_2^- generation in hyperglycemic environment (Mayhan & Patel, 1995). However, inhibitors of protein kinase C, calphostin C (300 nM) and staurosporin (2 μM), did not inhibit 23 mM D-glucose-induced O_2^- generation nor did they affect the actions of propofol on O_2^- (Table 1).

Propofol restores glucose overload-induced attenuation of NO production

ATP is released from endothelium as an endogenous mediator in response to mechanical stress (Oike *et al*.,

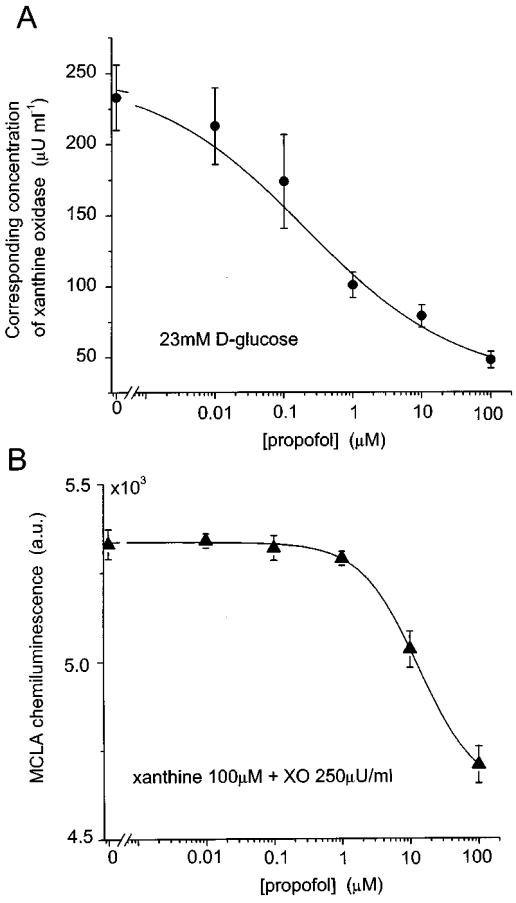

Figure 1 Effects of propofol on the generation of superoxide anion (O_2^-) induced by D-glucose overload, as assessed by MCLA chemiluminescence. (A) Concentration-response relationships between propofol and O_2^- generation in 23 mM D-glucose. Chemiluminescence is expressed as the equivalent concentrations of xanthine oxidase (XO). Continuous line was drawn with a logistic equation. Results are means$\pm$s.e.mean of seven experiments. (B) Effect of propofol on xanthine (100 μM) plus XO (250 μU ml^{-1}) induced O_2^- generation in BAEC. Continuous line was drawn using a logistic equation. Results are means$\pm$s.e.mean of five experiments.

Table 1 D-glucose-induced O_2^- generation

	Corresponding concentration of xanthine oxidase (μU ml^{-1})		
	Control	*+10 μM propofol*	*+150 U ml^{-1} SOD*
5.75 mM D-glucose	37 ± 12 (7)	40 ± 11 (7)	ND (6)*
11.5 mM D-glucose	45 ± 10 (7)	54 ± 20 (7)	ND (6)*
23 mM D-glucose	233 ± 23 (7)	79 ± 8 (7)*	ND (6)*
11.5 mM D-glucose +11.5 mM L-glucose	50 ± 10 (5)	NE	NE
23 mM D-glucose +300 nM calphostin C	233 ± 12 (12)	82 ± 9 (12)*	NE
23 mM D-glucose +2 μM staurosporine	242 ± 10 (12)	84 ± 9 (12)*	NE

Results are means$\pm$s.e.mean of n experiments where n is given in brackets against each value above. NE, not examined; ND, not detectable. Statistical analysis: *$P<0.01$ compared with control.

2000) and leads to NO production (Kimura *et al*., 2000). We therefore examined the effects of propofol on ATP-induced, Ca^{2+}-dependent NO production in BAEC with DAF-2. As shown in Figure 2A (open circles), ATP induced a gradual increase in DAF-2 fluorescence in control cells treated with 5.75 mM D-glucose for 4 h. This increase was abolished by L-NAME (Figure 2A, closed circles), suggesting that the increase in DAF-2 fluorescence reflects intracellular NO production. ATP-induced increase in DAF-2 fluorescence was virtually abolished in cells pretreated with 23 mM D-glucose (Figure 2A, open squares). This was not observed with L-glucose (Table 2). SOD prevented the effect of 23 mM D-glucose (Table 2), suggesting that the attenuation of NO production was due to O_2^- accumulation.

Propofol, applied during the incubation period with 23 mM D-glucose solution, restored ATP-induced NO production in a concentration dependent manner ($EC_{50}=0.60$ μM, Figure 2B). In contrast, propofol (10 μM) did not affect ATP-induced NO production in control cells that were treated with 5.75 mM or 11.5 mM D-glucose (Table 2). These results suggest that propofol restores ATP-induced NO production in a hyperglycemic environment.

Propofol does not reverse exogenous O_2^--induced attenuation of NO production

As shown in Figure 3A, ATP-induced NO production was impaired in xanthine/XO-treated BAEC. This was reversed by SOD, thereby indicating that NO production was attenuated by xanthine/XO-induced O_2^- generation (Figure 3A,B). In contrast, propofol (1 μM) did not restore the xanthine/XO-induced impairment of NO production (Figure 3A,B), and this was compatible with the fact that 1 μM propofol did not scavenge exogenous O_2^- (Figure 1B).

Propofol restores glucose overload-induced attenuation of Ca^{2+} oscillations

Fine control of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is essentially important for NO production in endothelium (Moncada *et al*., 1991). To address the cellular mechanism by which propofol restored NO production in high D-glucose-treated cells, we examined the effects of propofol on Ca^{2+} mobilizing properties in BAEC.

ATP (1 μM) induced Ca^{2+} oscillations in control cells that were pretreated with 5.75 mM D-glucose for 4 h (Figure 4A). In contrast, when cells were pretreated with 23 mM D-glucose solution for 4 h, 1 μM ATP did not induce Ca^{2+} oscillations but induced a small single Ca^{2+} transient (Figure 4B). L-glucose overload did not affect Ca^{2+} oscillations (Table 3). Cells treated with 23 mM D-glucose solution in the presence of 150 U ml^{-1} SOD showed Ca^{2+} oscillations in response to 1 μM ATP (Table 3), thereby indicating that the impairment of Ca^{2+} oscillations by D-glucose overload was due to the accumulation of O_2^-.

The inhibition by 23 mM D-glucose of ATP-induced Ca^{2+} oscillations was also prevented by 1 μM propofol (Figure 4C). Under these conditions, propofol restored the frequency of Ca^{2+} oscillations in a concentration dependent manner with an EC_{50} of 0.31 μM (Figure 4D). This was apparently not due to a direct activation of ATP-induced Ca^{2+} oscillations by propofol, since 10 μM propofol did not increase the frequency of Ca^{2+} oscillations in control cells (Table 3).

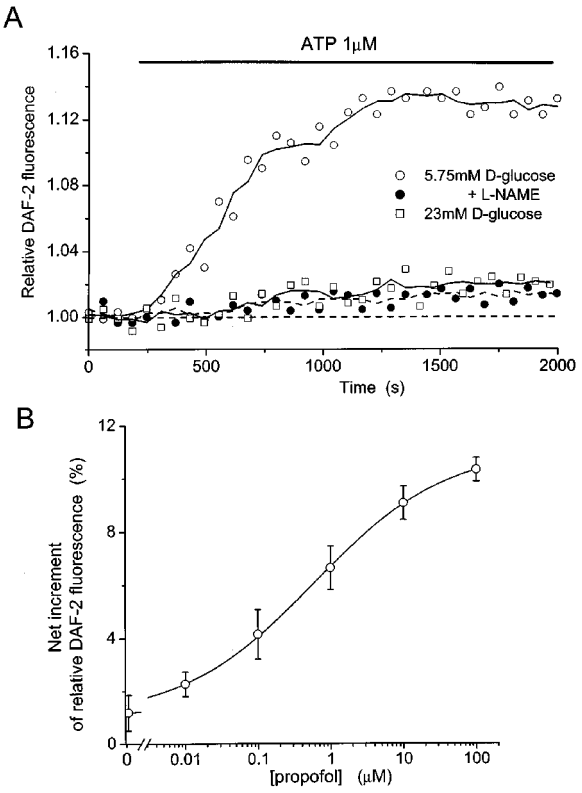

Figure 2 ATP-induced NO production in BAEC. (A) Effects of D-glucose and L-NMMA on ATP-induced changes in DAF-2 fluorescence. The results are the representative data from five experiments with 4–6 cells used in each experiment. Symbols indicate the measured values, and the lines were drawn by averaging six adjacent points. (B) Concentration-response relationships of propofol-induced restoration of NO production in 23 mM D-glucose-treated cells. Results are means ± s.e.mean of five experiments with 4–6 cells used in each experiment.

Propofol ameliorates glucose overload-induced attenuation of capacitative Ca^{2+} entry

Ca^{2+} entry from the extracellular space, rather than Ca^{2+} release from the intracellular Ca^{2+} stores, plays an essential role in endothelial constitutive NO production (Kimura *et al*., 2001b; Lantoine *et al*., 1998). We therefore examined the effects of propofol on the changes in capacitative Ca^{2+} entry (CCE, Berridge, 1995) induced by glucose overload.

Thapsigargin, an inhibitor of sarcoplasmic Ca^{2+} ATPase (Thastrup *et al*., 1990), induced an initial Ca^{2+} transient in Ca^{2+}-free solution in control cells pretreated with 5.75 mM D-glucose for 4 h. The subsequent application of extracellular Ca^{2+} evoked further $[Ca^{2+}]_i$ increase due to CCE (Figure 5A) (Kimura *et al*., 1998). The amplitude of this CCE-induced increase of $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_{i\text{-CCE}}$) was markedly reduced in cells treated with 23 mM D-glucose (Figure 5B), but this inhibition did not occur in cells treated with 11.5 mM D-glucose and 11.5 mM L-glucose (Table 4). SOD restored the amplitude of $\Delta[Ca^{2+}]_{i\text{-CCE}}$ (Table 4), suggesting that accumulation of O_2^- due to D-glucose overload was responsible for the reduction of $\Delta[Ca^{2+}]_{i\text{-CCE}}$.

Table 2 D-glucose-induced O_2 generation

	Net increment in DAF-2 fluorescence (%)			
	Control	*+10 μM propofol*	*+100 μM L-NAME*	*+150 U ml^{-1} SOD*
5.75 mM D-glucose	10.9±1.4 (4)	11.2±0.8 (4)	1.0±0.5 (5)*	NE
11.5 mM D-glucose	10.2±0.7 (5)	11.0±0.6 (5)	NE	NE
23 mM D-glucose	1.2±0.7 (5)	9.1±0.6 (6)*	NE	11.1±0.6 (4)*
11.5 mM D-glucose +11.5 mM L-glucose	10.8±0.5 (4)	NE	NE	NE

Results are means±s.e.mean of n experiments with 4–6 cells used in each experiment where n is given in brackets against each value above. NE, not examined. Statistical analysis: *$P<0.01$ compared with control.

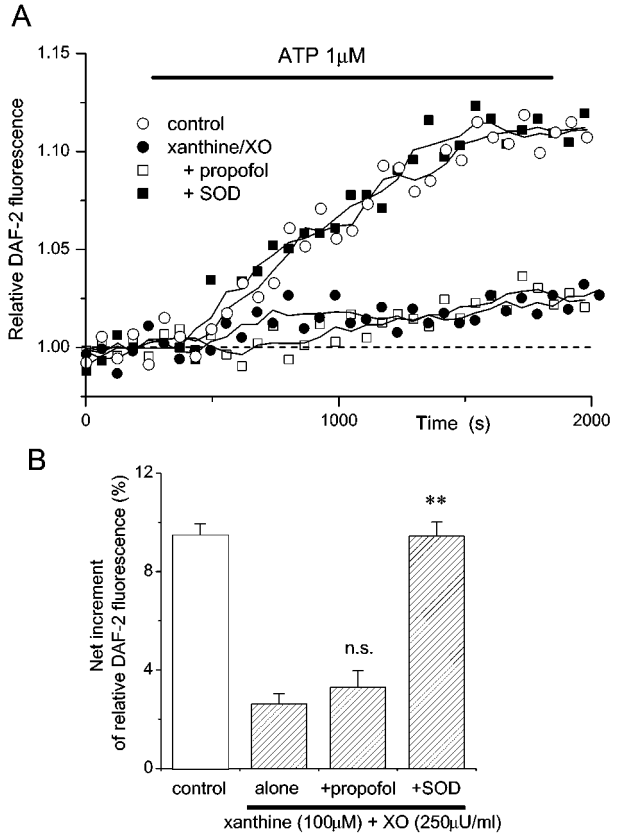

Figure 3 Effects of propofol on xanthine/XO-induced impairment of NO production. (A) Effects of propofol (1 μM) and SOD (150 U ml^{-1}) on xanthine (100 μM) plus xanthine oxidase (250 μU ml^{-1})-induced changes in ATP-stimulated DAF-2 fluorescence. The results are the representative data from 4–6 experiments with 4–6 cells used in each experiment. Symbols indicate the measured values, and the lines were drawn by averaging six adjacent points. (B) Statistical analysis of effects of propofol (1 μM) and SOD (150 U ml^{-1}) on xanthine (100 μM) plus xanthine oxidase (250 μU ml^{-1})-induced changes in ATP-stimulated DAF-2 Fluorescence. Results are means±s.e.mean of 4–6 experiments with 4–6 cells used in each experiment. Statistical analysis: n.s. $P>0.05$; **$P<0.01$ *vs* xanthine plus XO alone.

Propofol reversed the inhibition of CCE induced by D-glucose overload in a concentration-dependent manner ($EC_{50}=1.0$ μM; Figure 5C,D). Since 10 μM propofol did not affect $[Ca^{2+}]_{i\text{-}CCE}$ in control cells, we can exclude that the observed effect may be due to a direct activation of CCE by propofol (Table 4).

Propofol inhibits cellular glucose uptake in high glucose environment

To further address the cellular mechanisms responsible for its actions on O_2^-, we assessed the effects of propofol on intracellular glucose concentration in BAEC which was elevated from 4.6±0.6 mM ($n=9$) in cells treated with 5.75 mM D-glucose to 18.2±1.1 mM ($n=9$) in cells treated with 23 mM D-glucose. This increase was significantly inhibited by propofol, which at 1 μM reduced elevated intracellular glucose concentrations to 12.6±1.0 mM ($n=9$, $P<0.05$ *vs* 23 mM D-glucose without propofol). In contrast, propofol (1 μM) did not significantly alter intracellular glucose concentration in cells treated with 5.75 mM D-glucose (3.6±0.7 mM, $n=9$, $P>0.05$ *vs* 5.75 mM D-glucose without propofol).

Discussion

We described in this study that propofol inhibits D-glucose (23 mM) or xanthine (100 μM) plus XO (250 μU ml^{-1}) induced O_2^- generation in BAEC with IC_{50} values of 0.2 μM and 13.5 μM respectively. This finding confirms the antioxidative action of propofol reported in other systems, including chemically-stimulated leukocytes (>10 μM, Demiryurek *et al*., 1998), liver microsomes ($IC_{50}=9.5$ μM, Bao *et al*., 1998), and erythrocyte membranes ($IC_{50}=10$–300 μM, Tsuchiya *et al*., 2001). The marked difference in IC_{50} values determined in our studies using either high glucose or xanthine and XO suggests that inhibition of O_2^- by propofol under hyperglycemic conditions may occur by mechanisms independent of its antioxidant actions.

The cellular mechanisms associated with the generation of O_2^- by excess intracellular D-glucose may include (1) autooxidation of glucose and non-enzymatic protein glycation (Wolff *et al*., 1991), (2) the alteration of NADH/NAD^+ ratio by the activation of polyol pathway, the collateral metabolic pathway of glucose (Williamson *et al*., 1993), and (3) the activation of protein kinase C by excess glucose (Mayhan & Patel, 1995). However, protein kinase C is probably not involved in the effects of propofol on high D-glucose-induced O_2^- accumulation, because the inhibitors of protein kinase C did not affect the actions of propofol on O_2^- generation (Table 1). Whether propofol modulates D-glucose-induced O_2^- generation through effects on other systems such as glucose autoxidation, protein glycation or polyol pathway remains to be established. Another possibility would be that propofol may inhibit the cellular uptake of excess glucose in

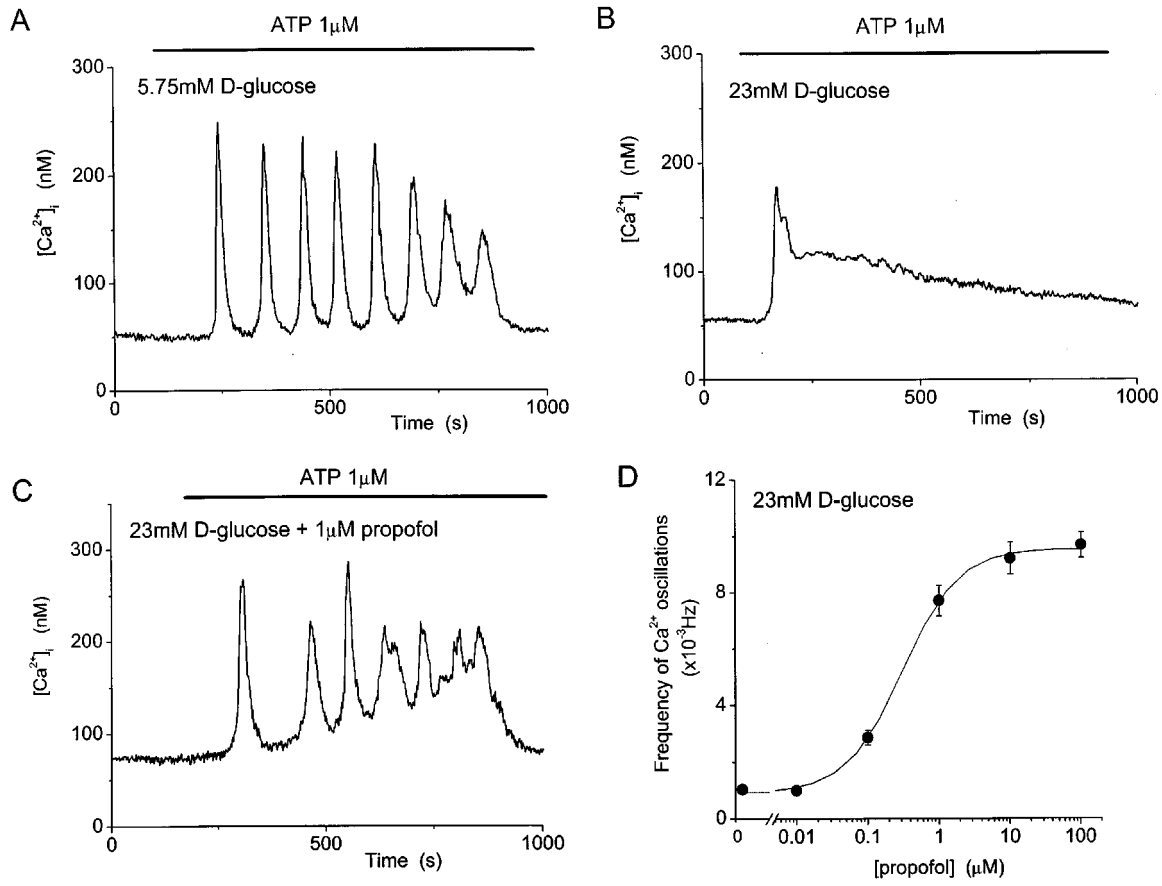

Figure 4 Effects of propofol on Ca^{2+} oscillations induced by ATP. (A) Representative tracing of Ca^{2+} oscillations induced by 1 μM ATP. (B) Inhibition of ATP-induced Ca^{2+} oscillations by 23 mM D-glucose. (C) Restoration of ATP-induced Ca^{2+} oscillations by propofol (1 μM) in the presence of 23 mM D-glucose. (D) Relationship between propofol concentration and the frequency of Ca^{2+} oscillations in 23 mM D-glucose-treated cells. Results are means$\pm$s.e.mean of 8–9 experiments with 4–6 cells used in each experiment.

Table 3 Frequency of ATP-induced Ca^{2+} oscillations

	Frequency of Ca^{2+} oscillations ($\times 10^{-3}$ Hz)		
	Control	*+10 μM propofol*	*+150 U ml^{-1} SOD*
5.75 mM D-glucose	9.3$\pm$0.5 (10)	9.7$\pm$0.4 (8)	NE
11.5 mM D-glucose	9.5$\pm$0.5 (9)	9.7$\pm$0.6 (8)	NE
23 mM D-glucose	1.0$\pm$0.2 (9)	9.2$\pm$0.6 (8)*	9.8$\pm$0.6 (8)*
11.5 mM D-glucose +11.5 mM L-glucose	9.5$\pm$0.4 (6)	NE	NE

Results are means$\pm$s.e.mean of *n* experiments with 4–6 cells used in each experiment where *n* is given in brackets against each value above. NE, not examined. Statistical analysis: *$P<0.01$ compared with control.

endothelium. Indeed, we observed that 1 μM propofol significantly reduced intracellular glucose concentration in 23 mM D-glucose but not in 5.75 mM D-glucose-treated cells. Such a suppression of glucose transport by propofol has been reported in 3T3-4C2 murine fibroblasts and human erythrocytes, although with much higher IC_{50} values (200–400 μM, Stephenson *et al*., 2000). We therefore propose that the endothelial glucose transporter may be highly sensitive to propofol, especially in a hyperglycemic environment, and this is probably one of the main mechanisms by which propofol is able to inhibit O_2^- generation in BAEC. This could explain the marked divergence in the IC_{50} values of propofol in inhibiting O_2^- generated by high glucose compared to that induced by xanthine and XO. However, 1 μM propofol reversed intracellular glucose concentration only partially whereas it virtually abolished the impairing actions of high glucose on O_2^- generation, NO production and Ca^{2+} mobilization. This may therefore indicate that other mechanisms in addition to the inhibition of glucose transport are involved in the actions of propofol.

The average blood concentrations of propofol in patients have been reported as being 4.05 μg ml^{-1} (23 μM) during

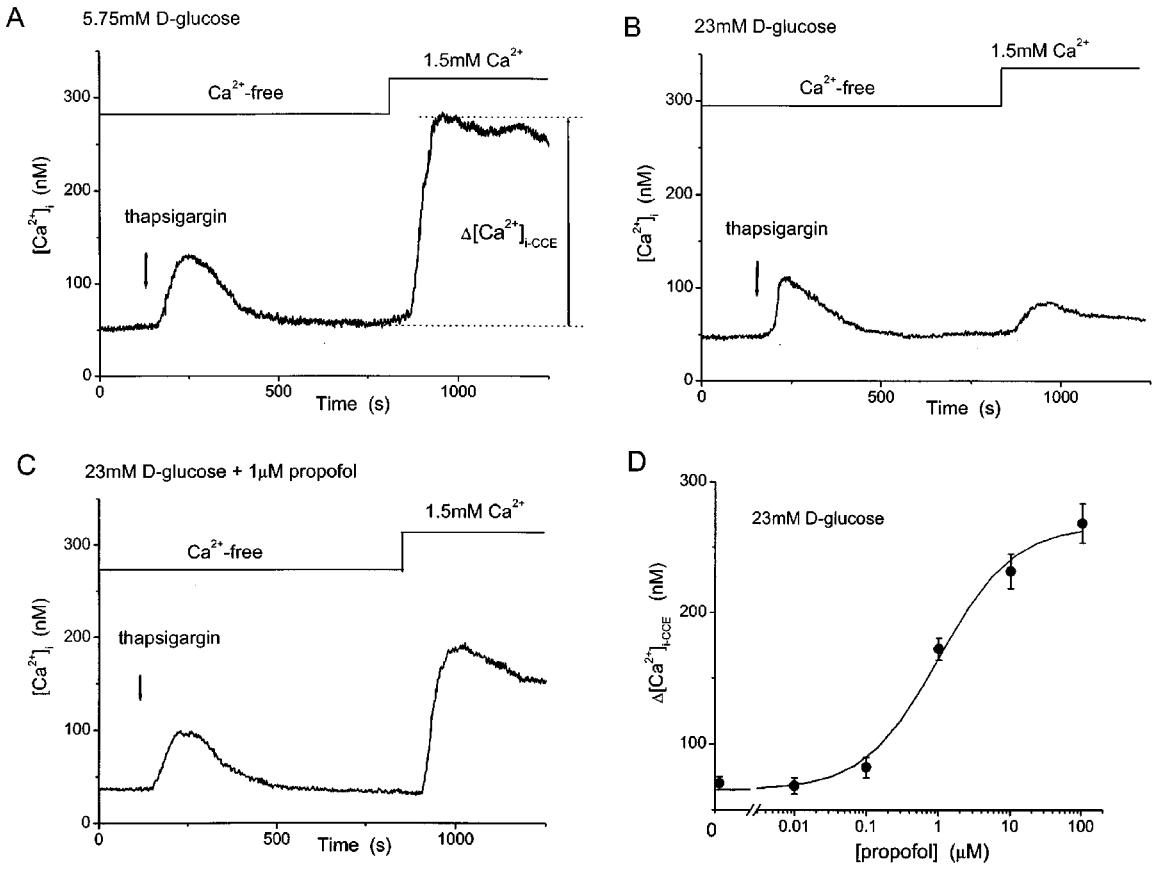

Figure 5 Effects of propofol and D-glucose on thapsigargin-induced Ca^{2+} mobilization. (A) Representative tracing of thapsigargin (1 μM)-induced Ca^{2+} mobilization in control cells. (B) Effect of 23 mM D-glucose on thapsigargin-induced Ca^{2+} mobilization. (C) Restoration of thapsigargin-induced Ca^{2+} mobilization by propofol in the presence of 23 mM D-glucose. (D) Concentration-response relationship between propofol and thapsigargin-induced $[Ca^{2+}]_{i\text{-}CCE}$ in 23 mM D-glucose-treated cells. Continuous line was drawn with an EC_{50} of 1.0 μM. Results are means$\pm$s.e.mean of 6–8 experiments with 4–6 cells used in each experiment.

Table 4 Net increase in $[Ca^{2+}]_i$ due to capacitative Ca^{2+} entry ($\Delta[Ca^{2+}]_{i\text{-}CCE}$)

	$(\Delta[Ca^{2+}]_{i\text{-}CCE})$ (nM)		
	Control	*+propofol 10 μM*	*+SOD*
5.75 mM D-glucose	277.2±13.5 (5)	309.6±16.2 (4)	NE
11.5 mM D-glucose	255.6±12.5 (6)	279.7±12.5 (6)	NE
23 mM D-glucose	70.2±4.9 (7)	231.7±13.4 (5)*	300.5±18.5 (5)*
11.5 mM D-glucose +11.5 mM L-glucose	261.2±20.5 (5)	NE	NE

Values were measured as described in Figure 5A. Results are means±s.e.mean of *n* experiments with 4–6 cells used in each experiment where *n* is given in brackets against each value above. NE, not examined. Statistical analysis: $*P<0.01$ compared with control.

generalized anaesthesia, 1.07 μg ml^{-1} (6.1 μM) during recovery and 0.95 μg ml^{-1} (5.4 μM) during orientation after surgery (Shafer *et al.*, 1988). Therefore, the present results indicate that propofol, at clinically used concentrations, completely inhibits endothelial O_2^- generation in a hyperglycemic environment throughout the peri- and post-operative periods.

We observed that ATP-induced NO production was impaired by O_2^- generated by the D-glucose overload (Figure 2). Propofol restored NO production in high D-glucose-treated cells in a concentration dependent manner with an EC_{50} of 0.6 μM, which is close to the IC_{50} value for inhibiting O_2^- generation. This is not due to a direct action of propofol on NO productivity, since propofol did not stimulate the ATP-induced NO production in control cells (Table 2). Furthermore, 1 μM propofol, which did not show apparent antioxidative action (Figure 1B), had no effects on exogenous O_2^--induced impairment of NO production (Figure 3B). Therefore, we propose that the effects of propofol in restoring endothelial NO productivity was mainly due to its action as an inhibitor of high D-glucose-induced O_2^- generation. This also indicates that acute D-glucose overload-induced endothelial dysfunction can be restored completely by inhibiting O_2^- generation.

It has been suggested that agonist-induced NO production in endothelium depends on Ca^{2+} entry (Kimura *et al*., 2001b; Lantoine *et al*., 1998) and that inhibition of ATP-induced Ca^{2+} oscillations by glucose overload may be due to impairments in CCE and Ca^{2+} extrusion in BAEC (Kimura *et al*., 1998). In our studies we found that ATP-induced Ca^{2+} oscillations and thapsigargin-induced CCE were abolished by O_2^- generated in high D-glucose-treated BAEC (Figure 4), confirming similar impairing actions of reactive oxygen species on Ca^{2+} entry pathways reported previously (Pieper & Dondlinger, 1997; Wesson & Elliott, 1994). We have also demonstrated that propofol (1 μM) is able to significantly restore both ATP-induced Ca^{2+} oscillations (Figure 4C) and thapsigargin-induced CCE (Figure 5C) without altering Ca^{2+} mobilizing properties in control cells (Tables 3 and 4). Taken together, we propose that propofol restores NO production by improving Ca^{2+} mobilization due to its inhibitory action on O_2^- generation in D-glucose overloaded BAEC.

Sufficient constitutive NO production in endothelium is critically important not only for fine tuning of vascular tone but also for the prevention of the development of thrombosis and coagulation. The impairment of endothelial NO production has been suggested to cause cardiovascular diseases (Dusting, 1996), and therefore the proper protection of endothelial function from peri-operative hyperglycemia would prevent operative cardiovascular complications. In conclusion, the present study provides the first evidence that anaesthetic concentrations of propofol inhibit O_2^- generation by D-glucose overload, and that this may occur via mechanisms that include, at least in part, the inhibition of cellular glucose uptake. This property of propofol would be highly beneficial in preventing endothelial dysfunction.

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