Caveolae and propofol effects on airway smooth muscle

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Editor’s key points
• The effect of propofol on intracellular calcium was investigated in human airway smooth muscle cells in vitro.
• Propofol decreased peak intracellular calcium responses to histamine.
• When caveolin-1 was blocked with small interference RNA, the effect of propofol was abolished.
• Propofol appears to affect airway relaxation via caveolin-1 effects on calcium.

Background. The i.v. anaesthetic propofol produces bronchodilatation. Airway relaxation involves reduced intracellular Ca2+ ([Ca2+]i) in airway smooth muscle (ASM) and lipid rafts (caveolae), and constitutively caveolin proteins regulate [Ca2+]i. We postulated that propofol-induced bronchodilatation involves caveolar disruption.

Methods. Caveolar fractions of human ASM cells were tested for propofol content. [Ca2+]i responses of ASM cells loaded with fura-2 were performed in the presence of 10 μM histamine with and without clinically relevant concentrations of propofol (10 and 30 μM and intralipid control). Effects on sarcoplasmic reticulum (SR) Ca2+ release were evaluated in zero extracellular Ca2+ using the blockers Xestospongin C and ryanodine. Store-operated Ca2+ entry (SOCE) after SR depletion was evaluated using established techniques. The role of caveolin-1 in the effect of propofol was tested using small interference RNA (siRNA) suppression. Changes in intracellular signalling cascades relevant to [Ca2+]i, and force regulation were also evaluated.

Results. Propofol was present in ASM caveolar fractions in substantial concentrations. Exposure to 10 or 30 μM propofol form decreased [Ca2+]i, peak (but not plateau) responses to histamine by ~40%, an effect persistent in zero extracellular Ca2+. Propofol effects were absent in caveolin-1 siRNA-transfected cells. Inhibition of ryanodine receptors prevented propofol effects on [Ca2+]i, while propofol blunted [Ca2+]i responses to caffeine. Propofol reduced SOCE, an effect also prevented by caveolin-1 siRNA. Propofol effects were associated with decreased caveolin-1 expression and extracellular signal-regulated kinase phosphorylation.

Conclusions. These novel data suggest a role for caveolae (specifically caveolin-1) in propofol-induced bronchodilatation. Due to its lipid nature, propofol may transiently disrupt caveolar regulation, thus altering ASM [Ca2+]i.

Keywords: bronchial smooth muscle; bronchodilatation; calcium regulation; caveolin; intravenous anaesthetic; signalling
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Since its invention in 1977, propofol has become the most frequently used i.v. anaesthetic, gaining rapid acceptance for use in sedation and general anaesthesia. Propofol is also known to produce substantial vasodilatation and bronchodilatation.1 Indeed, propofol-induced bronchodilatation has been demonstrated in both healthy and asthmatic patients.2 3 In spite of this clinical evidence, the cellular mechanisms by which propofol produces bronchodilatation are still under investigation.

Relaxation of the airway involves a reduction in intracellular Ca2+ concentration ([Ca2+]i) of airway smooth muscle (ASM). We and others have previously demonstrated that in ASM, [Ca2+]i regulation involves both Ca2+ release from sarcoplasmic reticulum (SR) stores and plasma membrane Ca2+ influx,4–8 with the latter via several mechanisms including controlled influx in response to SR Ca2+ depletion (store-operated Ca2+ entry (SOCE)).6 7 9 Force regulation involves myosin light chain (MLC) and Ca2+ sensitization via the RhoA/Rho kinase pathway.10 11 Thus, there are several potential targets for propofol-induced bronchodilatation. In this regard, propofol has been found to attenuate vagal and methacholine-induced bronchoconstriction, with possible direct actions on muscarinic receptors.12 13 Propofol also decreases ovalbumin-induced contraction of sensitized rat trachea through inhibition of serotonin.14 Whether propofol directly affects ASM has been examined only in a few studies (albeit in varied species). Propofol has been shown to decrease peak [Ca2+]i, response to agonists,15 perhaps

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through SR Ca\(^{2+}\) release via inositol trisphosphate (IP\(_3\)) receptor channels,\(^{16}\) but does not appear to affect Ca\(^{2+}\) sensitivity.\(^{17}\) On the other hand, one study suggested that all of the effects of propofol on the airway may be neurally mediated.\(^{12}\) Thus, the mechanisms underlying propofol-induced bronchodilatation remain unclear.

A particular feature of propofol (and indeed its preparation) is its highly lipid nature. There is now increasing evidence that the plasma membrane of almost all cell types (including ASM) contain specialized lipid rafts, including 50–100 nm invaginations called caveolae,\(^{18–24}\) likened to be more intricate T-tubules of the striated muscle. Caveolae can facilitate interactions between plasma membrane-regulatory elements and intracellular signalling cascades.\(^{18–26}\) We demonstrated in human ASM, that suppression of the constitutional protein caveolin-1 substantially blunts agonist-induced [Ca\(^{2+}\)]\(_i\) responses and alters [Ca\(^{2+}\)]\(_i\) signalling, indicating a role for lipid rafts in ASM contractility.\(^{26,27}\) In human, ASM substantially blunts agonist-induced [Ca\(^{2+}\)]\(_i\) responses and alters [Ca\(^{2+}\)]\(_i\) signalling, indicating a role for lipid rafts in ASM contractility.\(^{26,27}\)

Previous studies have shown that local and volatile anaesthetics act via interaction with lipid rafts.\(^{28,29}\) There is also evidence that caveolae specifically are involved in the actions of general anaesthetics.\(^{30,31}\) However, the role of caveolae in mediating the effects of propofol is not known. Given the lipid nature of propofol, we hypothesized that the bronchodilatory effects of this anaesthetic may be mediated (at least in part) by alterations in caveolar signalling. Accordingly, in the present study, we used human ASM cells to examine the potential role of caveolae and caveolin-1 in propofol-induced bronchodilatation.

**Methods**

**Human ASM cell preparation**

The techniques for isolation of human ASM cells have been described previously.\(^{27}\) Briefly, bronchi were obtained from lung tissues incidental to patient procedures in thoracic surgery at Mayo Clinic Rochester (de-identified tissues typically from lobectomies and pneumonectomies; approved by the Mayo Institutional Review Board and not considered Human Subjects Research). Tissues were initially placed in Hank’s balance salt solution (HBSS, Invitrogen, Carlsbad, CA, USA) with 2.5 mM extracellular Ca\(^{2+}\). ASM layers were isolated and enzymatically dissociated as described previously.\(^{13}\) Cells were plated in sterile culture flasks and grown in a 95% air/5% CO\(_2\) humidified incubator using DMEM F/12 supplemented with 10% fetal bovine serum (FBS). All experiments were performed in cells before the third passage to ensure cell phenotype as verified by immunostaining for smooth muscle actin and myosin and by expression of receptors for commonly used bronchoconstrictor agonists, such as the M3 muscarinic receptor and the H1 histaminergic receptor.

**Preparation of caveolar membranes**

Caveolin-rich membranes were prepared as described previously.\(^{27}\) Briefly, ASM strips were homogenized in cold buffer A (0.25 M sucrose, 1 mM EDTA, and 20 mM Tricine, pH 7.8) until fine emulsion was achieved. The homogenates were sonicated for 10 s each, centrifuged (1000g, 10 min, 4°C), the supernatant removed and brought to 5 ml, which was layered onto 30% Percoll, and re-centrifuged (84,000g, 30 min). The plasma membrane fraction was collected and brought to 2 ml with buffer A. A crude membrane fraction was then sonicated, resuspended in OptiPrep, and then placed in a centrifuge tube. A linear 20–10% OptiPrep gradient was layered on top before centrifugation at 52,000g for 90 min. The upper membrane layer containing the caveolae was then collected for analysis. The lower membrane layer was collected as a control.

**Measurement of propofol within caveolar membranes by gas chromatography**

Propofol was measured within caveolar membrane fractions to confirm the connection between lipid-rich caveolae and propofol. Propofol concentrations were measured by gas chromatography–mass spectrometry (GC/MS) using a modified method as described previously.\(^{33,34}\) Briefly, caveolar fractions contained in 0.5 ml sucrose buffer were mixed with 100 µl n-heptane for propofol extraction and the organic phase placed in 200 µl autosampler vials for analysis.

GC/MS analysis was performed using a Waters Quattro Micro triple quadruple GC/MS/MS system (Waters, Manchester, UK) operating under electron impact ionization conditions. The column used was a DB-5MS fused silica capillary column (30 m × 0.25 mm inside diameter, 0.25 µm film thickness, Agilent Technologies, USA). A temperature programme ramp was performed from 60 to 300°C at 20°C min\(^{-1}\). The injection temperature was set at 250°C and the transfer line temperature at 250°C. Helium was used as a carrier gas at a flow rate of 1.0 ml min\(^{-1}\), and 1 µl of the sample extract was injected. The [M-15]+ fragment ion of propofol at m/z 163.1 was monitored for quantification and the peak area compared with an external calibration curve of propofol, prepared in n-heptane over a concentration range of 0.015–1.8 ng µl\(^{-1}\), 1 µl being injected on-column.

**Fluorescence [Ca\(^{2+}\)]\(_i\) imaging**

ASM cells were incubated in 5 µM fura-2 AM (Invitrogen) for 45 min at room temperature and visualized with a fluorescence imaging system (MetaFluor, Universal Imaging, Downingtown, PA, USA) on a Nikon Diaphot inverted microscope. Cells were initially perfused with HBSS (2.5 mM Ca\(^{2+}\), 37°C), and baseline fluorescence established. [Ca\(^{2+}\)]\(_i\) responses of 10 cells per chamber were obtained for individual, software-defined regions of interest. Fura-2 was alternately excited at 340 and 380 nm, and the ratio of fluorescence emissions at 510 nm was obtained every 0.75 s (Photometric Cascade digital camera system, Roper Scientific, Tucson, AZ, USA).
Caveolin-1 small interference RNA
The technique for suppression of caveolin-1 expression in human ASM using small interference RNA (siRNA) caveolin-1 was recently described. Briefly, siRNA duplex corresponding to bovine caveolin-1 mRNA targeting against the open reading frame, 223-241 bases, and a negative control siRNA were selected for caveolin-1 knockdown (Dharmacon, Lafayette, CO, USA). ASM cells at 60% confluence were transfected using 50 nM siRNA and Lipofectamine 2000 (Invitrogen) in DMEM F/12 without FBS. Fresh growth medium was added 6 h after transfection and experiments performed after 48 h. Both Lipofectamine only (vehicle) and scrambled siRNA sequences were used as controls.

Western blot analysis
Proteins were separated by SDS–PAGE using the Criterion Gel System (Bio-Rad, Hercules, CA, USA) and 15% or 4–15% gradient gels. Proteins were transferred to polyvinylidine fluoride (PVDF) membranes (Bio-Rad) for 60 min and then blocked for 1 h with 5% milk in Tris-buffered saline containing 0.1% Tween (TBST). Membranes were then incubated with anti-caveolin-1 (1:1000), anti-ERK (1:1000), anti-eERK (1:100), anti-PI3K (1:1000), or anti-RhoA (1:1000) antibodies overnight at 4°C. Following three washes with TBST, primary antibodies were detected with horseradish peroxidase-conjugated secondary antibodies and signals developed by Supersignal West Pico Chemiluminescent Substrate (Pierce Chemical Co., Rockford, IL, USA).

Materials
Methyl-β-cyclodextrin (CD), Optiprep, and other chemicals were obtained from Sigma (St Louis, MO, USA) unless mentioned otherwise. All tissue culture reagents were obtained from Invitrogen. Primary and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Propofol DMSO formulation was obtained from Sigma; propofol emulsion was obtained from the Mayo Clinic Pharmacy.

Statistics
Propofol effects on [Ca^{2+}]_i, responses to histamine
In unstimulated human ASM cells, baseline [Ca^{2+}]_i was ~100 nM. Upon addition of 10 μM histamine, a typical rapid increase in [Ca^{2+}]_i was observed, with a peak response of 514 (43) nM. The subsequent plateau was 223 (53) nM. After a wash in HBSS for 20 min, exposure to 10 or 30 μM propofol for 5 min did not substantially decrease baseline [Ca^{2+}]_i levels. However, subsequent stimulation with 10 μM histamine resulted in an ~40% decrease in peak [Ca^{2+}]_i response (Fig. 2; P<0.05). The plateau [Ca^{2+}]_i response was not different between control and propofol-exposed cells.

Effect of caveolin-1 siRNA
In ASM cells transfected with caveolin-1 siRNA, exposure to histamine resulted in a considerably smaller [Ca^{2+}]_i response, consistent with our previous study. In these cells, the addition of 30 μM propofol also decreased peak [Ca^{2+}]_i responses to histamine; however, these effects were substantially blunted compared with cells transfected with Lipofectamine alone (Fig. 3; P<0.05 for significant effects of siRNA).

Propofol effect on SR Ca^{2+} release
Under conditions of zero extracellular Ca^{2+} exposure, baseline [Ca^{2+}]_i was ~80 nM and exposure to 10 μM histamine continued to result in a peak transient [Ca^{2+}]_i response [260 (15) nM]. Treatment with 10 μM or 30 μM propofol for 5 min substantially reduced the peak [Ca^{2+}]_i response to 10 μM histamine (P<0.05; Fig. 4A). The effects of 10 vs 30 μM propofol were the same.

Effect of propofol on IP_3 receptor channels
We have previously demonstrated that SR Ca^{2+} release in ASM involves both IP_3 receptor and ryanodine receptor (RyR) channels. In one set of experiments, IP_3 receptor channels were first blocked with 20 μM Xestospongin C.
Effect of propofol on RyR channels

We previously demonstrated that RyR channels are blocked by high concentrations of ryanodine. In this set of experiments, RyR channels were first blocked with 20 μM ryanodine for 15 min. Cells were stimulated with 10 μM histamine, thoroughly washed, treated with 30 μM propofol for 5 min, and re-stimulated with 10 μM histamine. Although histamine-induced [Ca²⁺]ᵢ responses were decreased by ryanodine, additional propofol did not further decrease this [Ca²⁺]ᵢ response (Fig. 4b).

Effect of propofol on Caveolin-1 expression

To further investigate the effects of propofol on SR Ca²⁺ release via RyR channels, ASM cells were exposed to 5 mM caffeine in the absence or presence of 30 μM propofol. Thirty micromoles of propofol blunted caffeine-induced SR Ca²⁺ release (Fig. 5; P<0.05).

Effect of propofol on SOCE

We have previously demonstrated SOCE in ASM. A similar protocol was used here. Extracellular Ca²⁺ was first removed by perfusion with zero-Ca²⁺ HBSS. Voltage-gated Ca²⁺ influx was further inhibited using 1 μM nifedipine and 10 mM KCl. SR Ca²⁺ stores were then depleted using 10 μM cyclopiazonic acid (an inhibitor of SR Ca²⁺ reuptake), resulting in a slow increase in [Ca²⁺]ᵢ levels due to continued leak from the SR. After 15 min, extracellular Ca²⁺ was rapidly re-introduced to trigger SOCE. Cells were then washed thoroughly to refill SR stores, and the protocol repeated with additional exposure to 30 μM propofol 5 min before re-introduction of extracellular Ca²⁺ (thus avoiding any effect of propofol on the SR release aspect). Exposure to propofol resulted in decreased SOCE in the propofol/DMSO formulation (P<0.05; Fig. 6), while the propofol emulsion showed no significant changes.

Since we postulated that propofol and caveolae or caveolin-1 interact, in an additional set of experiments, the experiments described above were repeated in cells treated with caveolin-1 siRNA. SOCE in cells treated with caveolin-1 siRNA was significantly reduced (P<0.05; Fig. 6a). The addition of propofol now did not further reduce SOCE (Fig. 6a).

Western analyses

Given the lipid nature of propofol, we tested whether this anaesthetic decreases caveolin-1 expression, offering an explanation for the observed effects. In whole-cell
homogenates of human ASM, treatment with either propofol DMSO or emulsion formulations did not significantly alter whole-cell caveolin-1 expression. However, in caveolar fractions enriched in caveolae, propofol decreased caveolin-1 expression (Fig. 7). Previous studies have demonstrated that intracellular mitogen-activated protein kinases such as extracellular signal-regulated kinase (ERK) are involved in [Ca\(^{2+}\)] regulation and the effects of inflammation.\(^{36}\) Other studies have shown that caveolin-1 can regulate ERK in smooth muscle.\(^{37-40}\) Accordingly, we examined whether ERK expression and phosphorylation are altered by propofol. Total ERK expression was unchanged after propofol treatment of only 5 min. However, the phosphorylated form of ERK (pERK) was significantly lower with propofol treatment.

Fig 4 Role of SR Ca\(^{2+}\) release channels in propofol effects on [Ca\(^{2+}\)]. (A) In human ASM cells exposed to zero extracellular Ca\(^{2+}\), propofol continued to decrease the peak [Ca\(^{2+}\)] response to histamine. (B) With inhibition of IP\(_3\) receptor channels by XeC, [Ca\(^{2+}\)], responses to histamine were decreased (as expected); however, additional exposure to propofol continued to inhibit [Ca\(^{2+}\)], responses to histamine. In contrast, inhibition of RyR channels with ryanodine decreased [Ca\(^{2+}\)], response to histamine, but prevented propofol-induced decrease in the response. Values are mean (SD) in the summary bar graphs of (A), n=3 patients, average of 60 cells per condition; *significant propofol effect; #significant inhibitor effect (P<0.05).
Additionally, there is some evidence that the PI3K/Akt pathway can indirectly affect ASM \([\text{Ca}^{2+}]_i\) and force regulation. Caveolin-1 is thought to activate PI3K. Accordingly, we tested whether this pathway is affected by propofol effects. However, the expression of PI3K was unchanged with propofol treatment, regardless of formulation (Fig. 7). Furthermore, Ca\(^{2+}\) sensitization during force production involves the RhoA/rho kinase pathway. RhoA expression was decreased with propofol treatment with propofol 30 \(\mu\)M concentrations (Fig. 7; \(P<0.05\)).

Discussion

Propofol is well known to produce bronchodilatation in different species.\(^{17,41,42}\) The results of the present study indicate that propofol-induced bronchodilatation involves direct effects on ASM, potentially via interference with caveolae (specifically the constituent caveolin-1) regulation of \([\text{Ca}^{2+}]_i\). In addition, by measuring propofol concentrations within caveolar fractions by GC, we suggest that propofol partitions into caveolae, disrupting caveolar regulation. Given the almost ubiquitous nature of caveolae in different mammalian cell types, these data suggest a potential common mechanism by which propofol mediates its effects across different cell types.

Caveolins and \([\text{Ca}^{2+}]_i\) and force regulation in ASM

In ASM, the elevation of \([\text{Ca}^{2+}]_i\) by bronchoconstrictors involves both SR Ca\(^{2+}\) release via IP\(_3\) and RyR receptor channels and plasma membrane Ca\(^{2+}\) influx.\(^{4,7,43}\) Ca\(^{2+}\) influx can occur through both voltage-gated\(^{44}\) and receptor-gated\(^{45}\) channels. Furthermore, SOCE in response to SR Ca\(^{2+}\) depletion also occurs,\(^{4,7}\) allowing for replenishment of intracellular Ca\(^{2+}\) stores. In a previous study,\(^{27}\) we demonstrated that caveolin-1 is present in the plasma membrane of human ASM and co-localizes with receptors of several bronchoconstrictor agonists. Furthermore, we demonstrated that the disruption of caveolae by CD and the inhibition of caveolin-1 expression via siRNA significantly disrupt baseline \([\text{Ca}^{2+}]_i\); and the \([\text{Ca}^{2+}]_i\) responses to agonists. Caveolae (and
Propofol and \([\text{Ca}^{2+}]\), regulation in ASM

Compared with the myriad of studies examining the effect of lipid, volatile anaesthetics on ASM structure and function, relatively few studies have examined the effect of propofol. Propofol has been shown to attenuate vagally induced bronchoconstriction\(^{12}\) and has been suggested to inhibit the actions of serotonin in the ovalbumin-sensitized rat trachea.\(^{16}\) Although one study suggested that propofol may not have any direct effects on ASM,\(^{12}\) in other studies, propofol has been shown to attenuate muscarinic receptor-mediated\(^{13}\) and ATP-induced ASM contraction.\(^{53}\) Thus, there is some, albeit conflicting, data on the effects of propofol on ASM. The results of the present study indicate that propofol does have effects on \([\text{Ca}^{2+}]\), regulation in ASM. The novel finding here is that perhaps due to its lipid nature, propofol interferes with lipid raft signalling (i.e. caveolae and caveolins). Indeed, our data suggest that while acute propofol exposure does not affect the total amount of caveolin-1 in the cell (which can be partly intracellular), but that propofol does draw out caveolin-1 from caveolae (as shown in the caveolar fractions), an effect similar to cholesterol chelating agents such as CD.

In this study, the finding that propofol decreases \([\text{Ca}^{2+}]\), responses to histamine in human ASM cells in vitro, that is, without the confounding effect on airway neurones, underlines a direct effect on ASM itself. In this regard, the relative lack of propofol effects under conditions of suppressed caveolin-1 expression would suggest that propofol effects on the ASM are mediated (potentially in a major way) through lipid rafts. Given our previous data showing the expression of histaminergic and other receptors within caveolae of human ASM, these present data would suggest that the depletion of plasma membrane caveolae may lead to altered receptor expression or potentially disruption of caveolar interactions with intracellular structures such as the SR. In this regard, our finding that propofol effects involve RyR channels, but less so IP\(_3\) receptor channels, is interesting. One previous study suggested that propofol affects SR \([\text{Ca}^{2+}]\) release via IP\(_3\) receptor channels.\(^{54}\) However, effects on RyR channels in ASM have not been reported previously. Again, given our previous finding of caveolar interactions with SR \([\text{Ca}^{2+}]\) regulation,\(^{27}\) perhaps some of these effects are mediated by disruption of such interactions. Further studies (potentially using real-time imaging of caveolin-1) will address this issue.

We have previously demonstrated that caveolae in ASM express multiple TRPC isoforms (putative SOCE channels).\(^{4}\) Accordingly, we expected propofol to decrease SOCE, and indeed, we found this to be the case. The mechanisms underlying this effect are not clear, but likely involve caveolae (based on the effect of caveolin-1 siRNA). On the one hand, propofol may simply alter the extent of influx by inhibiting caveolar regulation of SOCE. On the other hand, propofol may also alter the conductance of these channels, leading to increased SOCE. The lack of propofol effects under conditions of suppressed caveolin-1 expression would suggest that propofol effects on caveolar interactions with intracellular structures such as the SR are mediated via disruption of such interactions. Further studies (potentially using real-time imaging of caveolin-1) will address this issue.

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caveolin-1) also appear to be important in the regulation of SOCE in ASM. These previous data highlight the importance of caveolae and caveolin-1 in \([\text{Ca}^{2+}]\), regulation in human ASM. Force generation after \([\text{Ca}^{2+}]\), elevation can occur via \([\text{Ca}^{2+}]-\)dependent (MLC phosphorylation) and \([\text{Ca}^{2+}]-\)independent mechanisms (\([\text{Ca}^{2+}]\) sensitization).\(^{65}\) \([\text{Ca}^{2+}]\) sensitization involves a monomeric GTP-binding protein, RhoA, and its downstream target, Rho-kinase.\(^{47-49}\) Phosphorylation by Rho-kinase inhibits MLC phosphatase, thus maintaining MLC phosphorylation and promoting contraction. Rho-kinase inhibitors can attenuate the contraction of ASM in rats, which is further attenuated by the addition of propofol.\(^{50}\) The relevance of the RhoA/Rho-kinase pathway to the present study lies in the fact that caveolins are thought to be essential for the activation of this pathway,\(^{51}\) and caveolin effects on this pathway could modulate force in ASM. In a previous study using porcine ASM, we found that \([\text{Ca}^{2+}]\) sensitivity via RhoA/Rho-kinase is indeed altered by CD.\(^{52}\)

![Fig 7 Effect of propofol on signalling mechanisms in ASM. In human ASM cells, exposure to propofol did not decrease total cellular caveolin-1, but did decrease caveolar expression of caveolin-1. With prolonged propofol exposure, caveolar expression of caveolin-1 recovered partially (data not shown). While total ERK and PI3K levels were unaffected, phosphorylation of ERK was decreased by propofol. RhoA levels (involved in \([\text{Ca}^{2+}]\), sensitization) were decreased by propofol at 5 min. Values are mean (SD), n=3 patients; *significant propofol effect (p<0.05).](image-url)
by the XeC and ryanodine experiments), it may also modulate regulatory proteins such as STIM1 that are critical to SOCE. These novel mechanisms of propofol action remain to be examined.

In our study, we did not find an effect of propofol on baseline [Ca\(^{2+}\)]. However, we have previously shown that baseline [Ca\(^{2+}\)] is affected by caveolar disruption. It is possible that in the normal ASM, caveolar disruption by propofol is not sufficient enough to alter baseline [Ca\(^{2+}\)]. However, under conditions of inflammation and increased baseline ASM [Ca\(^{2+}\)], (and thus airway tone), propofol may very well decrease baseline [Ca\(^{2+}\)], thus contributing to the bronchodilator effect.

In addition to effects mediated by altered Ca\(^{2+}\) regulatory protein expression, caveolae and caveolin-1 may regulate intracellular signalling pathways. In human ASM, the disruption of caveolae using CD or caveolin-1 siRNA causes p42/p44 MAPK (ERK) activation and increased cell proliferation. However, studies in other cell types suggest that caveolin-1 actually activates the ERK pathway, which is consistent with our data of propofol effects on ERK. While ERK phosphorylation may be involved, propofol effects do not appear to involve the PI3/Akt pathway, although there are some data that caveolins can interact with this signalling pathway. Accordingly, the activation or inhibition of specific signalling pathways after propofol exposure is likely to be complex and needs further investigation.

Overall, the results of the present study suggest that propofol-induced reduction in [Ca\(^{2+}\)], of ASM (and resultant bronchodilatation) involve the disruption of caveolae and caveolin-1 regulation of both plasma membrane and intracellular Ca\(^{2+}\) regulatory mechanisms. Furthermore, propofol could interfere with signalling intermediates that are typically activated by bronchoconstrictors. While further research is needed to examine the molecular mechanisms of action for propofol, an important consideration is that given caveolar expression of muscarinic and purinergic receptors, caveolae could be important in different targets of propofol in producing bronchodilatation. Furthermore, given the ubiquitous expression of caveolae across cell types, the role of caveolae or caveolin-1 in mediating propofol effects in other smooth muscle types is also warranted. Finally, caveolae may be a common factor in anaesthetic action. For example, previous studies have shown that local anaesthetics and volatile anaesthetics can act via interaction with lipid rafts and that caveolae are involved in actions of general anaesthetics.

**Methodological issues**

One potential concern regarding whether propofol has effects on any cell type is the formulation of propofol used. In older studies, metabisulphite, the preservative used in propofol, had also been shown to have bronchodilatory properties. For example, metabisulphite decreases methacholine-induced bronchoconstriction. With the newer propofol formulation, these effects are unlikely. On the other hand, the lipid emulsion may potentially have effects on lipid rafts. However, since our data demonstrate that there is no significant difference in effect between the propofol emulsion and DMSO, this should not be an issue.

**Declaration of interest**

None declared.

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