Volatile anaesthetics have differential effects on recombinant m1 and m3 muscarinic acetylcholine receptor function†

G. W. NIETGEN, C. W. HÖNEMANN, C. K. CHAN, G. L. KAMATCHI AND M. E. DURIEUX

Summary
Muscarinic acetylcholine signalling plays major roles in regulation of consciousness, cognitive functioning, pain perception and circulatory homeostasis. Halothane has been shown to inhibit m1 muscarinic signalling. However, no comparative data are available for desflurane, sevoflurane or isoflurane, nor have the anaesthetic effects on the m3 subtype (which is also prominent in the brain) been studied. Therefore, we have investigated the effects of these compounds on isolated m1 and m3 muscarinic receptor function. Defolliculated Xenopus oocytes expressing recombinant m1 or m3 muscarinic or (for comparison) AT1A angiotensin II receptors were voltage clamped, and Ca\(^{2+}\) currents (\(I_{\text{Cl(Ca)}}\)) induced by acetyl-\(\beta\)-methylcholine (Mch) or angiotensin II were measured in the presence of clinically relevant concentrations of halothane, sevoflurane, desflurane or isoflurane. To determine the site of action of the volatile anaesthetics we compared anaesthetic effects on m1, m3 and AT1A receptor function and studied the effects of volatile anaesthetics on signalling induced by intracellular injection of the second messenger IP3. Desflurane had a biphasic effect on m1 signalling, enhancing at a concentration of 0.46 mmol litre\(^{-1}\) but depressing at 0.92 mmol litre\(^{-1}\). A similar, although not significant, trend was observed with m3 signalling. Isoflurane had no effect on m1 signalling, but profoundly inhibited m3 signalling. Sevoflurane depressed the function of m1 and m3 signalling in a dose-dependent manner. Halothane, similar to its known effect on m1 signalling, dose-dependently depressed m3 function. \(I_{\text{Cl(Ca)}}\) induced by intracellular injections of IP3 were unaffected by all four anaesthetics. Similarly, none of the anaesthetics tested affected AT1A signalling. Absence of interference with AT1A signalling and intracellular pathways suggest that the effects of anaesthetics on muscarinic signalling most likely result from interactions with the m1 or m3 receptor molecule. Multiple interaction sites with different affinities may explain the biphasic response to desflurane. Anaesthetic-specific effects on closely related receptor subtypes suggest defined sites of action for volatile anaesthetics on the receptor protein. (Br. J. Anaesth. 1998; 81: 569–577).

Keywords: theories of anaesthetic action, molecular; anaesthetics volatile; receptors, muscarinic

In addition to the well known actions in circulatory and respiratory regulation,12 muscarinic acetylcholine signalling plays an important role in the central nervous system (CNS). Level of consciousness is modulated significantly by brainstem muscarinic signalling.3 Cholinergic antagonists affect memory and learning abilities through actions on the basal forebrain, cortex and hippocampus.45 Spinal muscarinic receptors mediate antinociception, predominantly in the substantia gelatinosa of the dorsal horn, and also affect motor neurone areas.57

Anaesthetics cause CNS depression and alter bronchial tone and heart rate, effects which may be mediated in part by interactions with muscarinic signalling.8 9 Various anaesthetics are known to alter ligand binding to muscarinic receptors. Ether,10 halothane,11 chlorofom, enfurane and isoflurane12 were shown to increase muscarinic antagonist binding. However, these initial reports investigated mixed receptor populations and did not study receptor activation. More refined methods of investigating anaesthetic mechanisms became available when the members of the muscarinic receptor subfamily were cloned. Hence the influence of anaesthetics on the function of individual receptor subtypes can now be studied. Recently, we reported that halothane13 and ketamine14 inhibited m1 muscarinic signalling.

This study was designed to extend the initial findings with halothane. First, we wished to investigate if the effect of halothane on muscarinic signalling was subtype-specific. Therefore, we studied anaesthetic actions on the m3 muscarinic receptor, which is also expressed widely in brain.15 16 Second, we wished to investigate if the effects of halothane could be extrapolated to other volatile anaesthetics. Thus we studied the muscarinic inhibitory effects of sevoflurane, isoflurane and desflurane. The comparison between isoflurane and desflurane was of specific interest, as these anaesthetics are closely related structurally: desflurane differs from isoflurane only in the substitution of fluorine for chlorine on the \(\alpha\)-ethyl carbon. However, in contrast with isoflurane, desflurane has been noted to induce a relative sympathetic activation, resulting in significantly increased heart rate and mean arterial pressure compared with isoflurane.17–19

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We hypothesized that this effect might result in part from muscarinic inhibition, and that, despite their structural similarities, these anaesthetics might show divergent effects on muscarinic receptor function.

Therefore, we have determined the action of desflurane, isoflurane and sevoflurane on m1 and m3 muscarinic and, as a comparison, on angiotensin II receptor function. In addition, the effects of halothane on m3 signalling were investigated. Specifically, we investigated if the anaesthetics, at clinically comparable concentrations, influenced m1 and m3 muscarinic signalling, and where in the signalling pathway their effects were localized.

Materials and methods

XENOPUS OOCYTES

The study was approved by the Animal Research Committee of the University of Virginia. Adult female Xenopus laevis frogs were obtained from Xenopus I (Ann Arbor, MI, USA) and housed in an established frog colony. To obtain oocytes, frogs were anaesthetized with 1% tricaine (3-aminobenzoic acid ethyl ester) until unresponsive, and operated on ice. Oocytes were harvested via a 5-mm incision in the lower lateral abdominal wall and placed immediately in modified Barth’s solution containing (mmol litre⁻¹): NaCl 88, KCl 1, NaHCO₃ 2.4, CaCl₂ 0.41, MgSO₄ 0.82, Ca(NO₃)₂ 0.3, gentamicin 0.1, HEPES 0.1 mg ml⁻¹. The resulting cRNA was quantified by spectrophotometry, as these are translated more efficiently in the 570

m1, m3 or AT₄a receptor activation in oocytes leads to G protein-induced phospholipase C activity and release of inositoltrisphosphate (IP₃) from phosphoinositoldiphosphate (fig. 1A). IP₃ then binds to its specific receptor on intracellular Ca²⁺ stores to release Ca²⁺, which in turn opens an endogenous Ca²⁺-activated Cl⁻ channel. The resulting current can be measured by voltage clamp, and this Ca²⁺-activated Cl⁻ current (I(CaCl⁻)) is integrated and expressed as micro coulombs (µC), is a measure of intracellular Ca²⁺ release.20–23 The upward deflection during agonist application at times observed in the traces is a slight motion artefact. All experiments were performed at room temperature (approximately 22°C).

Microelectrodes were pulled in one stage from capillary glass (BBL with fibre; World Precision Instruments, Sarasota, FL, USA) on a micropipette puller (model 700C; David Kopf Instruments, Tujunga, CA, USA). Tips were broken to a diameter of approximately 10 µm, providing a resistance of 1–3 MΩ, and filled with KCl 3 mol litre⁻¹. A single oocyte was placed in a perfusible bath containing 3 ml of Tyrode’s solution containing (mmol litre⁻¹): NaCl 150, KCl 5, MgCl₂ 1, CaCl₂ 2, glucose 10, HEPES 10, pH adjusted to 7.4 with NaOH. Two microelectrodes were inserted into the oocyte, and a holding potential of −70 mV was applied to the membrane. The voltage clamp amplifier (OC725A; Warner Corporation, New Haven, CT, USA) was connected to a data acquisition and analysis system running on an IBM-compatible personal computer. The acquisition system consisted of a DAS-8 A/D conversion board (Keithly-Metrabyte, Taunton, MA) and analysis was performed with OoClamp software.24 Occasional cells that did not show a stable holding current of less than 1 µA during a 1-min equilibration period were excluded from analysis.

Membrane current was sampled at 125 Hz and recorded for 5 s before and 55 s after application of the agonist (acetyl-β-methylcholine (Mch) or angiotensin II (AT) delivered at appropriate EC₅₀ values: Mch 10⁻⁷ mol litre⁻¹ for m1 receptors13, Mch 10⁻⁶ mol litre⁻¹ for m3 receptors; AT 10⁻⁷ mol litre⁻¹ for AT₄a receptors). Agonists were delivered in 30-µl aliquots over 1–2 s using a micropipettor positioned approximately 1 mm from the oocyte. Responses were quantified by integrating the current trace by quadrature and are reported as µC as this reflects intracellular Ca²⁺-release better than peak current.25 Any motion artefact on agonist delivery was excluded from analysis. Each oocyte received a single agonist application only.

A 1.2-kbp cDNA in the CDM8 vector (Invitrogen, San Diego, CA, USA), encoding the rat AT₄a angiotensin II receptor, was obtained from Dr. K. R. Lynch (University of Virginia, Charlottesville, VA, USA). The construct was linearized with the nuclelease XhoI and transcribed in vitro by T7 RNA polymerase in the presence of a capping analogue. Oocytes were injected as described above. All injected cells were cultured for 72 h at 18°C in modified Barth’s solution before study.

ELECTROPHYSIOLOGY

The rat m1 and m3 muscarinic acetylcholine receptors were a gift from T. I. Bonner (National Institutes of Mental Health, Bethesda, MD, USA). Its complementary DNA (cDNA) consists of a 2.8-kilobasepair (kb) fragment in a commercial vector (pGEM1; Promega, Madison, WI, USA). The construct for m1 receptor expression was linearized by digestion with the restriction enzyme HindIII, and complementary RNA (cRNA) was transcribed in vitro using the bacteriophage RNA polymerase T7. A capping analogue (7mGpppG) was included in the reaction to generate capped transcripts.24 Occasional cells that did not show a stable holding current of less than 1 µA during a 1-min equilibration period were excluded from analysis.

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IP3 AND HEPARIN MICROINJECTION

To evaluate the influence of volatile anaesthetics on the second messenger system, we injected IP3 or heparin into the oocyte. Heparin inhibits the IP3 receptor on intracellular Ca2+ stores, thereby inhibiting Ca2+ release induced by IP3-signalling receptors. Heparin injections were performed 30 min before voltage clamping; heparin 120 ng (2 ng nl⁻¹) was microinjected using the same nanojector mentioned above. IP3, when injected into the cell, induces an ICl(Ca) similar to that observed after receptor activation in the oocyte. IP3-induced ICl(Ca) was evoked by injecting 50 nl of IP3 2 mmol litre⁻¹ in water into the oocyte. Each oocyte was injected only once with IP3; therefore, any kinetic effects observed were not caused by time-dependent effects or run-down after multiple injections. Control injections with water as vehicle were performed to assure a specific action of IP3.

STATISTICAL ANALYSIS

Results are reported as mean (SEM). As variability between batches of oocytes is common, responses were at times normalized to same-day controls for each batch. Differences between treatment groups were analysed using ANOVA and Student’s unpaired t test, appropriately corrected for multiple comparisons (Bonferroni). P < 0.05 was considered significant. Concentration–response curves were fit to the following logistic function, derived from the Hill equation:

\[ y = y_{\text{max}} + \left( y_{\text{max}} - y_{\text{min}} \right) \left( 1 - \frac{x}{x_0} \right)^n \]

where \( y_{\text{max}} \) and \( y_{\text{min}} \) = maximum and minimum response obtained, respectively, \( n = \) the Hill coefficient, and \( x_0 \) = concentration at which the half-maximal response occurs (EC50 for agonist, IC50 for anaesthetics). In figure 1D, curve fitting was performed on averaged raw data. In the other graphs, because of variability of oocyte batches from different frogs, data were normalized to the same day control value and then the fitting was performed on the averaged normalized values. All fitting values are given in table 1 and fits were used only if \( r^2 \) was greater than 0.95.

MATERIALS

Molecular biology reagents were obtained from Promega (Madison, WI, USA) and other chemicals were obtained from Sigma (St Louis, MO, USA). Volatile anaesthetics (desflurane and isoflurane from Ohmeda Pharmaceutical Products Division, Liberty Corner, NJ, USA, sevoflurane from Abbott Laboratories, North Chicago, IL, USA and halothane from Halocarbon Laboratories, River Edge, NJ, USA) were delivered using agent-specific vaporizers (carrier gas:air at 500 ml min⁻¹) and equilibrated in Tyrode’s solution by bubbling for at least 10 min. Oocytes were exposed to anaesthetics for approximately 5 min before testing. Bath concentrations of anaesthetics were verified by gas chromatography (Aerograph 940, Varian Analytical Instruments, Walnut Creek, CA, USA) using saline–gas partition coefficients (Psol/gas) at 22°C for isoflurane (1.08), sevoflurane (0.52), halothane (1.20) and desflurane (0.45) (estimations for desflurane and sevoflurane were based on the observation that Psol/gas is approximately twice as great at 22°C as at 37°C).26–28

Results

FUNCTIONAL EXPRESSION OF MUSCARINIC AND ANGIOTENSIN RECEPTORS IN XENOPUS OOCYTES

Control oocytes were unresponsive to Mch 10⁻⁷ mol litre⁻¹ or AT 10⁻⁶ mol litre⁻¹ (data not shown). Oocytes

Table 1  Curve fitting variables (Hill equation) for Mch concentration–response relationships in Xenopus oocytes expressing recombinant m1 or m3 muscarinic acetylcholine receptors and inhibition concentration relationship for isoflurane, sevoflurane and halothane on m1 or m3 muscarinic receptor signalling values. Values are given as mean (SEM).

<table>
<thead>
<tr>
<th>Concentration-response relationship</th>
<th>EC50 (mol litre⁻¹)</th>
<th>Hill coefficient</th>
<th>Emax (µC)</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1 muscarinic receptor</td>
<td>1.3 (0.15) × 10⁻⁷</td>
<td>0.83 (0.07)</td>
<td>8.77 (0.14)</td>
<td>0.99</td>
</tr>
<tr>
<td>m3 muscarinic receptor</td>
<td>1.02 (0.58) × 10⁻⁶</td>
<td>0.75 (0.27)</td>
<td>9.95 (0.88)</td>
<td>0.96</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Concentration–inhibition relationship</th>
<th>EC50 (mol litre⁻¹)</th>
<th>Hill coefficient</th>
<th>Emax (%)</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>m3 muscarinic receptor and isoflurane</td>
<td>0.45 (0.06)</td>
<td>1.92 (0.43)</td>
<td>99.12 (6.31)</td>
<td>0.98</td>
</tr>
<tr>
<td>m1 muscarinic receptor and sevoflurane</td>
<td>0.32 (0.01)</td>
<td>3.18 (0.32)</td>
<td>97.11 (2.25)</td>
<td>0.99</td>
</tr>
<tr>
<td>m3 muscarinic receptor and sevoflurane</td>
<td>0.75 (0.05)</td>
<td>7.25 (3.8)</td>
<td>103.7 (7.56)</td>
<td>0.96</td>
</tr>
<tr>
<td>m3 muscarinic receptor and halothane</td>
<td>0.88 (0.02)</td>
<td>5.4 (0.66)</td>
<td>98.61 (2.79)</td>
<td>0.99</td>
</tr>
</tbody>
</table>
inward current in a concentration-dependent manner (fig. 1A). Currents developed within several seconds after agonist application, and returned to baseline after approximately 30 s. Average response sizes were 3.4 (0.8) μC for the m1 receptor, 5.0 (1.2) μC for the m3 receptor and 1.6 (0.6) μC for the AT1A receptor. These findings are similar to those reported in our previous studies, where we showed that these currents were \( I_{\text{Cl(Ca)}} \).

To ensure that the responses were mediated by specific receptors, we attempted to inhibit them with antagonists. Atropine 1 μmol litre\(^{-1}\) completely inhibited responses to Mch in oocytes expressing m1 and m3 receptors (fig. 1A), and the m3-selective antagonist 4-DAMP 100 nmol litre\(^{-1}\) inhibited responses of m3 receptors (data not shown). Similarly, the non-peptide angiotensin receptor antagonist losartan 10 μmol litre\(^{-1}\) inhibited AT signalling in cells expressing AT1A receptors (fig. 1C).

**DESFLURANE AFFECT M1 AND M3 MUSCARINIC SIGNALLING IN A CONCENTRATION-DEPENDENT BIPHASIC MANNER**

We then tested the ability of desflurane at various concentrations to influence \( I_{\text{Cl(Ca)}} \) induced by appropriate Mch concentrations in oocytes expressing m1 or m3 receptors. Signalling of m1 receptors was enhanced significantly when desflurane 0.46 mmol litre\(^{-1}\) was present in the bath: responses were 71 (28) % greater than control (\( P < 0.001, t \) test). Desflurane, at a concentration of 0.70 mmol litre\(^{-1}\), induced no significant change in response (decrease of 12 (17) %; \( P = 0.47, t \) test) compared with control. At higher concentrations, desflurane inhibited muscarinic signalling: desflurane 0.92 and 1.66 mmol litre\(^{-1}\) reduced responses by 43 (11) % (\( P = 0.003, t \) test) and 75 (5) % (\( P < 0.001, t \) test), respectively (fig. 2A). Similar results were obtained for m3 signalling (fig. 2C). Thus desflurane had a biphasic, concentration-dependent effect on muscarinic receptor function.

**ISOFLURANE DID NOT AFFECT M1, BUT INHIBITED M3 MUSCARINIC SIGNALLING**

In contrast with desflurane, isoflurane had no effect on Mch-induced m1 muscarinic signalling (fig. 3A). At very high concentrations (0.75–0.96 mmol litre\(^{-1}\)) an inhibitory trend was noted (fig. 3B). However, these effects were not statistically significant. The effects of isoflurane on m1 signalling were therefore different from those of desflurane, especially when the actions of the two compounds were compared at MAC-equivalent concentrations. In contrast with its lack of effect on m1 signalling, isoflurane inhibited m3 signalling (IC\(_{50} 0.45 \text{ mmol litre}^{-1}\)) (fig. 3C).

**SEVOLURANE AND HALOTHANE INHIBITED M1 AND M3 SIGNALLING**

Sevoflurane and halothane inhibited m1 and m3 muscarinic signalling. We have reported previously that halothane inhibits m1 signalling with an IC\(_{50}\) of 0.3 mmol litre\(^{-1}\). In this study, we found that it inhibited m3 signalling with an IC\(_{50}\) of 0.88 mmol litre\(^{-1}\) (fig. 4C). Sevoflurane inhibited m1 and m3 signalling in a similar manner (IC\(_{50}\) 0.32 mmol litre\(^{-1}\) for m1 muscarinic inhibition (fig. 4A) and 0.75 mmol litre\(^{-1}\) for m3 muscarinic signalling (fig. 4B)).
the m1 receptor appeared to display higher sensitivity to the inhibitory effects of halothane and sevoflurane.

**ANGIOTENSIN II AND MUSCARINIC RECEPTORS SHARED THE SAME INTRACELLULAR SIGNALLING PATHWAY**

As we wished to study the effects of the anaesthetics on angiotensin signalling to localize their site of action, it was necessary to demonstrate that angiotensin and muscarinic receptors signal through the same pathway. We have shown previously that AT1A receptors expressed in *Xenopus* oocytes signal through the IP3 pathway, as signalling can be blocked specifically by intracellular microinjection of the IP3 receptor antagonist heparin.29 To confirm the experiments reported previously, and to determine if muscarinic signalling uses the same pathway, we evaluated the effect on signalling of microinjection of...
heparin (2 ng nl\(^{-1}\), 60 nl, 30 min before recording) into oocytes expressing m1 or AT\(_{1A}\) receptors. m1 muscarinic signalling was inhibited to the same degree as angiotensin signalling (fig. 5A). Heparin microinjection into the oocyte inhibited responses to Mch by 93% compared with controls (fig. 5B). In contrast, microinjected water had no effect on either muscarinic or angiotensin signalling (data not shown). As the oocyte expresses only a single isoform of phospholipase C, coupling receptor activation to intracellular Ca\(^{2+}\) release,\(^\text{21}\) our findings indicate that the muscarinic and AT\(_{1A}\) signalling pathways are identical downstream of the G protein (compare fig. 1A).

**SITE OF ACTION: VOLATILE ANAESTHETICS HAD NO EFFECT ON ANGIOTENSIN SIGNALLING**

As muscarinic and angiotensin signalling use the same intracellular second messenger pathways, we investigated the effect of desflurane, isoflurane and sevoflurane on AT\(_{1A}\) receptor function. In contrast with their variable effects on m1 and m3 signalling, the anaesthetics used had no effect on AT\(_{1A}\) signalling, even at high concentrations (fig. 5C). Similar results were reported for halothane.\(^\text{13}\) These results indicate that the actions of these anaesthetics on m1 and m3 signalling are not mediated by effects on the intracellular pathways, as these are shared by muscarinic and angiotensin receptors.

**SITE OF ACTION: VOLATILE ANAESTHETICS HAD NO EFFECT ON IP\(_3\)-INDUCED Cl\(^{-}\) CURRENTS**

To elucidate further the site of action of volatile anaesthetics on muscarinic signalling, we determined the effect of the anaesthetics on currents induced by microinjected IP\(_3\) (50 nl of 2 mmol litre\(^{-1}\), yielding approximately 100 \(\mu\)mol litre\(^{-1}\) intracellularly). IP\(_3\)-induced Cl\(^{-}\) currents resembled the responses induced by receptor activation (fig. 6A). In contrast, microinjected water was ineffective (fig. 6B). Desflurane, even at high concentrations, had no effect on IP\(_3\)-induced currents, indicating that this
Effects of volatile anaesthetics on muscarinic signalling

agent affects the signalling pathway before interaction between IP3 and its receptor (fig. 6B). Similarly, we have shown that halothane, isoflurane and sevoflurane have no effect on intracellular IP3 signalling or signalling induced by direct G-protein activation with GTPγS.30

REVERSIBILITY OF ANAESTHETIC ACTION

To complete this investigation, we determined the reversibility of anaesthetic inhibition of muscarinic signalling. Data are presented in figure 7. In all cases, anaesthetic inhibition was reversible on wash-out.

Discussion

Muscarinic signalling by m1 and m3 receptors was inhibited in a differential manner by the volatile anaesthetics studied. Halothane and sevoflurane, in clinically relevant concentrations, inhibited muscarinic signalling, with the m1 receptor displaying a higher sensitivity than the m3 receptor. Isoflurane had no effect on m1 muscarinic signalling, even at concentrations which would be anticipated to disrupt membrane structure,28 but significantly inhibited m3 signalling. Desflurane showed a biphasic effect on both receptors.

Differences in effect between desflurane and isoflurane are remarkable, as the two compounds differ only in a single halogen atom: a chlorine atom in isoflurane is replaced by a fluorine atom in desflurane. Thus minor changes in chemical structure can induce significant pharmacological effects.

The biphasic action of desflurane on muscarinic signalling is unusual, although not unprecedented. Ryanodine31 and adenosine32 are known to act similarly in various models. Our study was not designed to determine the molecular mechanism of this biphasic action. However, we could rule out interactions with the intracellular signalling pathway, as none of the anaesthetics had effects on both AT and IP3 signalling. Hence the site of action is most likely at the muscarinic receptor or (possibly) the associated G protein. Biphasic responses are explained most easily by the existence of two different binding sites at the receptor with different affinities and different agonistic–antagonistic effects when occupied. Mutagenesis studies may be able to delineate such various binding sites on the muscarinic receptor.

Our findings should be interpreted in view of the

Figure 6

Inositoltrisphosphate (IP3) signalling is not inhibited by high concentrations of desflurane. A: Left: 50 nl of IP3 injected (final intracellular concentration 100 μmol litre⁻¹) induced an IC50 resembling responses generated by Mch or AT (compare fig. 1A, 1C). Right: Desflurane 0.92 mmol litre⁻¹ had no effect on the oocyte response to IP3. B: Desflurane 0.46 and 0.92 mmol litre⁻¹ had no effect on IC50 generated by intracellular IP3 injection (average control response 1.3 μC; number of oocytes tested in parentheses). Injection of vehicle (H2O) into the oocyte did not induce currents.

Figure 7

Reversibility of anaesthetic actions on muscarinic receptor signalling. A: IC50 induced by Mch 10⁻⁸ mol litre⁻¹ to determine reversibility of inhibition of volatile anaesthetics on m1 muscarinic acetylcholine receptor signalling. The first bar represents control measurements (4.83 (0.86) μC) and the second bar shows the inhibitory effect of the volatile anaesthetic tested (2.5% halothane, 3.3% isoflurane, 5% sevoflurane and 18% desflurane). IC50 was reduced to 14% (0.7 (0.15) μC), 96.6% (4.64 (1.45) μC), 28.15% (1.36 (0.78) μC) and 24% (1.16 (0.27) μC) by halothane, isoflurane, sevoflurane and desflurane, respectively. The third bar shows recovery after 10 min of perfusion with Tyrode’s solution without anaesthetic (*P<0.05, t test). B: IC50 induced by Mch 10⁻⁸ mol litre⁻¹ to determine reversibility of inhibition of volatile anaesthetics on m3 muscarinic acetylcholine receptor signalling. Three consecutive measurements were made in different oocytes. The first bar represents control measurements (15.2 (0.67) μC) and the second bar shows the inhibitory effect of the volatile anaesthetic tested (2.5% halothane, 3.3% isoflurane, 5% sevoflurane, 18% desflurane). IC50 was reduced to 22% (3.43 (2.25) μC), 6.5% (0.93 (0.33) μC), 25% (3.8 (1.55) μC) and 37% (5.58 (1.88) μC) by halothane, isoflurane, sevoflurane and desflurane, respectively. The third bar shows recovery after 10 min of perfusion with Tyrode’s solution without anaesthetic (*P<0.05, t test).
artificial environment in which the receptors were expressed. However, muscarinic and angiotensin receptors have been expressed frequently in the *Xenopus* oocyte model system, both by ourselves and others, and it is well established that they function similarly in oocytes as they do in native cells. Although the receptors are expressed at a lower temperature than they are normally exposed to, this has not been shown to result in significant changes in kinetics or function.

Any attempt to correlate our findings to observed clinical effects of anaesthetics is arduous, as conclusions drawn from several receptor subtypes cannot be transferred to a complex organism and its response to anaesthesia. Although most clinical and experimental data comparing isoflurane and desflurane show a congruence in their CNS and circulatory effects, some unexplained differences exist. Desflurane is known to exhibit less negative inotropic activity and to induce smaller decreases in arterial pressure. When heart rate, mean arterial pressure and sympathetic nerve activity were compared after 10.9% desflurane induction and emergence from anaesthesia, all variables were enhanced significantly compared with isoflurane; after more prolonged anaesthesia there were no differences between the anaesthetics. This initial period of excitation, when anaesthetic concentrations are relatively low, could result potentially from interactions with muscarinic signalling pathways. It would be of great interest to study the interactions between desflurane and adrenergic receptor functioning, as excitatory effects of desflurane directly on the β-adrenergic receptor or on presynaptic ganglia might also explain its excitatory effects.

CNS muscarinic stimulation raises the level of consciousness and improves learning. It is interesting to speculate if stimulation of m1 signalling (the most prominent cerebral muscarinic subtype) by low desflurane concentrations might contribute to the rapid mental recovery observed with the anaesthetic. If so, this might be of particular importance in elderly patients with limited CNS muscarinic signalling reserve (e.g. patients with Alzheimer’s disease). The prominence of the m3 subtype in bronchial smooth muscle and glands could explain in part the irritating effect of desflurane during induction and emergence from anaesthesia, when anaesthetic concentrations are relatively low.

In brief, we have demonstrated that desflurane had a concentration-dependent biphasic effect on m1 and m3 muscarinic receptor functioning, whereas isoflurane had no effect on m1 signalling but pronounced effects on m3 signalling. We have also shown that halothane and sevoflurane inhibited m1 and m3 signalling in a dose-dependent manner. This action is most likely localized at the receptor level, as the anaesthetics did not influence intracellular signalling pathways. In addition, the anaesthetics had no effect on angiotensin signalling. In combination with our findings that halothane inhibits m1 signalling in a concentration-dependent manner, these results show that different volatile anaesthetics affect m1 and m3 muscarinic signalling in highly divergent ways, and that small changes in chemical structure of the volatile anaesthetic or the receptor can significantly affect anaesthetic–protein interactions.

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