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SPECIAL REPORT

Volatile general anaesthetic actions on recombinant $nACh_{\alpha 7}$, 5-HT_3 and chimeric $nACh_{\alpha 7}$-5-HT_3 receptors expressed in *Xenopus* oocytes

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The effect of halothane and isoflurane was studied on the function of recombinant neurotransmitter receptors expressed in *Xenopus* oocytes. Both anaesthetics inhibited nicotinic acetylcholine type α7 ($nACh_{\alpha 7}$) receptor-mediated responses, potentiated 5-hydroxytryptamine type 3 (5-HT_3) receptor-mediated responses at low agonist concentrations, and inhibited the function of a chimeric receptor (with the N-terminal domain from the $nACh_{\alpha 7}$ receptor and the transmembrane and C-terminal domains from the 5-HT_3 receptor) in a manner similar to that of the $nACh_{\alpha 7}$ receptor. Since the N-terminal domain of the chimeric receptor was from the $nACh_{\alpha 7}$ receptor, the observations suggest that the inhibition involves the N-terminal domain of the receptor.

Keywords: Anaesthetics; receptors; ion channels; molecular chimera; acetylcholine receptor; 5-hydroxytryptamine receptor

Introduction Traditionally, cell membrane lipids have been thought to be the primary target of volatile general anaesthetics, but recent studies have suggested that these anaesthetics may interact directly with certain neurotransmitter-gated ion channels (Franks & Lieb, 1993; 1994; Forman *et al*., 1995; McKenzie *et al*., 1995; Jenkins *et al*., 1996). However, the molecular region of the receptor involved in this anaesthetic action has not been established. Molecular chimeras have been found to be extremely useful for determining structure-function relationships of membrane proteins. A functional chimeric receptor-ion channel has been constructed with the N-terminal domain from the nicotinic cholinoceptor of the α7 type ($nACh_{\alpha 7}$) receptor and the transmembrane and C-terminal domains from the 5-hydroxytryptamine (5-HT)$_3$ receptor (Eiselé *et al*., 1993). Since both halothane and isoflurane potentiated 5-HT_3 receptor-mediated responses (Machu & Harris, 1994; Jenkins *et al*., 1996) and inhibited $nACh_{\alpha 7}$ receptor-mediated responses, we used the chimeric $nACh_{\alpha 7}$-5-HT_3 receptor to study whether the anaesthetic action involves the N-terminal or the transmembrane and C-terminal receptor domains.

Methods The $nACh_{\alpha 7}$ receptor cDNA was provided by Dr Jon Lindstrom (Schoepfer *et al*., 1990), and the 5-HT_3 receptor cDNA provided by Dr David Julius (Maricq *et al*., 1991). The chimeric receptor was constructed by using fragments cut by the enzymes *Bcl*I and *Sac*II, an N-terminal fragment of the $nACh_{\alpha 7}$ receptor was generated by the polymerase chain reaction (PCR) and ligated to a transmembrane and C-terminal fragment from the 5-HT_3 receptor at a junction of V201 on the $nACh_{\alpha 7}$ receptor, as previously described (Eiselé *et al*., 1993). Complementary RNAs were prepared by *in vitro* transcription. Preparation of, receptor expression using, and two-electrode voltage-clamp recording from, *Xenopus* oocytes, and data analysis, were as described previously (Zhang *et al*., 1995); membrane holding potential was −70 mV. In some experiments, 2.5 mM Ca^{2+} in the bathing solution was replaced by 2.5 mM Ba^{2+}. Halothane (Ayerst Laboratories) and isoflurane (Anaquest) solutions were prepared immediately before use and applied with a Hamilton gas-tight syringe connected to Teflon tubing. The bath concentrations of the anaesthetics were determined by gas chromatography and found to be the same as the calculated concentrations.

Results Figure 1a (i) illustrates that both halothane (5 mM) and isoflurane (5 mM) inhibited $nACh_{\alpha 7}$ receptor-mediated responses. In the standard extracellular bathing solution containing 2.5 mM Ca^{2+}, the inhibition was concentration-dependent between 0.1 and 10 mM for both halothane and isoflurane, with apparent Hill coefficients for the concentration-response curve of 1.3 ± 0.2 and 1.1 ± 0.2, and EC_{50} values of 1.2 ± 0.2 and 0.7 ± 0.2 mM, respectively. In Ca^{2+}-free bathing solution containing 2.5 mM Ba^{2+}, the inhibition was also concentration-dependent between 0.1 and 10 mM for both halothane and isoflurane, with apparent Hill coefficients for the concentration-response curve of 1.1 ± 0.3 and 1.4 ± 0.2, and EC_{50} values of 1.3 ± 0.5 and 0.9 ± 0.3 mM, respectively. The apparent Hill coefficient and EC_{50} values in Ca^{2+}-containing and in Ca^{2+}-free Ba^{2+}-containing bathing solutions were not significantly different (ANOVA, $P > 0.1$). In Figure 1b (i), both halothane (5 mM) and isoflurane (5 mM) decreased E_{max} of the agonist concentration-response curve (to 47 ± 4% and 41 ± 3% of the control response, respectively; ANOVA, $P < 0.001$), without significantly affecting either the EC_{50} or the apparent Hill coefficient of this curve (ANOVA, $P > 0.05$). Figure 1a (ii) illustrates that both halothane (5 mM) and isoflurane (5 mM) potentiated 5-HT_3 receptor-mediated responses activated by 0.1 μM 5-HT. The potentiation of current activated by 0.1 μM 5-HT was concentration-dependent between 0.1 and 10 mM for both halothane and isoflurane, with apparent Hill coefficients for the concentration-response curve of 1.1 ± 0.2 and 1.4 ± 0.3, and EC_{50} values of 2.2 ± 0.8 and 1.5 ± 0.3 mM, respectively. Figure 1b (ii) shows that the percentage increase of 5-HT_3 receptor-mediated responses by both halothane (5 mM) and isoflurane (5 mM) was maximal at the lowest agonist concentrations tested (0.03 and 0.1 μM 5-HT), and decreased with increasing agonist concentration. Potentiation was not observed with 5-HT concentrations $\geqslant 5$ μM. Halothane (5 mM) and isoflurane (5 mM) did not significantly affect the E_{max}, the EC_{50} or the apparent Hill coefficient of the 5-HT concentration-response curve (ANOVA, $P > 0.05$). Figure 1a (iii) illustrates that both halothane (5 mM) and isoflurane (5 mM) inhibited the chimeric receptor-mediated responses. The inhibition was concentration-dependent between 0.1 and 10 mM for both halothane and isoflurane, with apparent Hill

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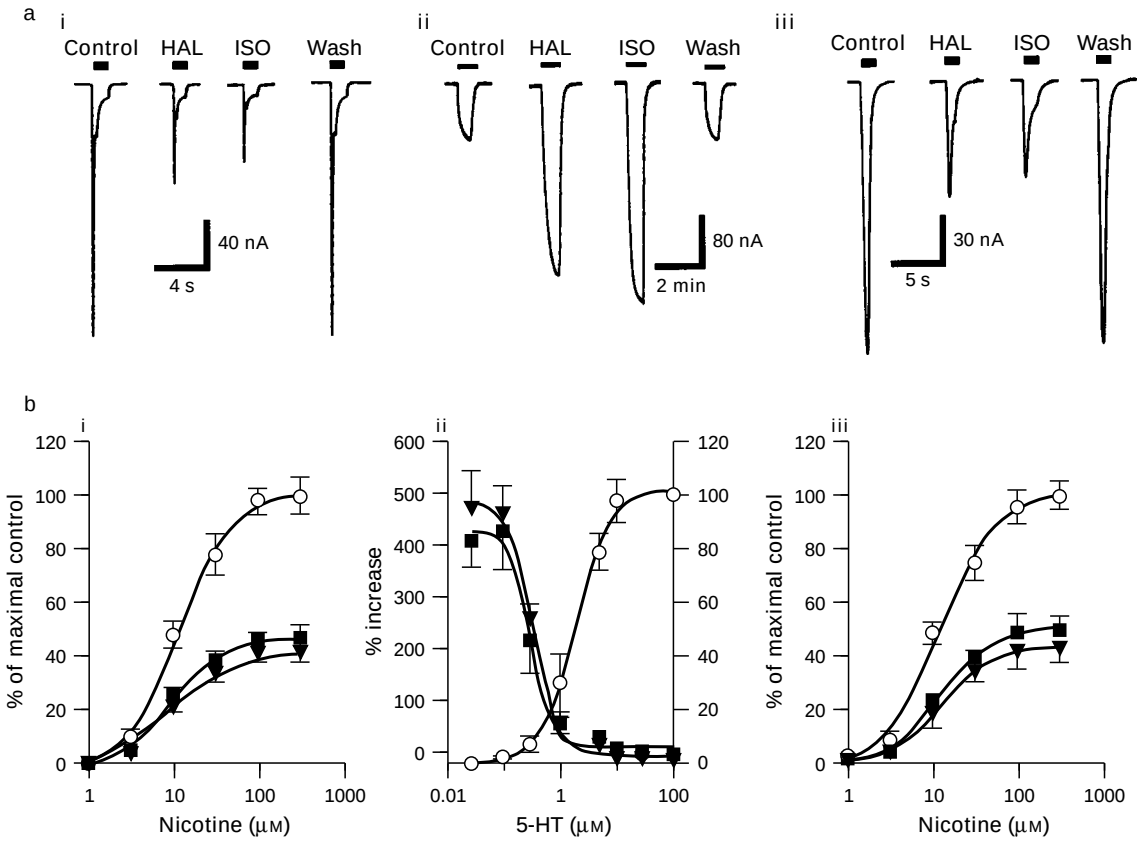

Figure 1 Effect of halothane (HAL) and isoflurane (ISO) on responses mediated by nicotinic acetylcholine type $\alpha 7$ receptors ($nACh_{\alpha 7}R$), 5-hydroxytryptamine type 3 receptors ($5\text{-}HT_3R$), and chimeric $nACh_{\alpha 7}$-$5\text{-}HT_3$ receptors (chimeric receptors). (a) Records illustrating the effect of 5 mM halothane and 5 mM isoflurane on currents mediated by $nACh_{\alpha 7}R$ (i), $5\text{-}HT_3R$ (ii), and chimeric receptors (iii). Bar above each record indicates agonist application. The $nACh_{\alpha 7}$ and chimeric receptor-mediated currents were activated by 10 μM nicotine; the $5\text{-}HT_3$ receptor-mediated currents were activated by 0.1 μM 5-HT. (b) Agonist concentration-response curves (○) for $nACh_{\alpha 7}R$ (i), $5\text{-}HT_3R$ (ii), and chimeric receptors (iii), and the effect of 5 mM halothane (■) and 5 mM isoflurane (▼) on the responses of these receptors. Each data point is the average of 5–7 cells (mean ± s.e.mean); error bars not visible are smaller than the size of the symbols. The control agonist concentration-response curves for the $nACh_{\alpha 7}$ and the chimeric receptor-mediated responses were normalized to the current activated by 500 μM nicotine, and that for $5\text{-}HT_3$ receptor-mediated responses was normalized to the current activated by 100 μM 5-HT.

coefficients for the concentration-response curve of 0.9 ± 0.3 and 0.8 ± 0.2, and EC_{50} values of 1.4 ± 0.4 and 0.8 ± 0.2 mM, respectively. In Figure 1b (iii), both halothane (5 mM) and isoflurane (5 mM) decreased E_{max} of the agonist concentration-response curve (to $51 \pm 4\%$ and $43 \pm 2\%$ of the control response, respectively; ANOVA, $P < 0.001$), without significantly affecting either the EC_{50} or the apparent Hill coefficient of this curve (ANOVA, $P > 0.05$). The E_{max}, EC_{50} and apparent Hill coefficient values for the concentration-response curves of both halothane and isoflurane effects on the chimeric receptor were not significantly different from those values for the $nACh_{\alpha 7}$ receptor (ANOVA, $P > 0.1$).

Discussion We found that both halothane and isoflurane inhibited $nACh_{\alpha 7}$ receptor-mediated responses, and this effect involved a decrease in the E_{max} of the agonist concentration-response curve without affecting the EC_{50} or the apparent Hill coefficient, indicating that the inhibition is non-competitive. The EC_{50} and apparent Hill coefficient values for inhibition by halothane and isoflurane were not altered by substituting Ba^{2+} for Ca^{2+} in the extracellular bathing solution, suggesting that the inhibition is not due to suppression of Ca^{2+}-activated Cl^- current resulting from Ca^{2+} influx through $nACh_{\alpha 7}$ channels. We also found that both halothane and isoflurane potentiated $5\text{-}HT_3$ receptor-mediated responses at low agonist concentrations, confirming previous findings that volatile anaesthetics can potentiate $5\text{-}HT_3$ receptor-mediated responses (Machu & Harris, 1994; Jenkins *et al*., 1996). The observations that both halothane and isoflurane had opposite effects on $nACh_{\alpha 7}$ and $5\text{-}HT_3$ receptors provided the opportunity to use the chimeric $nACh_{\alpha 7}$-$5\text{-}HT_3$ receptor to investigate whether the anaesthetic action involves the N-terminal domain or the transmembrane and C-terminal domains of the receptor. Since both halothane and isoflurane inhibited the chimeric receptor-mediated responses in a manner that was not significantly different from the inhibition of $nACh_{\alpha 7}$ receptor-mediated responses, and the $nACh_{\alpha 7}$ receptor contributed the N-terminal domain to the chimeric receptor, the observations suggest that the inhibition involves the N-terminal domain of the receptor. It would also be of interest to study the effect of the anaesthetics on the reverse chimera, *viz*. with the N-terminal domain from the $5\text{-}HT_3$ receptor and the $nACh_{\alpha 7}$ receptor. However, it has not been possible to obtain a reverse chimera that will functionally express (Eiselé *et al*., 1993; unpublished observations).

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