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Shape similarity indices are the best predictors of substituted fluoroethane and ether anaesthesia

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Summary — Activity relationships were calculated for 18 substituted fluoroethane and ether inhalational anaesthetics using molecular shape similarity indices as the independent variables. The correlation coefficients for the models based on shape similarity ranged from 0.939–0.984, whereas the coefficients based on oil/gas solubilities were 0.005–0.512. The predictive ability of these relationships, determined by cross-validation, was also found to be significantly higher than that of conventional lipid solubility models and of relationships based on Van der Waals (VdW) surface areas. The shape similarity models accurately predict the anaesthetic potencies of the enantiomers of a chiral ether, and activities of transitional compounds which deviate from the traditional Meyer–Overton rule. The results are discussed in terms of the molecular mechanisms of general anaesthetic action.

shape similarity / general anaesthetic / QSAR / mechanism

Introduction

The molecular mechanisms by which inhalational agents induce anaesthesia have not been established. The conventional model of anaesthetic action is based on the Meyer–Overton hypothesis [1, 2], which describes the proportional relationship between the non-polar solubilities of the agents and their anaesthetic activity. This relationship, which is effective for a chemically diverse range of gaseous and volatile compounds [3], implies that anaesthetics act in a hydrophobic phase of membrane lipids and/or proteins.

However, recent investigations have revealed a series of compounds which deviate from the Meyer–Overton hypothesis. These include the transitional agents [4–6], which are significantly less potent than predicted by the model; and the non-anaesthetics [6–8] which, despite their lipid solubility, exhibit no anaesthetic activity. Furthermore, the Meyer–Overton hypothesis is unable to account for potency differences in the stereoisomers of chiral anaesthetics. For example, the enantiomers of the ether isoflurane possess equal lipid solubilities, yet the *S*-isomer is 60% more active in vitro [9] and in vivo [10] than the *R*-isomer (although other chiral anaesthetics may not exhibit such marked potency differences [11]).

Stereospecificity implies that general anaesthetics interact with specific binding sites, possibly located on post-synaptic receptors [12, 13]. If this is the case, then anaesthetic potency would be determined by the long- and short-range intermolecular forces which influence ligand–receptor interactions. For example, the shape of an agent would have to be complementary to that of the binding site or steric repulsion will occur, resulting in a reduced potency and efficacy for the compound. Studies with conformational probes [14, 15] and connectivity indices [16, 17] have implied that shape may be important in determining anaesthetic activity. Furthermore, the calculation of molecular similarity indices [18] has shown that the shapes of some enantiomers of chiral agents are sufficiently dissimilar to account for their potency differences, although correlations with actual anaesthetic activities were not performed.

In this paper we develop the similarity approach, by investigating whether significant activity relationships can be formulated for general anaesthetics using shape similarity indices as the independent variables. The relationships are compared with lipid solubility models for their ability to predict anaesthetic activity, in particular for the transitional and chiral compounds which deviate from the Meyer–Overton rule. In this

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Abbreviations: MAC: minimum alveolar concentration; rCV^2: cross-validated correlation coefficient.

manner, we aim to determine whether shape is indeed an important aspect of the molecular mechanisms of anaesthetic action.

Pharmacology

The general anaesthetics used in this study are listed in table I. The compounds were divided into two groups, substituted fluoroethanes and ethers, with each group being considered separately for all the analyses. The pharmacological activities of the agents are expressed as the minimum alveolar concentration (MAC) required to abolish a movement response to an electrical stimulus applied to the tail of rats [19]. The anaesthetic potency data in table I are either taken from the literature [4–8] or are a personal communication from EI Eger. The MAC index of potency is now widely recognised as a definitive measure of anaesthesia, and has been applied to both clinical and experimental compounds. Each value of the MACs for the selected compounds represents a mean of 3 to 15 experiments. Two additional ether enantiomers (**i** and **ii**) were included in the calculation of the similarity matrices, although their individual potencies have not been conclusively established.

The agents are also classified as either Meyer–Overton anaesthetics or transitional compounds, depending on whether they agree or deviate from the conventional lipid solubility model of anaesthetic activity.

Table I. Activities of the anaesthetics considered in this study.

No	*Compound*	*Observed MAC (atm)*	*Activity class*[a]
Fluoroethanes			
1	CF_3-CF_2Br	2.32	T
2	CF_3-CF_2Cl	7.71	T
3	CF_3-CF_2H	1.25	A
4	CF_3-CFH_2	0.566	A
5	CF_3-CH_3	1.23	A
6	CFH_2-CH_3	0.244	A
7	CF_2H-CH_3	0.265	A
8	CF_2H-CF_2H	0.239	A
9	CF_2Cl-CF_2Cl	1.82	T
10	CF_2Cl-$CFCl_2$	0.0984	T
Ethers			
11[b]	CF_3-CClH-O-CF_2H (*R*-isoflurane)	0.0179	A
12[b]	CF_3-CClH-O-CF_2H (*S*-isoflurane)	0.0117	A
13	CF_3-$CH(CF_3)$-O-CF_2H	0.0375	A
14	CF_3-$CH(CF_3)$-O-CFH_2 (sevoflurane)	0.028	A
15	CF_3-$CH(CF_3)$-O-CH_3	0.046	A
16	CF_2H-CF_2-O-CFH_2	0.08	A
17	CF_3-CCl_2-O-CF_2Cl	0.132	T
18	$CFCl_2$-CF_2-O-CF_2Cl	0.214	T
i[b]	CHFCl-CF_2-O-CF_2Cl (*R*-isomer)	Not available	
ii[b]	CHFCl-CF_2-O-CF_2Cl (*S*-isomer)	Not available	

[a]Activity classes: A: anaesthetics obeying the Meyer–Overton hypothesis; T: 'transitional' compounds as described in the text; [b]chiral compound. MAC: minimum alveolar concentration.

Results and discussion

Activity models based on non-polar solubilities

Conventional activity relationships based on olive oil/gas partition coefficients were calculated to provide benchmarks for the subsequent analyses. Details of the regression equations with the best fits to the anaesthetic data are shown in table II. The first two models, A and B, were formulated using only the fluoroethanes and ethers which obey the Meyer–Overton hypothesis. These models accounted

Table II. Activity relationships based on olive oil/gas partition coefficients.

Models and compounds used	r^2	*n*	rCV^2
Model A: fluoroethanes **3** to **8** tested Log (MAC) = −1.229 × log(oil/gas) + 0.319	0.802	6	0.633
Model B: ethers **11** to **16** tested Log (MAC) = −0.960 × log(oil/gas) + 0.079	0.619	6	−0.345
Model C: all fluoroethanes (**1** to **10**) tested Log (MAC) = −0.820 × log(oil/gas) + 0.382	0.512	10	0.008
Model D: all ethers (**11** to **18**) tested Log (MAC) = + 0.121 × log(oil/gas) − 1.540	0.005	8	−0.722

rCV^2: Cross-validated correlation coefficient using the leave-one-out method.

for 80 and 62% of the variance in the anaesthetic activities of the Meyer–Overton fluoroethanes and ethers respectively. Cross-validation studies using the 'leave-one-out' method [20] indicated that the model for the fluoroethanes had some predictability, with a correlation coefficient (rCV^2) of 0.633; whereas the model for the ethers possessed no predictive power (rCV^2 less than 0).

Figure 1 illustrates how models A and B predict activity for the remaining compounds in table I. It can be seen that the lipid solubility model is unable to distinguish between the enantiomers of isoflurane (compounds **11** and **12**), and that the transitionals agents deviate significantly from the Meyer–Overton anaesthetics. Hence when the transitional compounds are included in the formulation of the regression models, the correlation between predicted and observed anaesthetic potencies decreases substantially. Model C explains only 51% of the variance in the observed activities of all the fluoroethanes, and has no predictability. A significant relationship could not even be formulated for the ether group, with model D accounting for less than 1% of the variance in observed activities of compounds **11** to **18**.

Activity models based on shape similarity indices

Single conformers representing the minimum energy structure of each anaesthetic were used for the calculation of the similarity indices. One anaesthetic was nominated as a lead compound, to which the other agents of the group were pre-aligned in a weighted fit based on atom size and charge. The agents were then translated and rotated to maximize their similarity in shape with the lead compound, calculated as Carbo indices by analytical integration [21]. The process was repeated until all of the anaesthetics had a turn at being the lead molecule. The similarity data was arranged as two N by N matrices [22, 23], which were correlated to observed potencies using the partial least squares (PLS) technique [24]. This reduced the data to equations which represent anaesthetic activity as a series of components, each of which consisted of weighted variables describing the shape similarity of a single anaesthetic to the other compounds in the group.

Table III summarizes the equations produced by the PLS analyses. Model E was a two-component equation explaining 94% of the variance in the observed activities of all the fluoroethanes, with substantial predictive power (rCV^2 = 0.899). The model for the ethers (F) consisted of a single component, explaining 76% of the variance with some predictability (rCV^2 = 0.605). Both of these models were formulated using all the available shape similarity variables, the weightings of which are shown in figure 2. The variables with the highest values indicate the compounds whose shape contributes the most to the activity models.

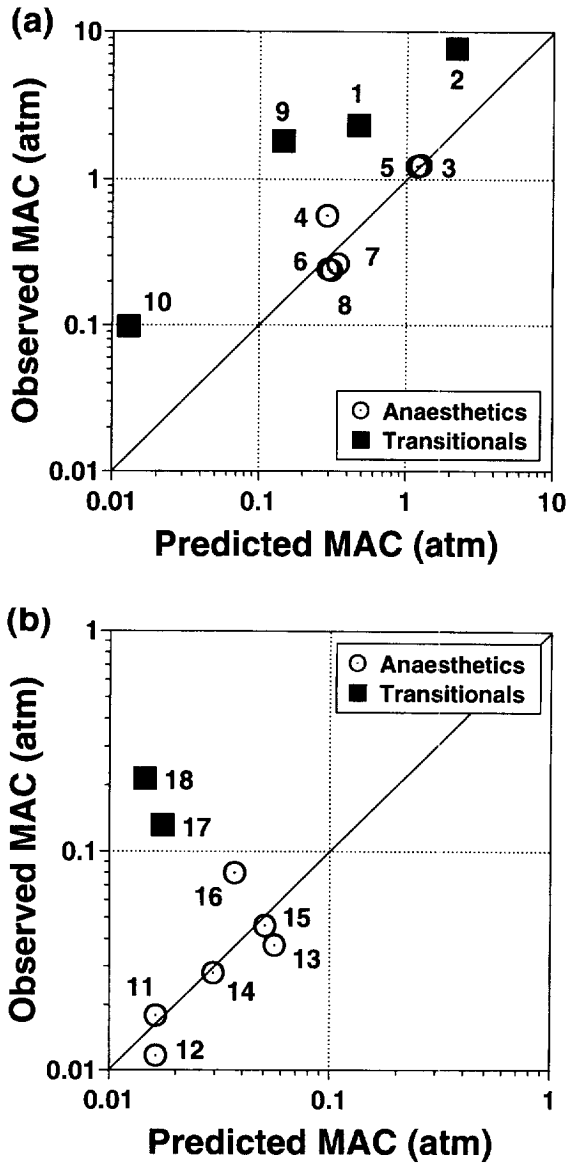

Fig 1. Correlation between observed MACs and values predicted using models A and B based on oil/gas partition coefficients for the fluoroethanes (**a**) and ethers (**b**). The regression models represent the best-fit for the Meyer–Overton anaesthetics (circles). Note that the transitional compounds (squares) deviate significantly from this relationship. The numbers refer to the agents listed in table I.

The minimum number of shape similarity variables required to adequately represent anaesthetic activity

Table III. Activity relationships based on shape similarity indices.

Models and compounds used	r^2	*n*	rCV^2
Model E: all fluoroethanes (**1** to **10**) tested Two-component model, using all the fluoroethane shape similarity variables	0.939	10	0.899
Model F: all ethers (**11** to **18**) tested One-component model, using all the ether shape similarity variables	0.763	8	0.605
Model G: all fluoroethanes (**1** to **10**) tested Two-component model, using shape similarity variables for fluoroethanes **1**, **2**, **3**, **5**, **8**, **9** and **10**	0.939	10	0.912
Model H: all ethers (**11** to **18**) tested Two-component model, using shape similarity variables for ethers **12** and **ii**	0.984	8	0.978

For abbreviations, see table II.

was determined by repeatedly reformulating the equations, whilst removing the variable with the lowest weighting at each stage. The model which possessed the highest rCV^2 value was retained. This 'noise reduction' procedure resulted in models G and H for the fluoroethanes and ethers respectively. Model G was based on the shape similarities of seven out of the ten fluoroethanes. Although it accounted for the same amount of variance in observed activities as model E, it did possess a higher predictability, with an rCV^2 of 0.912. The greatest improvement was shown for the ethers. Model H was based on the similarities for just two of the agents, *S*-isoflurane (CF_3-CClH-O-CF_2H) and ether **ii** (CHFCl-CF_2-O-CF_2Cl *S*-isomer). This model explained 98% of the variance in the activities of all the ethers considered, with a high level of predictability ($rCV^2 = 0.978$).

The ability of models G and H to predict anaesthetic activities is illustrated in figure 3. It can be seen that the models based on shape similarity correctly predict the potencies for the enantiomers of isoflurane and the activities of the transitional compounds – two aspects where the conventional non-polar solubility model fails.

Activity models based on molecular size

The possibility that the strong correlation described with the shape similarity indices may be a reflection of the size of the molecule rather than shape per se was tested by formulating activity models based on Van der Waals (VdW) surface areas. These models, described in table IV and illustrated in figure 4, show a low correlation between observed and predicted MACs, accounting for only 8 and 32% of the variance in the anaesthetic activities of the fluoroethanes and ethers respectively. This demonstrates that it is the

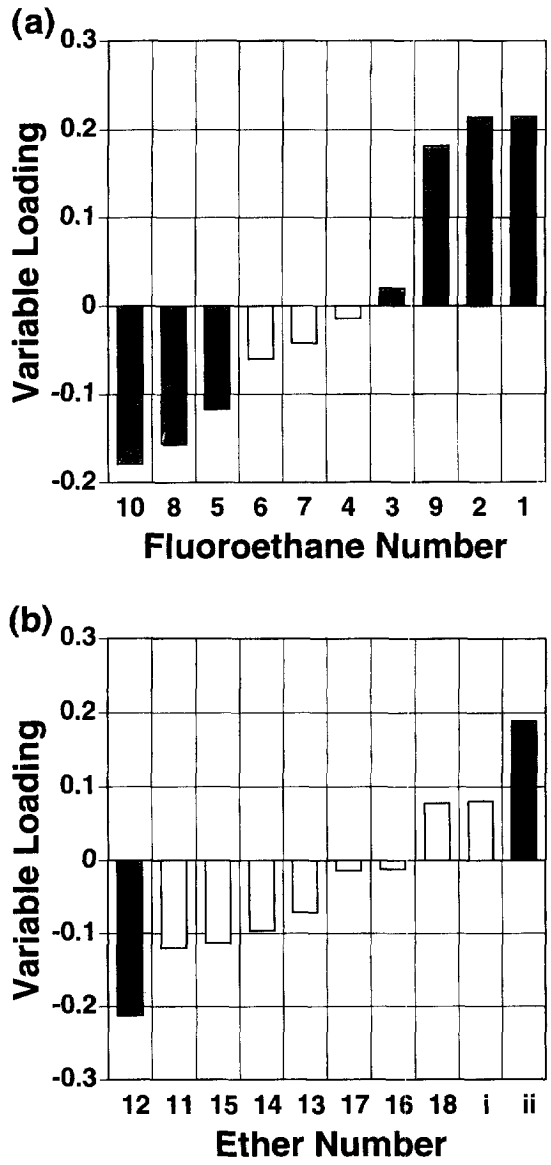

Fig 2. Shape similarity variable weightings for PLS models E and F. The filled columns indicate variables which were retained for models G and H.

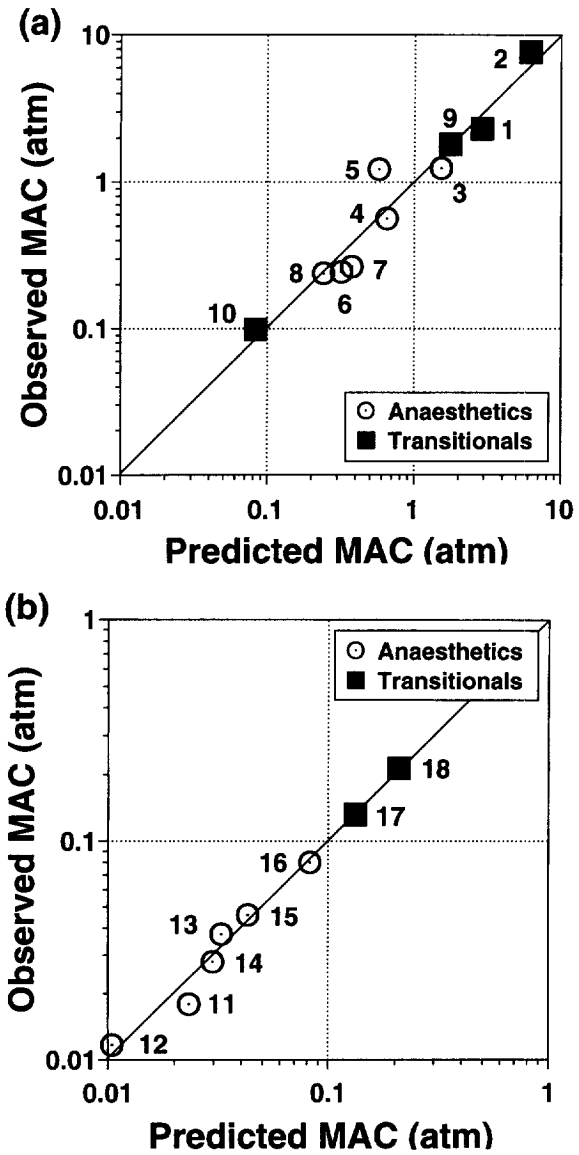

Fig 3. Correlation between observed anaesthetic activities and values predicted using shape similarity models G and H for the fluoroethanes (**a**) and ethers (**b**). The improved correlation compared with the oil/gas partition models is evident.

true three-dimensional shape and not the size of the molecules which determines their anaesthetic potency.

Molecular mechanism of action

The strong predictive power of the shape similarity models, especially for the compounds which deviate from the Meyer–Overton hypothesis, indicates that molecular shape is a major determinant of anaesthetic potency in the fluoroethanes and ethers studied. This finding is unexpected and exciting because although it has been implied by the previous reports mentioned in the *Introduction* [14–18], the effects of shape variations on anaesthetic potencies have not been so clearly defined as in this study. The importance of shape similarity is consistent with a molecular mechanism of action in which anaesthetics interact with specific protein binding sites. Any deviation from the ideal shape required to fit into these putative sites will result in a reduced potency for the agent. Further studies are required to fully characterise the nature and dimensions of this ideal shape.

In addition to molecular shape, there is also evidence that electrostatic interactions are a factor in determining the potencies of these compounds. The success of the activity relationships was found to be dependent on the initial orientation of the structures prior to the optimization of the shape similarity indices. The most effective protocol was one in which the molecules were pre-aligned by a weighted fit based on atom shape and charge, with the weighting ratios being 1:10. This emphasis on charge is consistent with the way in which a flexible molecule would align itself to interact with a binding site, since electrostatic forces act over a longer range compared with steric forces.

Conclusion

The use of shape similarity indices as independent variables enabled the formulation of significant anaesthetic activity relationships which did not require conventional oil/gas partition coefficient terms. However, this does not imply that non-polar solubility is of little importance in determining anaesthetic acti-

Table IV. Activity relationships based on VdW surface areas.

Models and compounds used	r^2	*n*	rCV^2
Model I: all fluoroethanes (**1** to **10**) tested Log(MAC) = + 0.009 × VdW surface area −1.030	0.075	10	−0.695
Model J: all ethers (**11** to **18**) tested Log(MAC) = + 0.016 × VdW surface area −3.781	0.324	8	−0.725

VdW: Van der Waals.

vity. After all, molecular shape itself is a contributing factor to the 'hydrophobic effect' [25]. The analysis of the fluoroethanes and ethers as separate homologous groups meant that other factors such as partitioning could be ignored. The success of the Meyer–Overton relationship in describing anaesthetic activities of chemically diverse agents suggests that an activity model formulated for heterologous compounds would need to include hydrophobicity in addition to electrostatic and steric terms. The similarity index approach can be applied equally to these three components, and we are currently undertaking studies of this type to resolve the question of whether chemically diverse general anaesthetics posses common or multiple sites of action.

Experimental protocols

Models of the anaesthetics were constructed using Chem-X [26]. Each structure was subjected to a rule-based conformational search, and the lowest energy conformer retained for geometry optimization using the AM1 Hamiltonian in MOPAC 6 [27]. Atomic partial charges were assigned by least-squares fitting to the molecular electrostatic potential [28].

The Carbo shape similarity indices were calculated by analytical integration with three Gaussian functions using the ASP software[1]. Compounds were pre-aligned to the lead structure and subjected to rigid optimization using the SIMPLEX algorithm. The resultant N by N matrix was analyzed using the PLS module in TSAR[2]. The number of significant components was determined by cross-validation, using the leave-one-out method with a fixed deletion pattern. A component was considered to be significant if the ratio of PRESS to the residual sum of squares was less than 1.0.

Regression models based on olive oil/gas partition coefficients and molecular surfaces areas were calculated using TSAR.

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[1]ASP v3.11b, Oxford Molecular Ltd, The Magdalen Centre, Oxford Science Park, Sandford-on-Thames, Oxford OX4 4GA, UK.

[2]TSAR v2.41a, Oxford Molecular Ltd, The Magdalen Centre, Oxford Science Park, Sandford-on-Thames, Oxford OX4 4GA, UK

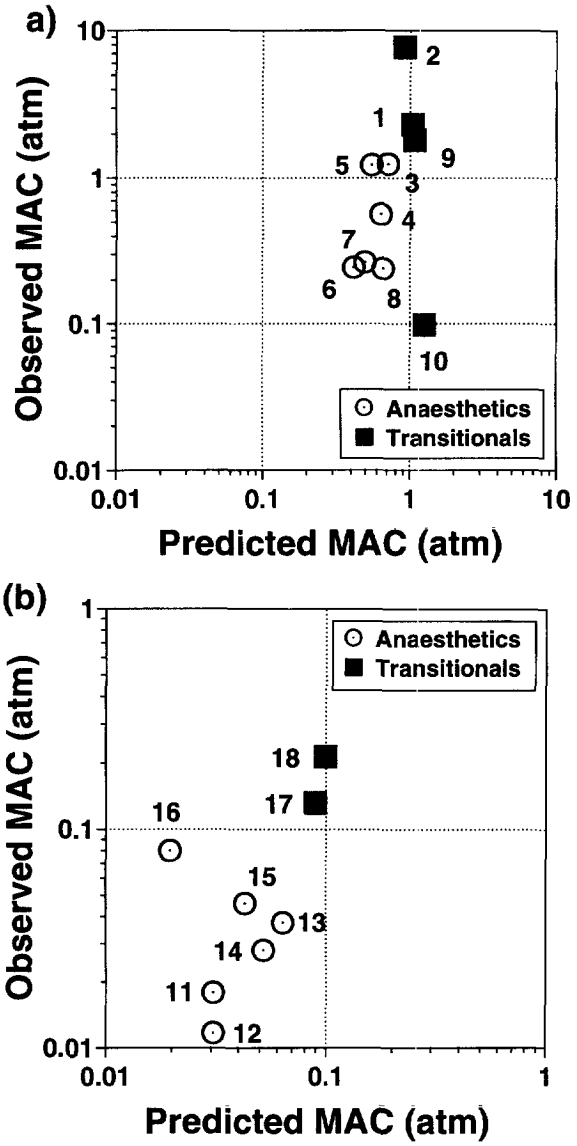

Fig 4. Activities predicted from the surface area models I and J for the fluoroethanes (**a**) and ethers (**b**). The low correlation between observed and predicted MACs demonstrates that molecular size is not a determinant of potency for these structures.

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