Derivation of preliminary three-dimensional pharmacophoric maps for chemically diverse intravenous general anaesthetics

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Background. The molecular basis of i.v. general anaesthetic activity was investigated using comparative molecular field analysis (CoMFA).

Methods. The free plasma concentrations that abolish movement to a noxious stimulus for 14 structurally diverse i.v. anaesthetics were obtained from the literature. The compounds were randomly divided into a training set \((n=10)\) to derive the activity model, and a separate test set \((n=4)\) used to assess its predictive capability. The anaesthetic structures were aligned so as to maximize their similarities in molecular shape and electrostatic potential to conformers of the most active agent in the group, eltanolone. The conformers and alignments that showed the maximum similarity (calculated using combined Carbo indices) were retained, and used to derive the CoMFA models.

Results. The final model explained 94.0% of the variance in the observed activities of the training set \((n=10, P<0.0001)\) and was a good predictor of test set activity \((n=4, r^2=0.799)\). In contrast, a model based on non-polar solubility (LogP) explained only 78.3% of the variance in the observed activities of the training set \((n=10, P=0.0007)\) and was a poor predictor for the test set \((n=4, r^2=0.272)\). Further analysis of the CoMFA results identified the spatial distribution of key areas where steric and electrostatic interactions are important in determining the activity of the 14 anaesthetics considered.

Conclusions. A single activity model can be formulated for i.v. general anaesthetics and preliminary pharmacophoric maps derived, which describe the molecular basis of their in vivo potency.

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We have demonstrated previously the importance of molecular shape and electrostatic potential (a measure of the charge distribution around a structure) in determining the activity of chemically diverse i.v.\(^1\) and inhalation general anaesthetics.\(^2\)–\(^4\) Whereas our previous activity models have considered only global similarities in molecular shape and partial charge, we now investigate the relationship between the spatial distribution of these molecular properties and the in vivo potencies of the anaesthetics. For this purpose, we have used the computer-aided drug design technique of Comparative Molecular Field Analysis (CoMFA).\(^5\)–\(^6\)

In CoMFA, the molecular structures are aligned and placed in a grid consisting of regularly spaced lattice points. The steric and electrostatic interaction energies between the molecular structures and a charged probe atom are calculated at each point, and correlated with potency to formulate an activity model. By identifying which lattice points make the greatest contribution to the model, three-dimensional pharmacophoric ‘maps’ describing the location of areas where steric and electrostatic interactions are important in determining activity can be formulated.

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The aim of the present study was to derive such pharmacophoric maps for chemically diverse i.v. general anaesthetics, and to identify the spatial distribution of the key steric and electrostatic features of the anaesthetic molecules that determine their in vivo potency (assessed by lack of response to the noxious stimulus of the initial surgical incision).

**Methods**

**Compounds studied**

A structurally diverse group of 14 i.v. general anaesthetic agents was considered, consisting of steroid anaesthetics (Althesin as alphaxalone, minaxolone, eltanolone (5β-pregnanolone), ORG 21465), barbiturates (pentobarbital, thiopental, thiamylal, methohexital) and miscellaneous agents (R(−) and S(+) ketamine, propofol, R(+)-etomidate, chlormethiazole, ORG 25435). ORG 21465 ((2β, 3α, 5α)-3-hydroxy-2-(2,2-dimethylmorpholin-4-yl) pregnane-11,20-dione) is a water-soluble steroid anaesthetic, which potentiates GABA\(_A\) receptor chloride currents in vitro. Recent studies have shown the α-amino acid phenolic ester derivative ORG 25435 ((R)-2-(N-Bis(2-methoxyethyl)amino) butyric acid, 2',6'-dimethoxy-4'-methylphenyl ester hydrochloride) to be an i.v. anaesthetic in man, with rapid onset of action and recovery from anaesthesia.\(^9\) \(^10\) In vitro data confirm its modulatory action on GABA\(_A\) receptors.\(^11\)

Data for protein binding and EC\(_{50}\) plasma drug concentrations that abolish movement to a noxious stimulus were obtained from the literature.\(^7\) \(^8\) \(^12\) \(^13\) \(^14\) Where possible, the potency data were taken from studies in which no other adjuvant drugs were administered up to the time of the stimulus (the initial surgical incision). In cases where nitrous oxide 67% was given as part of the anaesthetic, this was assumed to equate to 0.6 MAC.

The anaesthetics were divided into a training set of 10 agents, which were used to formulate the CoMFA activity model; and a test set of four anaesthetics, which were used to independently evaluate the model’s predictive capability. The selection process was random, with the following exceptions. First, eltanolone, the most active agent in the group, was retained in the training set because this compound was used as an alignment template for the other anaesthetic molecules. Secondly, the larger steroids, minaxolone and ORG 21465, were also included in the training set as their size determined the dimensions of the lattice grid used in the CoMFA analyses. To select the test set agents, the anaesthetics were ranked in order of increasing potency and divided into four activity bins. One anaesthetic was randomly chosen from each bin and added to the test set. This process ensured that the test set compounds represented the full range of potencies exhibited by the 14 i.v. anaesthetics.

**Molecular model construction**

A Silicon Graphics O2 R10000 workstation was used for the construction of computer-based representations of the anaesthetic structures, the calculation of the anaesthetics’ three-dimensional physico-chemical properties, and the derivation of the CoMFA activity model and pharmacophoric maps.

Full details of the molecular modelling procedures used to derive representative structures of the anaesthetics were outlined in our previous publication.\(^1\) In brief, starting structures were constructed for each compound using the molecular modelling software SYBYL v6.7 (Tripos Inc., St Louis, MO, USA). All of the anaesthetics considered exhibit some degree of molecular flexibility, and so exist as a series of dynamically interchanging molecular configurations or conformers. This flexibility was incorporated into the model by deriving a set of low energy conformers for each anaesthetic, identified by a random search procedure in SYBYL. In this process, the torsion angles of the molecules were randomly perturbed, and the resulting structures geometry optimized using molecular mechanics minimization (in which the atoms of the molecules are represented as spheres, and the bonds as springs). Only the optimized conformers with a potential energy within +4 kcal mol\(^{-1}\) of the lowest energy conformer of a given anaesthetic were retained. Since the probability of a specific conformer occurring is related to the potential energy of the structure, this limit ensures that only realistic configurations of the anaesthetics were considered. The process was repeated until each anaesthetic had been subject to 10 000 random structure perturbations or until each of the low energy conformers had been found at least 12 times.

The geometry of the conformers was further refined using semi-empirical quantum mechanics in vacuo and the MOPAC 6 software package (Quantum Chemistry Program Exchange, IA, USA). The AM1 Hamiltonian was used and atomic partial charges assigned using the Coulson method. This process optimizes the molecules at the valence electron level. After geometry optimization, duplicate conformers (defined as conformers with an RMS difference of <0.2 Å) were removed. The final training set consisted of 1176 unique conformers for the 14 anaesthetics.

**Structure alignment**

The alignment of the molecular structures is a critical stage in CoMFA analyses. However, the chemical diversity of the anaesthetics precludes their alignment by a common substructure. After extensive testing, suitable alignments were generated using an unbiased molecular similarity approach,\(^15\) based on the local minimum method.\(^16\) \(^17\) This procedure aligns the structures so as to maximize their similarity in shape and electrostatic potential (which describes the distribution of charge around the structure) with the most active agent in the group, the ‘lead
CoMFA analysis was performed for each of the four conformers of the lead compound. Molecular similarity was quantified by calculating combined shape and electrostatic potential Carbo indices (see 1), which range in value from 0 (totally dissimilar molecular shapes and electrostatic potentials) to 1 (totally identical). The Carbo indices were calculated using an analytical method with the ASP v.3.22 software (Automated Similarity Package, Accelrys Inc., Cambridge, UK). The anaesthetics were initially aligned to the lead compound by weighted molecular extent and atomic partial charge (using the default weighting ratio of 1:10), before being translated and rotated in a rigid search (30° increment) with SIMPLEX optimization to maximize their molecular similarity. The conformers and alignments of the anaesthetics with the maximum similarity to each of the eltanolone conformers were retained for the CoMFA modelling, a separate set of alignments being obtained for the four conformers of the lead compound.

**CoMFA formulation**

The spatial distribution of the steric and electrostatic features that determine biological activity was identified using CoMFA, calculated with SYBYL. In this procedure, the aligned molecules were placed in a rectangular grid consisting of lattice points at 1 Å intervals. The grid extended at least 4 Å beyond the surface of all the molecules, and consisted of a total of 7344 lattice points. A probe atom (an sp³ carbon of unitary positive charge) was placed at each of the points, and the steric and electrostatic interaction energies calculated between this probe and the anaesthetic molecules. These energies mimic the steric and electrostatic interactions that occur between the anaesthetics and their site(s) of action (see 18). The steric interaction energies were determined using Lennard-Jones potentials, which describe both the attraction between molecules because of van der Waals forces (dispersion interactions, dipole-induced dipole and dipole–dipole interactions) and the repulsion as a result of steric clashes. The electrostatic interaction energies reflect the sum of the Coulombic interactions. A distance-dependent dielectric constant was used, and standard cut-offs were applied to both the steric and electrostatic energies at 30 kcal mol⁻¹. A separate CoMFA analysis was performed for each of the four alignment sets.

**Activity model formulation**

The values of the steric and electrostatic interaction energies at each lattice point were block scaled to unit variance, and used as independent variables in the formulation of an activity model. Because of the high number of independent variables produced (7344 steric and 7344 electrostatic variables) and their co-linearity, partial least squares (PLS) regression was used for this purpose. In this process, orthogonal latent variables are formed from a weighted combination of the steric and electrostatic energies at each lattice point. The latent variables are subsequently correlated with in vivo potency to derive the activity model. The magnitude of the individual weighting coefficients within the latent variables indicates the relative importance of the steric or electrostatic energy at each lattice point in determining anaesthetic activity. Thus, specific regions in three-dimensional space where steric and electrostatic interactions are important can be identified, and plotted to derive pharmacophoric maps. As only 10 compounds were present in the training set, a single latent variable was calculated for each CoMFA model (this conservative approach ensures against potential over-modelling of the data).

**Model validation**

The intrinsic predictive power of the CoMFA models was determined using leave-one-out cross-validation. In this process, the activity models were repeatedly re-formulated, but with one compound left out from the training set at each stage. The model obtained was used to predict the activity of the excluded compound, and the process repeated until each member of the training set had been excluded once, and once only. The CoMFA model with the greatest cross-validated $r^2$ was retained as the final model. The extrinsic predictive power of this final model was assessed by predicting the anaesthetic potencies of the randomly excluded test set agents.

The possibility of obtaining a chance correlation was tested by randomly re-assigning the observed potency data to different anaesthetics in the training set, and repeating the CoMFA analysis. A total of 100 cycles of random perturbation were used, and the intrinsic and extrinsic predictive powers of the distorted data sets evaluated. For benchmark purposes, a conventional activity model based on non-polar solubility was also formulated using published octanol/water partition coefficients (LogP).

**Results**

**I.V. general anaesthetic potencies**

The anaesthetic potencies expressed as the plasma drug concentrations associated with no response to a noxious stimulus or surgical incision in 50% of patients (EC₅₀), the average plasma protein binding and the calculated equivalent free drug concentrations are shown in Table 1. Note that the EC₅₀ value for alphaxalone differs from that presented in our earlier publication. Further data on the plasma protein binding for alphaxalone are now available with a revised value of 96.8% rather than the 37.7% published by Child and colleagues. These new data indicate a greater anaesthetic potency for alphaxalone than thought previously, with a revised $–\log$(EC₅₀) of 6.335 compared with the earlier value of 5.062.
Table 1 Typical values for the in vivo potencies of the intravenous general anaesthetics considered in this study. *Chiral anaesthetic, potency evaluated using racemate; † values differ from our earlier models, following update of protein binding data

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>MW</th>
<th>Octanol/water partition coefficient</th>
<th>Total plasma concentration (μg ml⁻¹)</th>
<th>Fraction protein bound (%)</th>
<th>Free plasma concentration (μM)</th>
<th>Refs.</th>
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<tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1. Eltanolone</td>
<td>318.5</td>
<td>3.97</td>
<td>3</td>
<td>99</td>
<td>0.094</td>
<td>1</td>
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<td>2. Minaxolone</td>
<td>405.6</td>
<td>3.7</td>
<td>1.6</td>
<td>95</td>
<td>0.197</td>
<td>1</td>
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<td>3. ORG 21465</td>
<td>445.7</td>
<td>3.7</td>
<td>3</td>
<td>97‡</td>
<td>0.202</td>
<td>7 8</td>
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<tr>
<td>4. Methohexital*</td>
<td>262.3</td>
<td>2.4</td>
<td>10</td>
<td>75</td>
<td>9.53</td>
<td>1</td>
</tr>
<tr>
<td>5. Thiouyl*</td>
<td>254.4</td>
<td>3.23</td>
<td>26</td>
<td>85</td>
<td>15.33</td>
<td>1</td>
</tr>
<tr>
<td>6. Thiopental*</td>
<td>242.3</td>
<td>2.59</td>
<td>40</td>
<td>85</td>
<td>24.76</td>
<td>1</td>
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<tr>
<td>7. R(+) Etoimdate</td>
<td>224.3</td>
<td>3.05</td>
<td>1</td>
<td>75</td>
<td>1.02</td>
<td>1</td>
</tr>
<tr>
<td>8. ORG 25435</td>
<td>369.5</td>
<td>2.98</td>
<td>8.75</td>
<td>84</td>
<td>3.79</td>
<td>9 10</td>
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<td>9. R(−) ketamine</td>
<td>237.7</td>
<td>2.18</td>
<td>4.2</td>
<td>27</td>
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<tr>
<td>10. Chlorimethazloe</td>
<td>161.6</td>
<td>2.12</td>
<td>13.7</td>
<td>65</td>
<td>29.75</td>
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<td>11. Alphaxalone</td>
<td>332.5</td>
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<td>4.8</td>
<td>96.8†</td>
<td>0.46†</td>
<td>1 12 13</td>
</tr>
<tr>
<td>12. Pentobarbital*</td>
<td>226.3</td>
<td>2.07</td>
<td>28</td>
<td>61</td>
<td>48.26</td>
<td>1</td>
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<tr>
<td>13. Propofol</td>
<td>178.3</td>
<td>3.79</td>
<td>14</td>
<td>98</td>
<td>1.57</td>
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<tr>
<td>14. S(+) Ketamine</td>
<td>237.7</td>
<td>2.18</td>
<td>1.4</td>
<td>27</td>
<td>4.30</td>
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</table>

CoMFA activity model

The training set consisted of 10 anaesthetics: three steroid agents (eltanolone, minaxolone, ORG 21465), three barbiturates (methohexital, thiouyl, thiopental), and four other chemical entities (R(+) etomidate, ORG 25435, R(−) ketamine, chlorimethazloe). By orienting the anaesthetic structures to maximize their molecular similarity to the conformers of eltanolone (the most potent agent in the group), suitable alignments were obtained for effective CoMFA analysis. The final CoMFA activity model was based on the alignments of the anaesthetics to the eltanolone conformer shown in Figure 1A. Also shown are the maximum similarity alignments for thiopental (Fig. 1B) and R(−) ketamine (Fig. 1C). It can be seen that these anaesthetics have been aligned to maximize their similarity in shape and electrostatic potential (which is colour-coded onto the surface of the molecules) to the lead compound. Files containing the atomic co-ordinates of the aligned anaesthetics used in the CoMFA modelling are available from the authors on request.

The aligned anaesthetics were placed in a lattice grid, and the steric and electrostatic interaction energies between the anaesthetics and a probe atom calculated at each lattice point. The magnitudes of these energies were correlated with in vivo potency using PLS regression to formulate an activity model. The final activity model, which was based on a single latent variable, explained 94.0% of the variance in the observed activities of the training set compounds ($F_{1,8}=125.843, n=10, P<0.0001$). A plot of the observed and predicted activities for the training set agents (circles, Fig. 2) suggests that the model is effective across the full range of i.v. anaesthetic potencies.

Model validation and predictive capability

Cross-validation experiments demonstrate that the CoMFA activity model has good intrinsic predictive power, with a cross-validated $r^2$ of 0.768. However, a more rigorous test is to see how well the model predicts the potencies of ‘unknown’ compounds. This was assessed by using the CoMFA model to predict the potencies of four test set agents, randomly excluded from the model’s formulation. Overall, the activity model was found to be an adequate predictor of anaesthetic potency for the test set, explaining 79.9% of the variance in the observed activities. Comparison of the predicted and observed activities for the test set (squares, Fig. 2) indicates that very accurate predictions were obtained for alphaxalone (compound 11) and S(+) ketamine (compound 14). In the latter case, note that the model also predicted the greater potency of the S(+) isomer (compound 9) of the training set.

The possibility of a chance correlation being obtained was assessed by randomly perturbing the potency data and repeating the CoMFA analyses. Activity models for these distorted data sets were found to have no intrinsic predictive power, resulting in a negative mean (SEM) cross-validated $r^2$ of −0.468 (0.041) ($n=100$). The perturbed models were also poor predictors of the activities of the test set agents, with a mean correlation coefficient ($r$) of −0.102 (0.072) ($n=100$).

Octanol/water partition coefficient model

The performance of a conventional physicochemical activity model based on octanol/water partition coefficients (LogP) is shown in Figure 3. The LogP activity model explains only 78.3% of the variance in the observed activities of the training set agents ($F_{1,8}=28.914, n=10, P=0.0007$), and has lower intrinsic (cross-validated $r^2$ of 0.701) and extrinsic (test set $r^2$ of 0.272) predictive power compared to the CoMFA model. Furthermore, this activity model is unable to predict the different in vivo potencies for the ketamine enantiomers (compounds 9 and 14).
The relative contributions of the electrostatic and steric fields to the overall activity model were approximately equal, at 50.7 and 49.3%, respectively. Preliminary pharmacophoric maps, which describe the spatial distribution of the key areas where electrostatic and steric interactions are important in determining in vivo potency, were derived using isocontour plots. These plots were obtained by linking together lattice points in the CoMFA grid where the standard deviation of the interaction energies multiplied by the PLS weighting coefficients at that point (stddev×coeff) exceed a certain value. Hence the plots highlight areas where the differences in either the steric or electrostatic interaction energies are strongly associated with differences in anaesthetic potency.

Frequently, two isocontours are used in CoMFA pharmacophoric maps. One links together the lattice points with high negative stddev×coeff values (indicating areas
where either negative electrostatic potential or the absence of molecular bulk is favoured for high potency), and the other connects lattice points with high positive values (indicating areas where either positive electrostatic potential or the presence of molecular bulk are favoured). By default, the thresholds for these isocontours are set by the SYBYL software to values that represent a contribution of 20 and 80%, respectively, of the absolute integral of the stdev^3 coeff values for all the grid points. However, such default isocontours based on the absolute integral assume a symmetrical distribution for the number of negative and positive stdev^3 coeff values over the whole CoMFA grid. Whilst this was found to be the case for the electrostatic interaction energies of the anaesthetics, this was not valid for the steric energies (presumably because of the alignment method used). However, such default isocontours based on the absolute integral assume a symmetrical distribution for the number of negative and positive stdev^3 coeff values over the whole CoMFA grid. Whilst this was found to be the case for the electrostatic interaction energies of the anaesthetics, this was not valid for the steric energies (presumably because of the alignment method used). The steric isocontour thresholds were therefore modified to reflect this uneven distribution, and were set to values that represent approximately two-fifths of the individual contributions of the negative and positive terms (equivalent to an overall contribution of 3.9 and 62.7% of the absolute integral of the values for all the grid points). To avoid possible confusion, we have subsequently specified the thresholds for the isocontours in terms of absolute stdev^3 coeff values for both the electrostatic and steric maps.

The electrostatic pharmacophoric map is shown in Figure 4A. Areas where negative potential are favoured for high anaesthetic potency are shown in red (stdev^3×coeff values less than ±0.002) and areas where positive potential is favoured in blue (stdev^3×coeff values greater than +0.001). It can be seen that there are three main zones (A, B, and C) where positive electrostatic potential is favoured and two areas (D and E) where negative potential is favoured. The positioning of these areas in relation to the lead compound eltanolone is shown in Figure 4B. The arrows provide a qualitative indication of where the electrostatic potential of eltanolone (cf. Fig. 1) fits the pharmacophoric map. Hence atoms with a positive potential (e.g. hydrogens) coincide with the blue areas (A, B, and C), and atoms with a negative potential (such as the oxygen atom) coincide with one (E) of the two main red areas. For comparison, Figure 4C shows the equivalent match for thiopental, a compound which is less potent than eltanolone. It is evident that there are fewer regions where the electrostatic potentials match.

The equivalent pharmacophoric map for the steric interactions is shown in Figure 5A. Areas where molecular bulk is favoured are shown in green (stdev^3×coeff values greater than +0.0018), and areas where bulk is disfavoured (i.e. if the molecular bulk extends into this zone, anaesthetic potency will be reduced) are shown in magenta (stdev^3×coeff values less than −0.0008). The orientation of the steric map is identical to the electrostatic equivalent in Figure 4A. There is a ‘C-shaped’ zone consisting of three centres (F, G,
and H) where molecular bulk is favoured, and two areas (I and J) where molecular bulk is disfavoured. The positioning of the disfavoured regions in relation to eltanolone is shown more clearly in Figure 5B, which has been rotated 85° about the x-axis. It can be seen that the two areas where molecular bulk is disfavoured (I and J) are located above and below the plane of the steroid ring of eltanolone.

**Discussion**

We have confirmed that non-polar solubility (expressed as LogP) is a poor predictor of anaesthetic potency (the EC50 associated with no response in 50% of patients to a noxious stimulus) for this diverse group of i.v. anaesthetic agents. This is in agreement with the results obtained by Krasowski and colleagues23 for a series of structurally homologous propofol analogues. We have therefore sought other physicochemical properties to explain the molecular basis of i.v. general anaesthetic activity. Our previous model for i.v. agents demonstrated that in vivo anaesthetic potency was related to global molecular shape and electrostatic...
logous ligands. Furthermore, it is unlikely that the good models, derived using structurally homologous or heterologous ligands, are consistent with data from other CoMFA training set agents within ±0.4. This error range is consistent with data from other CoMFA models, derived using structurally homologous or heterologous ligands. Moreover, it is unlikely that the good predictive power of the CoMFA model is a result of a chance correlation, as models derived after random perturbation of the activity data exhibit limited predictive capability.

Secondly, how valid are the molecular alignments used to derive the model? The conventional approach used for CoMFA is to align the compounds by a common substructure. This was not possible for the anaesthetics considered. Alignments were therefore obtained using an unbiased molecular similarity approach. This procedure has been shown to be successful for other structurally diverse series. During our extensive testing of alignment procedures, we found that different alignment protocols using molecular similarity produced consistent alignments for the steroids, barbiturates and ketamine stereoisomers. However, the positioning of propofol, R(+) etomidate and clormethiazole was more variable, depending on the technique used. The alignments used to formulate the final model presented here represent the orientations that were frequent and repeatable.

It may be possible to improve the alignments further by incorporating more molecular flexibility. In this study, we have used a rigid-alignment approach. A range of low energy conformers were identified for each anaesthetic and the conformers aligned to the lead compound without any further structural changes. Each conformer was given an equal weighting in the fitting process, and the conformers were not biased according to their probability of occurrence (although the use of an energy cut-off when selecting the conformers ensured that only feasible configurations were considered). It is possible to use an alternative flexible fit, in which the geometry of the molecule is also modified to maximize similarity to the lead compound. Further experiments are required in this area.

Two compounds were poorly predicted by the activity model: eltanolone (the lead structure) and propofol. It is possible that the poor prediction for propofol reflects a less than favourable molecular alignment, or the need to explicitly model the effects of hydrogen bonding as described by Krasowski and colleagues. It is not clear why eltanolone is weakly predicted by the model. This may be because of the fact that it was the only 5β-steroid considered, and the inclusion of related compounds (if such potency data become available) would improve the predictive power of the model. It should be noted that alphaxalone is no longer an outlier in our model, following revision of the potency data. Our earlier estimations were based on the apparently low protein plasma binding of 37.7% reported by Child and colleagues. The likely explanation for this low binding may lie with the methodology used in that study; namely the binding of radio labelled compound in contrast to the selective measurement of alphaxalone by HPLC in the recent study of Visser and colleagues. One explanation for the difference in alpha-xalone by HPLC in the recent study of Visser and colleagues has found no dose, concentration, or time dependence on the plasma protein binding of alphaxalone. These revised data are also in keeping with estimates for total progesterone binding to albumin and cortisol binding globulin to be up to 98%.

What are the implications of this study on the mechanisms of anaesthesia? The CoMFA activity model was formulated by calculating the steric and electrostatic interaction energies between the anaesthetic structures and a probe atom at specific points, and correlating the magnitudes of these energies with in vivo potency. The high correlation obtained and the good predictive capability of the model demonstrate that the spatial distribution of molecular bulk and electrostatic potential are important in determining the activity of anaesthetic agents. Furthermore, the fact that a single activity model could be formulated for chemically and structurally diverse i.v. general anaesthetics suggests that the spatial arrangement of the key steric and electrostatic regions may be common for the compounds studied. It should be noted that this does not imply that there is a single site of action; rather it suggests that there is a common molecular basis for anaesthetic activity, determined by the spatial distribution of physicochemical molecular properties that can be represented as steric and electrostatic pharmacophoric maps.

What is the importance of the pharmacophoric maps to clinical anaesthesia? Maps of this type may be of value in the development of novel anaesthetic agents. Since the maps are based on the spatial distribution of physicochemical properties rather than a specific chemical structure, it may be possible to identify new chemical entities with anaesthetic activity that are structurally dissimilar to existing i.v. agents. Furthermore, by deriving additional pharmacophoric maps for other end points and characteristics of general anaesthesia, it may be possible to rationally design
chemical entities with more desired properties at the expense of the undesirable side-effects.

Further studies incorporating a larger number of agents are required to test this hypothesis. Our present study has been limited by the availability of comparable free plasma concentration data for i.v. anaesthetic agents. We aim to circumvent this difficulty by using dose data (mol kg⁻¹), which is available for a much wider range of anaesthetic analogues.

References