Comparative molecular field analysis to derive pharmacophore maps for induction doses of intravenous anaesthetic agents†

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Editor’s key points

- I.V. hypnotic agents have two separate anaesthetic therapeutic effects—use by single dose for induction of anaesthesia and use by continuous infusion for the maintenance of anaesthesia.
- Use of comparative molecular field analysis allows derivation of pharmacophore maps based on electrostatic and steric molecular interactions. Sets of maps for both therapeutic endpoints have been determined.
- Comparison of the maps for induction and maintenance of anaesthesia shows significant differences, suggesting that these effects may be mediated by different neurophysiological mechanisms or through interactions with different receptor sub-types.

Background. The present study examines the molecular basis of induction of anaesthesia by i.v. hypnotic agents using comparative molecular field analysis (CoMFA).

Methods. ED50 induction doses for 14 i.v. anaesthetics in human subjects (expressed as molar dose per kilogram body weight) were obtained from the literature. Immobilising potency data for the same 14 agents (expressed as the EC50 plasma free drug concentrations that abolish movement in response to a noxious stimulus in 50% patients) were taken from our previous publication. These data were used to form CoMFA models for the two aspects of anaesthetic activity. Molecular alignment was achieved by field-fit minimization techniques. The lead structure for both models was eltanolone.

Results. The final CoMFA model for the ED50 induction dose was based on two latent variables, and explained 99.3% of the variance in observed activities. It showed good intrinsic predictability (cross-validated $q^2 = 0.849$). The equivalent model for immobilizing activity was also based on two latent variables, with $r^2 = 0.988$ and $q^2 = 0.852$. Although there was a correlation between –log ED50 and –log EC50 ($r^2 = 0.779$), comparison of the pharmacophore maps showed poor correlation for both electrostatic and steric regions when isocontours were constructed by linking lattice grid points, making the greatest 40% contributions; the relative contributions of electrostatic and steric interactions differing between the models (induction dose: 2.5:1; immobilizing activity 1.8:1).

Conclusions. Comparison of two CoMFA activity models shows only small elements of commonality, suggesting that different molecular features may be responsible for these two properties of i.v. anaesthetics.

Keywords: anaesthetics i.v.; comparative molecular field analysis; indices of potency; model, computer simulation

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We have previously reported studies identifying the molecular bases of immobilizing activity of i.v.1 and inhalation general anaesthetics,2 3 and also the cardiovascular effects of i.v. agents by continuous infusion,4 using Comparative Molecular Field Analysis (CoMFA). In CoMFA, the molecular structures are placed in a rectangular grid consisting of regularly spaced lattice points. The steric and electrostatic interaction energies between the compounds and a charged probe are calculated at each point and correlated with potency of the anaesthetics to formulate an activity model. By identifying those grid points making the greatest contributions to the activity model, the key regions where steric and electrostatic interactions are important in determining the activities of the compounds can be identified and expressed as three-dimensional pharmacophoric maps.

I.V. hypnotic agents have two separate and distinct therapeutic effects—use as a single dose for induction of anaesthesia and use by continuous infusion either alone or as supplement to nitrous oxide or opioid, or to regional analgesia to maintain anaesthesia. The two corresponding measures of drug potency are the ED50 dose (the dose needed for induction of anaesthesia in 50% subjects) and the EC50 (the free drug concentration needed to prevent response to noxious surgical stimuli in 50% of patients).
CoMFA to derive pharmacophore maps

Methods

A group of 14 structurally diverse i.v. anaesthetic hypnotic agents were considered [eltanolone, alphaxalone, minaxalone, ORG 21465, thiamyllal, thiopental, methohexital, pentobarbital, (S)-ketamine, (R)-ketamine, R- etomidate, clomethiazole, propofol, and ORG 25435]. These have all been used to both induce and maintain i.v. anaesthesia. The measure of induction dose activity used to develop the CoMFA model was the ED50 dose—the dose which will induce anaesthesia in 50% of patients within 30 s.

Induction dose and immobilizing potency data

ED50 values for induction of anaesthesia in men (expressed as molar doses per kilogram bodyweight) were obtained from the literature together with additional unpublished data of the author, and immobilization potency data (expressed as the ECM50 plasma free drug concentration that abolishes movement in 50% patients in response to noxious stimuli) from our previous publications. For both endpoints, results were taken from studies involving ASA I and II unpremedicated patients, except where otherwise stated.

Modelling

Anaesthetic structures were formulated using the molecular modelling software SYBYL v7.3 (Tripos Inc., St Louis, MI, USA) on a Silicon Graphics O2 R10000 workstation, with compounds modelled in their un-ionized state. The starting structures were geometry optimized using molecular mechanics optimization so as to minimize the total potential energy of the structure. The default Tripos molecular force field was used as implemented in SYBYL v7.3. Gasteiger–Huckel partial charges were assigned to each atom, and non-bonded electrostatic interactions were calculated using a distance-dependent dielectric function. The latter simulates the electrostatic screening effect of the solvent without explicitly including the solvent molecules in the calculation.

For each anaesthetic, a set of low energy conformers were identified by a random search procedure in SYBYL. Only geometry-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, the applied limit ensured that only realistic configurations of the anaesthetics were considered. The process was repeated until each anaesthetic had been subjected to 10 000 random structure perturbations or until each of the low energy conformers had been found at least 12 times.

The geometries of the compounds were further refined using quantum mechanics. In this approach, a mathematical model of molecular structure is formed in terms of the nuclei and electron distributions, providing a more accurate representation of molecular geometry. This quantification was carried out using the MOPAC 6 software package (Quantum Chemistry Program Exchange, IN, USA) with the AM1 Hamiltonian with atomic partial charges assigned using the Coulson method. After geometry optimization, duplicate conformers (defined as conformers with an RMS difference of <0.2 Å) were removed. The final set consisted of 1176 unique conformers for the 14 anaesthetics.

Structure alignment

Because the chemical diversity of the i.v. agents precluded their adequate alignment by a common substructure, the low energy conformers were aligned for CoMFA by field-fit minimization to provide the best correlation between the steric and electrostatic fields of the molecules and that of a single lead structure. The same conformer of eltanolone (the most potent agent with regard to immobilization) was used as in the previous studies.

CoMFA formulation

A separate CoMFA model was derived for these two aspects of i.v. anaesthetic activity. The aligned structures were placed in a grid consisting of lattice points at 1 Å intervals. An sp3 carbon atom with a charge of +1.0 was used as a probe to generate the steric and electrostatic interaction energies between the probe and the anaesthetic molecules at each lattice point. Steric energies were calculated using a Leonard-Jones 6–12 potential, which describes attraction between molecules due to van der Waals forces (dispersion, dipole–induced dipole, and dipole–dipole interactions) and repulsion due to steric clashes. Electrostatic interaction energies were calculated using Coulomb potentials with a distance-dependent dielectric function. A default cut-off value of 30 kcal mol−1 was applied to both steric and electrostatic energies. The orientations of the anaesthetics yielding CoMFA models with the greatest predictive capability were retained. The use of 1 Å grid intervals represented a compromise between accuracy and the introduction of ‘noise’ from sampling irrelevant data. The grid extended at least 4 Å beyond the surface of the molecules and consisted of 9025 lattice points. The interaction energies at each point were correlated with molar induction dose and immobilizing potency using partial least squares regression to formulate the two activity models.

Activity model formation

This was carried out using partial least squares regression analysis to correlate the interaction energies at each lattice point with the induction dose or immobilizing activities of the anaesthetics. The same modelling approach was used as in previous publications.
Two approaches were used to determine the intrinsic predictive power of the CoMFA models. First, the models were assessed using partial least squares regression to derive leave-one-out cross-validation. In this process, activity models were repeatedly reformulated by leaving one compound out of the model at each stage. The model with the greatest cross-validated $r^2$ ($q^2$) was retained as the final model. The activity models were further assessed using cross-validation with four compounds randomly excluded at each stage. These cross-validation studies were each run for 10 cycles and mean values ($M_{CV4q2}$) were calculated. Linear correlation was used to examine the relationship between log $ED_{50}$ dose and log $EC_{50}$ plasma free drug concentration.

Results

The mean $ED_{50}$ values, $EC_{50}$ free plasma drug concentrations for immobilization, and log $P$ (octanol–water partition coefficient) are shown in Table 1.

The final CoMFA model for induction dose potency was based on two latent variables. It explained 99.3% of the observed variance ($F_{2,21} = 745.313, \ P < 0.0001$; $q^2 = 0.869$; and $mCV4q^2 = 0.808 (\ 0.032)$) with a range of values 0.748–0.850. The mean absolute log residual (calculated as predicted–observed $pLog$) was 0.041 (0.030). Overall, the model was a good predictor of the $ED_{50}$ molar dose; the two drugs showing the greatest absolute difference being pentobarbital and $R(+) $-etomidate. The first of these is now rarely used for induction of anaesthesia; while the dose quoted for etomidate varies between studies and subjects due partly to a tendency for the drug to be accompanied by marked excitatory activity, so making the exact definition of the endpoint more variable.

If ketamine was excluded from the $ED_{50}$ CoMFA model, there was no significant difference in the $r^2$ value (0.975), whereas the values for $q^2$ and $mCV4q^2$ were decreased (0.773 and 0.734, respectively).

In contrast, the performance of a conventional activity model for induction dose potency based on log $P$ (octanol–water partition coefficient) explained only 28.8% of the variance in observed activities; in comparison, the log $P$ model for the $EC_{50}$ immobilizing activity of the same 14 agents explained 60.2% of the variance.

The relative contributions of the electrostatic and steric interactions in the molar induction dose model were 71.3% and 28.7%, respectively. Analysis of the individual partial least squares regression weightings for each lattice point enables the identification of regions where steric and electrostatic interactions are important in determining activity, and pharmacophoric maps can be derived by using isocontours linking together the lattice points in the CoMFA grid making the greatest contributions to the activity model.

The model for the $EC_{50}$ immobilizing activity of the same 14 i.v. agents was also based on two latent variables, and explained 98.8% of the variance between the observed

<table>
<thead>
<tr>
<th>$EC_{50}$ (µM)</th>
<th>$ED_{50}$ (mg kg$^{-1}$)</th>
<th>$p$ log $ED_{50}$ (mol kg$^{-1}$)</th>
<th>log $P$</th>
<th>Refs</th>
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<td>0.63</td>
<td>5.704</td>
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</tr>
<tr>
<td>AX</td>
<td>0.46</td>
<td>0.35</td>
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<tr>
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<td>0.52</td>
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<tr>
<td>ORG 21465</td>
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<tr>
<td>THIA (rac)</td>
<td>15.33</td>
<td>5.00</td>
<td>4.707</td>
<td>3.23</td>
</tr>
<tr>
<td>THIO (rac)</td>
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</table>

The quoted references refer to studies from which estimates for the $ED_{50}$ values were taken; in the cases where several papers are cited, a mean value has been used. Data for the $EC_{50}$ immobilizing concentrations for the same 14 agents was also based on two latent variables, and explained 60.2% of the variance.
activities of the agents ($F_{2.11} = 454.271$, $p < 0.0001$); $q^2 = 0.852$; and mCV$\text{q}^2 = 0.765$ ($0.070$) with a range of values from $0.644$ to $0.843$. The relative contributions of the electrostatic and steric interactions in the immobilizing activity model were $64.0\%$ and $36.0\%$, respectively. There was a significant correlation between molar induction dose ($-\log ED_{50}$) and immobilizing activity ($-\log EC_{50}$) for the $14$ i.v. anaesthetics, $r^2 = 0.7793$ ($p < 0.0001$). There were no significant effects on the $EC_{50}$ CoMFA model parameters if the enantiomers of ketamine were excluded ($r^2 = 0.987$ and $q^2 = 0.823$).

**Comparison of the pharmacophore maps**

The pharmacophoric maps for molar induction dose and immobilizing activity are shown in Figure 1A–D, with the lead compound eltanolone as the reference molecule, with its four-ring steroid structure. The induction dose model has two main electrostatic areas—a negative-favoured area (coloured red) related to the D ring of the steroid structure, and a large positive potential favoured area (coloured blue) engulfing above and below the plane of the A and B rings. The corresponding steric map shows two bulk-favoured areas (coloured green) at each end of the molecule and a central bulk-disfavoured area (coloured magenta) located above and below the plane of the molecule.

**Relative contributions**

There are differences in the relative contributions that steric and electrostatic interactions make to the CoMFA activity models, although the electrostatic component makes the larger contribution to both models.

**Commonalities**

There are several key regions which are common to both models; these include the negative charge favoured region to the right of the D ring of the electrostatic model, and...
the positive-favoured regions at the other end of the molecule. However, the immobilizing potency model has a second negatively favoured area not seen in the induction dose model. Both models have similar steric features.

**Regional differences**

There are some regional differences between the two models. The electrostatic maps indicate two distinct negative potential favoured areas for immobilizing activity, but only one in the induction dose map. In the latter, there is also extension of the positive potential favoured area—engulfing the whole of the A and B rings of the steroid nuclear structure of the lead compound, eltanolone. When isocontours are drawn linking lattice points making the greatest 40% of the individual negative or positive contributions for each model, there were low correlations between the models for both steric ($r = 0.554$) and electrostatic ($r = 0.221$) energies.

**Discussion**

This paper has examined the molecular basis for two different actions of i.v. hypnotic agents—first, induction of anaesthesia, and secondly, infusion of the drugs to maintain anaesthesia either alone, in combination with an opioid drug, or as supplement to nitrous oxide. Separate CoMFA models have been formulated and comparison has been made between the models using electrostatic and steric isocontour analysis.

In clinical studies, various endpoints have been used as a surrogate marker for induction of anaesthesia. These include loss of verbal contact, loss of the eyelash reflex, dropping a filled syringe held at arm’s length, and a number of EEG parameters. A number of factors influence the required dose of an anaesthetic induction agent, such as patient age, weight and lean body mass, rate of drug administration, co-administered drugs, the drug’s lipid solubility $P_l$, cardiac output, plasma protein binding (and hence free drug fraction), and blood–brain equilibration time ($t_{1/2K_{eo}}$). $^{26-31}$ Furthermore, there are different timescales from the start of drug administration for achieving each one of the surrogates endpoints for induction of anaesthesia. In the present study, the ED$_{50}$ has been taken as that dose of drug given over 20–30 s that will induce anaesthesia within a further 30 s when defined as loss of verbal contact.

The EC$_{50}$ value for the immobilizing potency of hypnotic agents depends partly on systemic drug clearance, the presence (or not) of other agents having anti-nociceptive effects, and the intensity of the noxious stimulus. In all studies included in this analysis, the applied noxious stimulus was the initial surgical incision. Because hepatic drug clearance for most i.v. agents (thiopental and thiamylal excepted) is liver blood flow-dependent, the significant relationship between pharmacophores for the EC$_{50}$ concentration for immobilization and cardiovascular depression can be understood. However, although there was no such correlation between the pharmacophores for induction dose potency and immobilization (see later), there was a relationship between the ED$_{50}$ dose and EC$_{50}$ free drug concentration.

Previous studies compared the molecular bases of immobilization with noxious stimuli and cardiovascular depression, and found some similarities and differences between the two CoMFA models. $^6$ There was a similarity between the pharmacophores with regard to the spatial distribution of positive- and negative-charged regions, and also bulk-favoured and -disfavoured areas. These similarities and differences were reflected in two ways—in the significant correlation between the EC$_{50}$ free drug concentrations and cardiovascular depression (when calculated as the free drug concentration associated with a 20% reduction in the pre-anesthetic arterial pressure) ($r^2 = 0.861$); and secondly, in that the two models showed 72.2% equivalent points for electrostatic fields and 70.9% for steric fields. When isocontours were constructed by linking lattice points making the 40% greatest contributions to each activity model, there were 87.6% of equivalent electrostatic points and 86.2% steric points. However, there were also some electrostatic and steric regions where spatial conflict existed.

In contrast, the two activity models described in this paper differ significantly. Pharmacophoric maps representing the molecular bases of induction dose potency and immobilizing activity showed only poor similarity, with one region where there is conflict—namely, a positive-favoured area in the induction dose potency model and a negative-favoured area in the immobilization potency model. These regional differences between the maps may relate to the physiological basis of their respective activities. There were poor correlations between the ED$_{50}$ and EC$_{50}$ models when using isocontour analysis.

What do these differences in CoMFA models indicate? Does it mean that hypnosis and immobilization are mediated by different neurophysiological mechanisms? Recent studies in mice and rats suggest that there may indeed be distinct molecular sites associated with these two properties of i.v. hypnotic agents—with differences in the GABA$_A$ subtypes associated with sedation (hypnosis) and immobilization (namely the $\beta_2$ and $\beta_3$ subtypes, respectively). $^{32-34}$ I.V. anaesthetics (ketamine partly excepted) act almost exclusively to produce potent hypnosis (=anaesthesia) via GABA$_A$ receptors containing either $\beta_2$ or $\beta_3$ subunits. However, the ability of these i.v. anaesthetics to depress spontaneous and evoked movements is limited (such that they can be deemed to have a low intrinsic immobilizing capacity). The studies of Jurid and colleagues, $^{32}$ Reynolds and colleagues, $^{35}$ Rudolph and Antkowiak, $^{33}$ and Zeller and coworkers $^{36,37}$ have all shown immobilizing activity to be dependent on $\beta_3$ GABA$_A$ receptor subunits, while sedation was mediated via the $\beta_2$ subunits. The ability to produce immobility is believed to be mediated by spinal cord circuits. It has been shown that $\beta_3$ subunits are the predominant GABA$_A$ receptor subtype expressed in the dorsal root ganglia, superficial dorsal horns of the spinal cord, and motor neurones. The action of barbiturate pentobarbitral on these circuits has been confirmed by Zeller and colleagues. $^{38}$
The amnestic effects of i.v. anaesthetics are probably due to other receptor interactions, so supporting the concept of a ‘multisite model for general anaesthetic action’. Although the effects of ketamine anaesthesia are mediated mainly through the NMDA receptor, there is some evidence from mice studies that anaesthesia (=immobilization) may also be partly mediated by GABA<sub>A</sub> receptor interactions.

A limitation of both models in this study has been the need to use concentration data and dose data for the racemic mixtures of the barbiturates rather than enantiomer-specific values. Differences in dose potency and pharmacokinetics have been described for three of the barbiturates (methohexitol excluded) both in vitro and in vivo. However, there are no data relating to enantiomer-specific concentration data for immobilization in men. We cannot state whether inclusion of such values would significantly affect the CoMFA models.

This paper therefore supports the concept of a separation of the mechanisms for induction of anaesthesia and immobilization for the 14 i.v. agents studied. A CoMFA activity model can be formulated which accurately predicts induction dose potency for i.v. anaesthetics, based on the three-dimensional spatial distribution of key steric and electrostatic regions. This model differs from that previously formulated for immobilization in response to noxious stimuli.

Apart from these main aims of the present study, the derived CoMFA models could also be used to predict the likely behaviour of new and novel compounds. We have previously used this approach when formulating the EC<sub>50</sub> CoMFA models for immobilization of both i.v. and volatile agents by randomly sorting the agents into training and test sets, where the former gave a good predictive estimate for the latter compounds. In the present study, we opted to use an all-in field-fit minimization approach for the alignment of the molecules in the CoMFA models.

At the present time, there are no available data for both ED<sub>50</sub> and EC<sub>50</sub> values for other new i.v. anaesthetic agents to test the predictive ability of our models. We await these with interest.

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Conflict of interest
None declared.

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