Response Surface Modeling of Alfentanil–Sevoflurane Interaction on Cardiorespiratory Control and Bispectral Index

ONE OF THE ADVANTAGES of combining an opioid and an anesthetic over the use of single agents is the synergistic increase in desired anesthetic effect, such as absence of movement in response to a painful stimulus as defined by the minimal alveolar concentration (i.e., anesthetic potency). The consequence of this mechanism is the need for less drugs with possibly less side effects. Anesthesiologists make use of these fortuitous interactions by combining opioids and anesthetics during anesthesia. Because respiratory depression is a serious side effect of anesthetics, hypnotics, and opioids, even at low doses, it is surprising that few studies in humans addressed the issue of the impact of drug combinations on respiration. Especially, the nature of the interaction (additive versus synergistic) of an anesthetic–opioid combination on the control of breathing remains unknown. Therefore, we studied the influence of the opioid alfentanil and the inhalational anesthetic sevoflurane on ventilatory control, heart rate (HR), bispectral index (BIS), as measure of the hypnotic state of the subjects, and the ventilatory and HR responses to hypoxia. The ability of the ventilatory control system to cope adequately with hypoxic episodes is of importance because hypoxic periods frequently occur perioperatively.

The alfentanil–sevoflurane interaction on ventilatory control, HR and BIS was assessed by response surface modeling. This approach enables us to construct three-dimensional representations of the concentration-response relation among combinations of alfentanil and sevoflurane and assess the nature of the interaction (additive, synergistic or antagonistic) over the whole surface area (it is possible for the response surface to include all of these interactions in different regions). This approach is superior to the construction of isoboles (or iso-effect curves), which allows assessment of the interaction at drug combinations yielding a constant effect, such as 25% or 50% reduction in effect parameter (i.e., C_{25} and C_{50}).

In this study we used the dynamic end-tidal forcing technique. This technique enables us to assess the effect of drug combinations on ventilatory control at identical end-tidal partial pressures of carbon dioxide (P_{ETCO_2}'s). Consequently, this makes a comparison among the respiratory effects of different drug combinations possible because the results are not confounded by changes in P_{ETCO_2}.

METHODS

Subjects and Apparatus
Nine healthy male volunteers (aged 18–25 yr) participated in the protocol after approval was obtained from the local human ethics committee (Commissie Medische Ethiek, Leiden Univer-
Table 1. Total Number of Paired Hypoxic Studies at Each of the Treatment Concentrations

<table>
<thead>
<tr>
<th>Target Alfentanil Conc. (ng/ml)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>4</td>
<td>9</td>
<td>3</td>
<td>9</td>
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<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
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</tr>
<tr>
<td>0.1</td>
<td>9</td>
<td>5</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>9</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.4</td>
<td>2</td>
<td></td>
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</tr>
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</table>

sity Medical Center, 2300 RC Leiden, The Netherlands). Oral and written consent was obtained from all volunteers. The subjects were healthy and did not have a history of tobacco or illicit drug use.

After arrival at the laboratory, two intravenous catheters were inserted in the left and right antecubital vein (one for alfentanil administration and one for blood sampling). Subsequently, electrodes for electroencephalographic measurement were placed on the head as specified by the manufacturer and the subjects rested for 20–30 min. Next, a face mask was applied over the mouth and nose and the experiments started.

See METHODS section Apparatus of Chapter 2 for a description of the procedure and apparatus. The electroencephalograph was recorded using a monitor that computed the BIS over 2-s epochs. We averaged the BIS values over 1 min-intervals and used data points obtained at 3-min intervals.

**Study Design**

The ventilatory response to isocapnic hypoxia (acute hypoxic response) was assessed during inhalation of sevoflurane, infusion of alfentanil and the combined administration of both agents. Initially, two hypoxic control studies (i.e., studies without the administration of any agent) were obtained. Next, inhalation of sevoflurane was started, and hypoxic studies were performed during inhalation of sevoflurane. To achieve blood-brain equilibrium, sevoflurane hypoxic experiments were preceded by a 12-min equilibration period.137 In Table 1, the various imposed target end-tidal sevoflurane concentrations are given. After this set of studies, the subject rested for 30–45 min, and another two control studies were obtained. Next, intravenous infusion of alfentanil was started, and hypoxic studies were performed at various blood target concentrations (Table 1). Subsequently, hypoxic studies during the combined administration of sevoflurane and alfentanil were performed (Table 1). The sevoflurane and alfentanil target concentrations were chosen in such a way that at least 60% depression but preferably 80% depression, of the ventilatory response to hypoxia was achieved. All experiments were performed on a single day, starting at 08:30 AM.

**The Isocapnic Hypoxic Study**

The $P_{ET}O_2$ waveform was as follows: (1) 10 min at 110 mmHg; (2) a rapid decrease to 50 mmHg; (3) 3 min at 50 mmHg; and (4) 4–5 min at 110 mmHg. At each treatment level (control, various concentrations of sevoflurane, alfentanil, and sevoflurane plus alfentanil), two hypoxic studies...
Figure 1. LEFT. The influence of plasma alfentanil concentration (x-axis) at fixed concentrations of end-tidal sevoflurane concentration on the hypoxic ventilatory response (y-axis) in one subject (subject 926). Note the parallel displacements of the alfentanil dose-hypoxic response curve caused by sevoflurane. RIGHT. A three-dimensional graph of the individual Bayesian estimate of the response surface of subject 926 for the hypoxic ventilatory response. The surface gives an impression of the nature of the interaction at all possible drug combinations, which in this case is additive.

were obtained.

All hypoxic studies were performed at identical end-tidal $PCO_2$ concentrations, $\sim 5–7$ mmHg more than awake resting values. This high value was chosen to offset an increase in end-tidal $PCO_2$ caused by the alfentanil-sevoflurane-induced ventilatory depression.

**Alfentanil Administration, Blood Sampling, and Assay.**
A target controlled infusion was used for the administration of alfentanil. A palm-top computer programmed with the population pharmacokinetic data set, reported by Maitre et al. was connected to a syringe pump, which was filled with alfentanil (0.5 mg/ml). This system allows a theoretical plasma concentration of alfentanil to be achieved rapidly and maintained. Hypoxic studies were performed 5–10 min after blood alfentanil concentrations reached their target levels. Because this equals 5–10 times the alfentanil blood-brain equilibration half-life of 1 min, we assume that brain and blood alfentanil concentrations were in equilibrium.

Before and after changes in target alfentanil concentration, 3 ml blood samples for the measurement of alfentanil were collected. A capillary gas chromatographic technique was used to determine the plasma concentration.

**Data Analysis and Statistics**
Averaged values of the breath-to-breath data were chosen over identical time segments. Normoxic data points were the mean of the 10 breaths before the 3-min hypoxic period. Hypoxic data points were mean of the last 10 breaths of the hypoxic episode. Analysis was performed on the following variables: normoxic $V_t$, normoxic $VT$, normoxic RR, normoxic HR, normoxic BIS, normoxic and hypoxic end-tidal $PCO_2$, normoxic and hypoxic end-tidal partial
pressure of oxygen \((PO_2)\); and the change in \(\dot{V}_i\), \(VT\), RR and HR from normoxia to hypoxia relative to the absolute changes in arterial oxygen saturation \((i.e., \Delta \dot{V}_i/\Delta \dot{S}_P O_2, \Delta V_T/\Delta \dot{S}_P O_2, \Delta RR/\Delta S_P O_2\) and \(\Delta HR/\Delta S_P O_2\)). Hypoxic sensitivities usually are defined as follows: \([E(\text{hypoxia}) - E(\text{normoxia})]/\Delta S_P O_2\), where \(\Delta S_P O_2\) is \([100 - S_P O_2\) (hypoxia)\], and \(E\) the value of a measured parameter. Instead of 100, we used the actual \(S_P O_2\)-value measured during normoxia to calculate \(\Delta S_P O_2\). This definition results in positive hypoxic sensitivity values when the measured parameter \(E\) increases upon the introduction of hypoxia \((i.e., E(\text{hypoxia}) > E(\text{normoxia}))\). Only when the effect parameter decreases because of hypoxia \((i.e., E(\text{hypoxia}) < E(\text{normoxia}))\), will the value of the hypoxic sensitivity be negative. The data sets (sevoflurane concentration, alfentanil concentration, \(E\) parameter) were analyzed using a pharmacodynamic model which can be visualized in three-dimensional space as a ‘response surface’. We used the following pharmacodynamic model:

\[
f(x) = \alpha \cdot (1 - x^\gamma)
\]

By substituting \(E_0\), which is the baseline value \((i.e., \text{pre-drug administration value})\) of a variable, for \(\alpha\) and substituting \(U^\gamma/2\) for \(x^\gamma\) and \(U = C/C_{50}\), where \(C_{50}\) is the concentration causing 50% decrease of \(E\), \(E\) a measured variable and \(C\) the concentration of one drug we obtain:

\[
E(C) = E_0 \cdot (1 - U^\gamma/2)
\]

Pure additive interactions of two drugs (alfentanil and sevoflurane) are modeled as follows:

\[
E(C_A, C_S) = E_0 \cdot (1 - (U_A + U_S)^\gamma/2)
\]

\[
U_A = C_A/C_{50,A}
\]

\[
U_S = C_S/C_{50,S}
\]

\(C_A\) is the alfentanil plasma concentration, \(C_S\) the end-tidal sevoflurane concentration, \(C_{50,A}\) is the alfentanil concentration causing a 50% decrease of \(E\), \(C_{50,S}\) is the sevoflurane concentration causing a 50% decrease of \(E\).

To include non-additive interactions in the model, an interaction, term \(I(Q)\), is introduced:

\[
E(C_A, C_S) = E_0 \cdot (1 - [(U_A + U_S)^\gamma]/2 \cdot I(Q))
\]

\(I(Q)\) is a smooth function (spline) of \(Q\) (see Appendix 1). Following Minto \textit{et al.},

\[
Q = U_A/(U_A + U_S)
\]

\(Q\) ranges from 0 (sevoflurane only) to 1 (alfentanil only) and is the drug concentration ratio of alfentanil and sevoflurane normalized by their respective \(C_{50}\)s or potencies (equations 4 and 5). The smooth function has two parameters, \(I_{\text{max}}\) and \(Q_{\text{max}}\). \(I_{\text{max}}\) is the maximum value of the interaction term, and \(Q_{\text{max}}\) is the value of \(Q\) \((i.e., \text{concentration ratio})\) for which \(I\) attains \(I_{\text{max}}\). When \(I_{\text{max}}\) equals 1, the interaction is purely additive. An \(I_{\text{max}}\) of less than 1 denotes antagonism and an \(I_{\text{max}}\) of more than 1 denotes synergy.
The model was fitted to the data with NONMEM (conditional estimation method), version V, level 1.1 (a data analysis program for non-linear mixed effects modeling)* using a population approach. The interindividual variability of each of the model parameters is characterized by the percentage coefficient of variation, which is a parameter derived from the variance of the logarithm of the individual model parameters. Likelihood-ratio tests were performed to determine whether \( y \) did not equal 1 and whether \( I_{max} \) did not equal 1. The intraindividual variability was quantified by the standard deviation of the residuals. \( P \)-values of less than 0.01 were considered significant.

RESULTS

A total of 109 paired hypoxic responses were obtained at various treatment levels (table 1). The duration of the experiments ranged from 5 to 6 hours. In figure 1, an example of the various drug combinations applied in a single subject is shown. This graph further illustrates that the maximum depression of the hypoxic drive (= \( \Delta \dot{V}/\Delta S_pO_2 \)) was 70 to 80\%, obtained at multiple drug combinations. This level of depression was one of the aims of the study protocol, which was met in all subjects.

End-tidal carbon dioxide values were equal in normoxia and hypoxia (\( P_{ET}CO_2 \) normoxia 48.3 ± 0.5 mmHg (mean ± SEM) versus hypoxia 48.2 ± 0.6 mmHg). These values are 6–7 mmHg more than individual resting values. The measured plasma alfentanil values averaged to 12.7 ± 0.9 ng/ml, 21.3 ± 1.6 ng/ml, 27.4 ± 1.2 ng/ml and 35.6 ± 2.4 ng/ml for target concentrations of 10, 20, 30 and 40 ng/ml, respectively.

A three dimensional graph of the conditional Bayesian estimate of the response surface of one subject for \( \Delta \dot{V}/\Delta S_pO_2 \) is given in figure 1 (right). The value of \( y \) was not different from 1. This indicates a linear relation between alfentanil, sevoflurane, and effect. The surface gives an impression of the nature of the interaction at all possible drug combinations, at least within the dose ranges tested, which, in this case, is additive in all regions of that part of the surface. It further predicts the interaction at concentrations higher than those we applied. Assuming that the concentration-effect relation remains linear, the model predicts the complete loss of the hypoxic response (i.e., \( \Delta \dot{V}/\Delta S_pO_2 = 0 \)) in this subject at combinations of alfentanil and sevoflurane on a line on the surface connecting the points (alfentanil = 55 ng/ml, sevoflurane = 0 \( ET\% \)) and (alfentanil = 22 ng/ml, sevoflurane = 0.4 \( ET\% \)).

The population estimates of the response surfaces are given in table 2 and figures 2–4. The model fit the data well. For all variables, the model fits yielded values of \( y \) not significantly different from 1. This indicates that alfentanil and sevoflurane, and combinations of alfentanil and sevoflurane at fixed ratios, cause changes of the measured variables in a linear manner. The \( C_{25} \) and \( C_{50} \) values (the concentrations causing 25\% and 50\% reduction of effect) are given in table 2. Note the absence of effect of alfentanil on BIS (table 2 and fig. 4) and of sevoflurane on normoxic breathing frequency (table 2;

see also below).

Pure additive interactions were found for the following parameters: \( \Delta \dot{V}_i/\Delta SPO_2 \), \( V_T \), \( \Delta V_T/\Delta SPO_2 \), \( \Delta RR/\Delta SPO_2 \), \( \Delta HR/\Delta SPO_2 \). Inert interactions were observed for RR and BIS. This leads to straight isoboles or iso-effect curves (the isoboles are shown in figs. 2–4 Right). Alfentanil, over the dose range studied, caused a modest reduction of normoxic breathing frequency. The \( C_{25} \) was 81.5 ng/ml, clearly outside the dose range we studied (table 2). Sevoflurane had no effect on RR when increasing from 0 to 0.4 \( ET\% \). Sevoflurane caused a linear reduction of BIS with an \( EC_{25} \) of 0.45 \( ET\% \) (table 2). The effect of sevoflurane on the BIS was independent of the alfentanil concentration.

Synergistic interactions were found for normoxic \( \dot{V}_i \) and HR (table 2 and figs. 2 and 3). In figure 5, the relations between \( Q \) (i.e., the alfentanil–sevoflurane concentration ratio) and \( I(Q) \) (i.e., the interaction) are given. For \( \dot{V}_i \) and HR, synergistic interactions were observed, with maximum synergistic interactions (\( I_{\text{max}} \) in table 2) occurring at \( Q \) values of approximately 0.7 (\( Q_{\text{max}} \) in table 2). Depression of \( \dot{V}_i \) by 25% occurred at an alfentanil concentration of 38 ng/ml and at a sevoflurane concentration of 0.7 \( ET\% \). The combinations of sevoflurane and alfentanil causing 25, 50, and 75% reduction in \( \dot{V}_i \) are given in figure 2 (TOP Right). The synergy is obvious from the concave form of the isoboles. One possibility for 25% reduction is 13.4 ng/ml alfentanil plus 0.12 \( ET\% \) sevoflurane (\( I(Q) \sim 1.9 \) at a \( Q \) of \( \sim 0.7 \)).

**DISCUSSION**

The main findings in this study are as follows: (1) Alfentanil, (up to a plasma concentration of 50 ng/ml), and sevoflurane (from 0 to 0.4 \( ET\% \)), when administered separately, depress ventilation, HR, and the ventilatory and HR responses to acute hypoxia in a dose-dependent linear manner; (2) When combined, their depressant effect on ventilation and HR is synergistic, whereas their effect on the hypoxic responses is additive; (3) Relative to normoxic baseline parameters (\( \dot{V}_i \), \( V_T \), RR and HR), the responses to hypoxia, show greater sensitivity to the effects of alfentanil and sevoflurane (i.e., depression occurs at lower drug concentrations), when drugs are administered separately and when combined (\( C_{25} \) values differ by 2–8 times); (4) The bispectral index is sensitive to sevoflurane but not alfentanil, even when these agents are combined (inert interaction).

*The Pharmacodynamic Model*

Although the relation between drug concentration and respiratory effect has been modeled previously using an inhibitory sigmoid \( E_{\text{max}} \) model,\(^{42}\) we refrained from such an approach. In contrast, we chose the function \( f(x) = a(1 - x^γ) \), which allows for linear and non-linear concentration-effect relationships (see fig. 6). This was done for the following reasons:

1. Asymmetric sigmoidal relations between drug and effect may occur in complex systems, such as the ventilatory control system. For example, at high drug concentrations (higher than those studied by us), nonlinear threshold values may cause
Table 2. Model Parameter Estimates for Ventilation ($\dot{V}_i$), Tidal Volume ($V_T$), Respiratory frequency (RR), heart rate (HR), the hypoxic sensitivities ($\Delta E/\Delta S_pO_2$) and bispectral index (BIS)

<table>
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<tr>
<th></th>
<th>$\dot{V}_i$</th>
<th>$\Delta \dot{V}_i/\Delta S_pO_2$</th>
<th>HR</th>
<th>$\Delta$ HR/ $\Delta S_pO_2$</th>
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<tr>
<td></td>
<td>L/min</td>
<td>L min$^{-1}$ %-%</td>
<td>min$^{-1}$</td>
<td>min$^{-1}$ %-%</td>
</tr>
<tr>
<td>Baseline</td>
<td>18.4 ± 1.5</td>
<td>1.04 ± 0.27</td>
<td>60.7 ± 2.4</td>
<td>1.10 ± 0.10</td>
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<tr>
<td>%CV</td>
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<td>74</td>
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<td>24</td>
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<tr>
<td>$C_{25}$ Alfentanil$^a$</td>
<td>37.7 ± 11</td>
<td>15.7 ± 1.4</td>
<td>155 ± 90</td>
<td>19.1 ± 3.0</td>
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<tr>
<td>%CV</td>
<td>48</td>
<td>17</td>
<td>108</td>
<td>20</td>
</tr>
<tr>
<td>$C_{50}$ Sevoflurane$^b$</td>
<td>0.73 ± 0.44</td>
<td>0.14 ± 0.05</td>
<td>1.92 ± 1.35</td>
<td>0.25 ± 0.11</td>
</tr>
<tr>
<td>%CV</td>
<td>106</td>
<td>41</td>
<td>*</td>
<td>70</td>
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<tr>
<td>$I_{max}$</td>
<td>1.92 ± 0.28</td>
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<td>$Q_{max}$</td>
<td>0.68 ± 0.11</td>
<td>0.69 ± 0.13</td>
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<tr>
<td>SD of Residuals</td>
<td>2.99</td>
<td>0.21</td>
<td>3.67</td>
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<tr>
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<th>$V_T$</th>
<th>$\Delta V_T/\Delta S_pO_2$</th>
<th>RR</th>
<th>$\Delta$ RR/ $\Delta S_pO_2$</th>
<th>BIS</th>
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<tr>
<td></td>
<td>ml</td>
<td>ml %-%</td>
<td>min$^{-1}$</td>
<td>min$^{-1}$ %-%</td>
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<tr>
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<td>0.25 ± 0.07</td>
<td>96.3 ± 0.8</td>
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<td>16</td>
<td>86</td>
<td>2.1</td>
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<tr>
<td>$C_{25}$ Alfentanil$^a$</td>
<td>37.0 ± 6.5</td>
<td>18.4 ± 2.6</td>
<td>81.5 ± 24.7</td>
<td>11.6 ± 0.4</td>
<td>*</td>
</tr>
<tr>
<td>%CV</td>
<td>30</td>
<td>28</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>$C_{50}$ Sevoflurane$^b$</td>
<td>0.39 ± 0.18</td>
<td>0.15 ± 0.05</td>
<td>*</td>
<td>0.24 ± 0.02</td>
<td>0.45 ± 0.08</td>
</tr>
<tr>
<td>%CV</td>
<td>80</td>
<td>70</td>
<td>*</td>
<td>*</td>
<td>53</td>
</tr>
<tr>
<td>SD of Residuals</td>
<td>80</td>
<td>10.9</td>
<td>2.49</td>
<td>0.18</td>
<td>3.32</td>
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</table>

Values are population estimate ± SE and the percentage coefficient of variation (%CV), which is a measure of the interindividual variability, as derived from the NONMEM analysis; Baseline is the control parameter value (i.e., before drug administration); $a$ and $b$: $C_{25}$ and $C_{50}$ denote concentrations of alfentanil (in ng/ml) or sevoflurane (in end-tidal concentration or $ET\%$) giving 25% and 50% reductions in $E$; * parameter not included in the statistical model; $I_{max}$ and $Q_{max}$ are interaction parameters: $I_{max}$ values not significantly different from 1 are not included in the table (in these cases the alfentanil–sevoflurane interaction is additive) and $I_{max}$ values greater than 1 indicate synergy; SD (standard deviation) of the residuals is a measure of goodness of fit.
the respiratory oscillator to stop abruptly and may cause irregular or cyclic breathing and apnea. In our model effect becomes zero at the concentration $C_{50} \cdot 2^{\gamma}$ (fig. 6).

2. Some respiratory parameters, such as hypoxic sensitivity ($\Delta \dot{V}_i/\Delta S_pO_2$), may become negative above certain drug concentrations. For example, we previously observed in one male subject that although hypoxia caused an increase in $\dot{V}_i$ during alfentanil infusion (target, 40 ng/ml) in the awake state, it caused an immediate decrease in ventilation when the subject was asleep (i.e., $[\dot{V}_i(\text{hypoxia}) - \dot{V}_i(\text{normoxia})]/[S_pO_2(\text{normoxia}) - S_pO_2(\text{hypoxia})] < 0$). Our model predicts such behavior at concentrations $> C_{50} \cdot 2^{\gamma}$ (figure 6). In some of the subjects in this study negative responses were observed for $\Delta V_T/\Delta S_pO_2$, $\Delta RR/\Delta S_pO_2$ and $\Delta HR/\Delta S_pO_2$ at high alfentanil concentrations.

3. Within a limited dose range (such as the range studied by us) some respiratory dose-response relations appear linear or almost linear. For example, halothane and isoflurane, over the dose range from 0 to 0.2 MAC, cause a linear decrease of the hypoxic ventilatory response (at 0.2 MAC $\Delta \dot{V}_i/\Delta S_pO_2$ is approximately 20% of control). In our model, a linear function is obtained when $\gamma = 1$ (fig. 6). Interestingly, for all parameters our model, fits yielded values of $\gamma$ not significantly different from 1. This indicates that, at least within the dose range we tested, the data are well-described by a linear relation between drug (sevoflurane or alfentanil) and effect. This is also true for combinations of both agents, but only at fixed concentration ratios ($Q$). Incorporation of higher doses might have yielded values of $\gamma$ significantly different from 1. This may be especially true for the BIS-sevoflurane relation.

Response Surface Modeling The response surface modeling method, as recently reported by Minto et al., is based on two ideas. First, there is the notion that the combination of two drugs should be regarded as a new drug with its own properties, with the concentration-effect relation $E = f(C_A/C_{50,A} + C_S/C_{50,S}; \psi)$ where the parameter vector $\psi$ (in our model $I(Q)$) controls the properties of the interaction and specifically how it deviates from pure additivity. Second, $\psi$ is assumed to depend only on the ratio of the concentrations of the two administered drugs. These two ideas are crucial because they allow for a greatly reduced number of parameters necessary to describe a surface and thus make them estimable from a study of reasonable size. A proper choice of $\psi$ and $f$ may further reduce the number of parameters while describing the concentration-effect relation in the range measured. We made two modifications to the model specified by Minto et al. First, interaction was taken into account by the function $I(Q)$ for which we chose a spline with two, interpretable parameters (see Appendix 1 for details). Second, for $f$ we chose equation 1.

Although visual inspection of the residuals did not show remaining structure, other possibilities of modeling the concentration-effect relation and the nature of the inter-
Figure 2. **TOP.** Left: Response surface modeling of the influence of plasma alfentanil concentration and end-tidal sevoflurane concentration on normoxic ventilation ($\dot{V}_i$) at a fixed $P_{ET}CO_2$. Right: Individual data points and isoboles of 25, 50, and 75% depression of $\dot{V}_i$. Open circles denote data points above the surface, closed circles denote data points below the surface (control data points not shown). The area of the circles is proportional to the distance from that data point to the surface area. A 25% reduction of $\dot{V}_i$ occurred at: an alfentanil concentration of $\sim 38$ ng/ml when no sevoflurane was present, at a sevoflurane concentration of $\sim 0.73\%$ when no alfentanil was present, and at $\sim 0.1\%$ sevoflurane when 15 ng/ml alfentanil was present. This indicates synergistic interaction ($I(Q) \sim 1.9$ at $Q$ of $\sim 0.7; P < 0.01$). **BOTTOM.** Left: Response-surface modeling of the influence of alfentanil and sevoflurane on the ventilatory response to acute hypoxia at a fixed $P_{ET}CO_2$ (i.e., the hypoxic drive). Right: Individual data points and isoboles of 25, 50, 75 and 100% reduction of hypoxic drive. A 25% reduction of hypoxic drive occurred at an alfentanil concentration of $\sim 16$ ng/ml when no sevoflurane was present, at a sevoflurane concentration of $\sim 0.14\%$ when no alfentanil was present, and at $\sim 0.05\%$ sevoflurane when 10 ng/ml alfentanil was present. This indicates additive interaction.
Figure 3. **TOP.** Left: Response surface modeling of the influence of plasma alfentanil concentration and end-tidal sevoflurane concentration on normoxic heart rate (HR) at a fixed $P_{ETCO_2}$. Right: Individual data points and isoboles of 10, 20, and 30% reduction of HR. Open circles denote data points above the surface, closed circles denote data points below the surface (control data points not shown). The area of the circles is proportional to the distance from that data point to the surface area. **BOTTOM.** Left: Response-surface modeling of the influence of alfentanil and sevoflurane on the HR response to acute hypoxia at a fixed $P_{ETCO_2}$. Right: Individual data points and isoboles of 25, 50, 75, and 100% reduction of the HR response to acute hypoxia at a fixed $P_{ETCO_2}$. 
action could be explored, for example, by using the function:

\[
f(x) = \frac{\alpha}{(1 + \delta + x^\gamma)^{1/\delta}}
\]

Now a spectrum of linear, sigmoidal, and asymmetric sigmoidal concentration-effect relations is possible with varying values of \( \gamma \) and \( \delta \). However, because in our analysis \( \gamma \) never significantly differed from 1, inclusion of additional nonlinear parameters, such as in equation 8, does not seem necessary. Finally, when synergistic interactions were detected (i.e., for \( \dot{V}_i \) and HR), the standard deviations of the estimated parameters did not warrant a more complex form of \( I(Q) \).

\[\dot{V}_i \text{ and HR versus } \Delta\dot{V}_i/\Delta S_{\text{PO}_2} \text{ and } \Delta \text{ HR}/\Delta S_{\text{PO}_2}\]

We observed synergistic alfentanil–sevoflurane interactions on \( \dot{V}_i \) and HR. This indicates that the effect of the drug combination was greater than expected from the dose-response curves of sevoflurane alone and alfentanil alone. Our analysis shows that for both variables, maximum synergy occurred at \( Q \) values of approximately 0.7. This indicates that, taking into account their respective \( C_{50} \) values, concentrations that give maximal synergy on reduction of \( \dot{V}_i \) are at fractions (or multiples) of 26.7 ng/ml for alfentanil and 0.24 \( \text{ET\%} \) for sevoflurane (maximum synergy causing 25% reduction of ef-

\[\text{Note that when } \delta \text{ is fixed to } -1, \text{ equation. } (8) \text{ reduces to eqn. } 1.\]

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**Figure 4.** LEFT. Response surface modeling of the influence of plasma alfentanil concentration and end-tidal sevoflurane concentration on the Bispectral Index (BIS). RIGHT. Individual data points and isoboles of 10, and 20% reduction of the BIS. Open circles denote data points above the surface, closed circles denote data points below the surface (control data points not shown). The area of the circles is proportional to the distance from that data point to the surface area. A 25% reduction of the BIS occurred at a sevoflurane concentration of \(~0.43\%\) (table 2), but no decrease was seen with increasing doses of alfentanil, even in the presence of sevoflurane.
Figure 5. The relations between $Q$ (i.e., the alfentanil–sevoflurane concentration ratio) and $I(Q)$ (i.e., the interaction) for ventilation (left) and heart rate (right). For both effects, synergistic interactions were observed over the range of concentration ratios from 0 to 1, with maximum synergistic interactions ($I_{\text{max}}$ in table 2) occurring at $Q$ values of about 0.7 ($Q_{\text{max}}$ in table 2).

Figure 6. Three examples of the concentration–effect relation predicted by the pharmacodynamic model $f(x) = E_0(1 - x^\gamma)$ where $x^\gamma = U^\gamma/2$ and $U = C/C_{50}$, obtained at three fixed values of $\gamma$ (0.2, 1 and 4). When $\gamma = 1$, the model becomes linear. At all three $\gamma$ values, negative effects are possible at drug concentrations $x > C_{50} \cdot 2^{\gamma}$. Note that the values for $C_{50}$ and $\gamma$ were chosen in such a way that the zero crossing for all three examples occurred at the same value of C. As a consequence the values of C causing 50% of the effect (i.e., $C_{50}$) occur at different values of C ($C_1$ through $C_3$ are the $C_{50}$ values for the three different curves). Different values for $C_{50}$ and $\gamma$ would result in the zero crossing at different values of C. $E_0 =$ baseline effect, $E_{50} = 50\%$ depression of effect.
fect occurs at $13.4\, \text{ng/ml alfentanil} + 0.12\, \text{ET}\%\, \text{sevoflurane}$, maximum synergy causing 50% reduction occurs at $26.7\, \text{ng/ml alfentanil} + 0.24\, \text{ET}\%\, \text{sevoflurane}$, and maximum synergy causing 100% reduction occurs at $53.4\, \text{ng/ml alfentanil} + 0.48\, \text{ET}\%\, \text{sevoflurane}$.\(^4\) For HR these concentrations are fractions of $86.6\, \text{ng/ml for alfentanil}$ and $0.48\, \text{ET}\%\, \text{for sevoflurane}$ ($= C_{50}$ values). Because we explored only a relatively small part of the response surface for $\dot{V}_i$ and HR, $C_{50}$ and $C_{100}$ values are extrapolations. As indicated above, nonlinearities of the ventilatory controller may cause apnea at concentrations higher than those explored in our protocol but lower than those estimated from our analysis. Moreover, we may have underestimated the magnitude of synergy in our study. Therefore, our results apply to the portion of the response surface analyzed and extrapolation should be performed with caution (especially for those surfaces that are explored only marginally, e.g., HR). The mechanism of the observed synergistic interactions remain elusive. Further studies (e.g., identifying shared central effect-sites for opioid- and anesthetic-induced respiratory depression) are needed to understand the synergistic effects of alfentanil and sevoflurane on ventilation and heart rate.

We observed additive alfentanil-sevoflurane interactions on $\Delta \dot{V}_i/\Delta S_P \text{O}_2$ and $\Delta \text{HR}/\Delta S_P \text{O}_2$. This indicates that the effect of the sevoflurane-alfentanil combination is expected from the concentration-response curve of the individual agents. The absence of synergy may be related to the different pathways through which sevoflurane and alfentanil depress the hypoxic response (inhalational anesthetics depress hypoxic response at sites within the peripheral chemoreflex loop,\(^4\)\(^7\)\(^2\)\(^0\) and opioids depress the hypoxic response through effects at the brainstem). Another possibility is that because of the great sensitivity of the hypoxic response to both agents and consequently the early loss of the hypoxic response (i.e., $\Delta \dot{V}_i/\Delta S_P \text{O}_2 = 0$ occurring at $60\, \text{ng/ml alfentanil}$ and $0.5\, \text{ET}\%\, \text{sevoflurane}$), we are unable to unearth any synergy from the data.

The observation that the hypoxic drive, relative to resting $\dot{V}_i$, is more sensitive to the effects of an opioid and an inhalational anesthetic, suggests that the hypoxic test is the more sensitive tool to assess the effects of anesthetics and opioids on ventilatory control.

One of the many determinants of HR at rest and during hypoxia is stimulation and depression of lung receptors by increases/ and decreases in $\dot{V}_i$ (i.e., HR effects are secondary to $\dot{V}_i$-effects).\(^5\) This may explain the agreement in the nature of alfentanil-sevoflurane interactions for $\dot{V}_i$ and HR and $\Delta \dot{V}_i/\Delta S_P \text{O}_2$ and $\Delta \text{HR}/\Delta S_P \text{O}_2$.

**Bispectral Index and Respiration**

In contrast to sevoflurane, alfentanil, in the absence and presence of sevoflurane, had no effect on BIS. This indicates that the algorithm for calculating the BIS is not sensitive to the changes in arousal level from opioids and the combination of opioids and anesthetics (at least within the dose-ranges we evaluated). Because we did not obtain other measures of sedation during the study (such as the subjective observers’ assesseme...
Figure 7. The influence of alfentanil on ventilation (left) Values are % of control) and hypoxic drive (right) Values are % of control) at constant bispectral index (BIS) values (so called iso-BIS curves). The BIS values are in italic and range from 95 to 65. The BIS values were fixed by inhalation of constant concentration of sevoflurane. Note that the hypoxic drive is more vulnerable than $\dot{V}_i$ for the effects of alfentanil at all tested BIS values.

ment of alertness and sedation score) we remained uninformed on the level of arousal during the alfentanil-sevoflurane studies. However, because clinicians and researchers use BIS monitoring, we constructed iso-BIS curves (equivalent to iso-effect curves, fig. 7). We now are informed about the steady-state relation between alfentanil and $\dot{V}_i$ and $\Delta\dot{V}_i/\Delta S_{PO2}$ at varying BIS levels. Further studies are needed to examine whether these steady-state relations are independent of sevoflurane and also apply to other agents that affect the BIS (such as propofol, isoflurane, dexmedetomidine, midazolam), or that separate BIS-opioid-$\dot{V}_i$-relations exist for each anesthetic/hypnotic.

Study Limitations
Some methodologic issues deserve further comment. We used an isohypercapnic design ($P_{ET}CO_2$ fixed to $\sim$48 mmHg). This enables the comparison of the effect of various drug concentrations on $\dot{V}_i$ without the confounding influence of variations in $P_{ET}CO_2$. However, because every drug effect on $\dot{V}_i$ is conditional on a certain $P_{ET}CO_2$ value, other results might be seen at different carbon dioxide concentrations. Furthermore, because we performed a steady-state study (drug concentrations, $P_{ET}O_2$, and $P_{ET}CO_2$ were clamped during hypoxic testing), the time course of drug-induced variations in blood gases (such as those that may occur during bolus opioid infusions) and their translation into $\dot{V}_i$ and oxygen delivery were not modeled. Further studies are needed to examine the blood gas and $\dot{V}_i$ dynamics caused by the administration of opioids and anesthetics occurring in the clinical setting.
APPENDIX

For the function $I(Q)$ we use a spline with a piece $g_1(x)$ between knots at $x = 0$ and $x = Q_{\text{max}}$ and a piece $g_2(x)$ between knots $x = Q_{\text{max}}$ and $x = 1$, constrained by the following eight conditions:

\begin{align*}
(9) & \quad g_1(0) = g_2(1) = 1 \\
(10) & \quad g_1(Q_{\text{max}}) = g_2(Q_{\text{max}}) = I_{\text{max}} \\
(11) & \quad \frac{dg_1(x)}{dx} \bigg|_{x=Q_{\text{max}}} = \frac{dg_2(x)}{dx} \bigg|_{x=Q_{\text{max}}} = 0 \\
(12) & \quad \frac{d^2g_1(x)}{dx^2} \bigg|_{x=0} = \frac{d^2g_2(x)}{dx^2} \bigg|_{x=1} = 0
\end{align*}

The first four constraints (eqns. 9 and 10) deal with the values of $I(Q)$ at the three knots, constraints five and six (eqn. 11) make the second knot a maximum (or minimum) and constraints seven and eight (eqn. 12) are natural boundary conditions at the first and last knot and assure the absence of values outside $(1, I_{\text{max}})$. Because we specify eight conditions, we can utilize a cubic spline so that each piece is given by a third-order polynomial:

\begin{equation}
\begin{aligned}
g(x) &= a_0 + a_1 x + a_2 x^2 + a_3 x^3 \\
\end{aligned}
\end{equation}

The parameters $a_i$ are such that the above constraints are satisfied. Note that they need not be interpreted, but are merely functions of $I_{\text{max}}$ and $Q_{\text{max}}$. For $g_1(x)$ we have:

\begin{align*}
a_0 &= 1 \\
a_1 &= -3(1 - I_{\text{max}})/(2Q_{\text{max}}) \\
a_2 &= 0 \\
a_3 &= (1 - I_{\text{max}})/(2Q_{\text{max}}) / Q_{\text{max}}^2
\end{align*}

For $g_2(1-x)$, the parameters $a_i$ are obtained by substituting $(1 - Q_{\text{max}})$ for $Q_{\text{max}}$. 