Impact of peripheral elimination on the concentration–effect relationship of remifentanil in anaesthetized dogs

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Background. This study elucidates the impact of sampling site when estimating pharmacokinetic-pharmacodynamic (PK-PD) parameters of drugs such as remifentanil that undergo tissue extraction in the biophase. The interrelationship between the concentrations of remifentanil predicted for the effect compartment and those measured in arterial, venous, and cerebrospinal fluid were investigated under steady-state conditions.

Methods. Following induction of anaesthesia with pentobarbital, an arterial cannula (femoral) and two venous catheters (jugular and femoral) were inserted. Electrodes were placed for EEG recording of theta wave activity. Each dog received two consecutive 5-min infusions for the PK-PD study and a bolus followed by a 60-min infusion was started for the steady-state study. Cerebrospinal fluid, arterial and venous blood samples were drawn simultaneously after 30, 40, and 50 min. At the end of the infusion, arterial blood samples were collected for pharmacokinetic analysis.

Results. Remifentanil PK-PD parameters based on theta wave activity were as follows: apparent volume of distribution at steady-state (Vss) (231–37 ml kg⁻¹), total body clearance (Cl) (63–16 ml min⁻¹ kg⁻¹), terminal elimination half-life (t1/2b) (7.71 min), effect compartment concentration at 50% of maximal observed effect (EC50) (21–13 ng ml⁻¹), and equilibration rate constant between plasma and effect compartment (ke0) (0.48–0.24 min⁻¹). The mean steady-state cerebrospinal fluid concentration of 236 ng ml⁻¹ represented 52 and 74% of that in arterial and venous blood, respectively.

Conclusions. Our study re-emphasizes the importance of a sampling site when performing PK-PD modelling for drugs undergoing elimination from the effect compartment. For a drug undergoing tissue elimination such as remifentanil, venous rather than arterial concentrations will reflect more exactly the effect compartment concentrations, under steady-state conditions.

Keywords: model, dog; pharmacodynamics; pharmacokinetics, remifentanil

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Remifentanil was designed to be short acting by incorporating a methyl ester group susceptible to enzymatic hydrolysis.¹ Unlike other opioids, remifentanil undergoes widespread metabolism by blood and tissue non-specific esterases.²³ Chism and Rickert have shown previously that amongst all tissues examined in dogs, the muscle, intestines, and brain exhibited the highest extraction ratios.⁴ The pioneering work of Chiou and colleagues emphasized the marked dependence of drug concentration on blood sampling site.⁵⁶ In non-steady-state pharmacokinetic-pharmacodynamic (PK-PD) studies, the time lag between the maximum concentration in arterial and venous blood has always been a major concern.⁷⁻¹¹ It was stated that the concentration–effect relationship could be best described by measuring arterial blood, as it carries the drug to various parts of the body to produce the pharmacological response. However, for drugs undergoing tissue extraction the question remains unclear.

Several investigators pointed out the implications of having an arteriovenous gradient present at the sampling site on the concentration–effect relationship of a drug. Parameters such as the effect compartment concentration at 50% of maximal observed effect (EC50), equilibration rate constant between plasma and effect compartment (ke0), and slope factor (gamma) have been shown to differ

¹Declaration of interest. Remifentanil was provided by Abbott Laboratories.
for atracurium.\textsuperscript{12} This effect would be amplified for a drug such as remifentanil that is rapidly eliminated within the biophase itself. Recently, Hermann and coworkers pointed out that inappropriate conclusions such as acute tolerance could have been inferred if venous samples were used for the estimation of remifentanil PK-PD parameters in female volunteers.\textsuperscript{13}

The primary objective of our study was to elucidate the impact of sampling site when estimating PK-PD parameters of drugs undergoing tissue extraction in the biophase. For doing so, we investigated the interrelationship between the predicted effect compartment concentrations of remifentanil, a drug undergoing tissue elimination, and the concentrations measured in arterial, venous, and cerebrospinal fluid sampling sites under steady-state conditions. A PK-PD study of remifentanil in pentobarbital-anaesthetized dogs was thus undertaken.

Materials and methods

Chemicals

Remifentanil hydrochloride and the internal standard (I.S.) (GI 97559) were provided by Glaxo-Wellcome (Stevenage, UK). The commercial preparation Ultiva\textsuperscript{®} (5 mg-vial) was supplied by Abbott Laboratories (Montréal, Québec, Canada). All solvents were of high performance liquid chromatography (HPLC) grade and purchased from Anachemia (Montréal, Québec, Canada).

Animal preparation

Experimental procedures in this study adhered to the Canadian Council on Animal Care and were approved by the Université de Montréal Animal Care and Ethics Committee before animal experimentation. The in\textit{vivo} experiment conducted in anaesthetized dogs is shown in Figure 1.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Animal preparation & PK-PD period & Washout period & Steady state period & PK period \\
\hline
\end{tabular}
\end{table}

Animals. Mongrel (\textit{n}=5) and beagle (\textit{n}=2) dogs (8.8–20 kg) were obtained from Biolab (Montréal, Québec, Canada). The animals were fasted overnight but allowed free access to water. On the day of the experiment, each dog was anaesthetized with an i.v. dose of sodium pentobarbital (Somnotol\textsuperscript{®}, 30 mg kg\textsuperscript{-1}). The level of anaesthesia was monitored and adjusted according to haemodynamic parameters as well as corneal reflex. Intermittent bolus doses (4 mg kg\textsuperscript{-1}) were administered for maintenance of deep anaesthesia. Respiration was controlled through a tracheal tube, with room air delivered by a respirator (model 607, Harvard, South Natick, MA). Body temperature was monitored and kept constant at 38°C throughout the experiment by a thermoregulator (model 74; Yellow Springs Instruments, Yellow Spring, OH) connected to a heating pad.

\textbf{Implantation of electrodes and recording.} Before surgery, four surface electrodes (circular silver/silver chloride, SAFELEAD\textsuperscript{®} model) were placed bilaterally on the skin for centro-parietal EEG recording using a four-channel polygraph (Grass Model 7400, Astro-Med, West Warwick, RI). The low pass filter was set at 60 Hz, the time constant at 0.3 s, and the gain at 50 µV cm\textsuperscript{-1}. The EEG waveform was allowed to stabilize in each dog and 5-min recording periods were taken at 90, 60, and 30 min before drug administration to verify the adequacy of the signal. Grass Polyview\textsuperscript{TM} software (version 2.1, Astro-Med, West Warwick, RI) was used for data acquisition and off-line analysis.

\textbf{Animal surgery.} The right femoral vein was cannulated with a polyethylene tube for remifentanil and pentobarbital administration and the right femoral artery was cannulated for blood sampling. A pressure transducer was connected to the arterial line cannula. A three-way stopcock was placed between the dog and the pressure transducer to allow a switch between arterial pressure measurement and arterial blood sampling. ECG leads were inserted subcutaneously into the right and left hindlimbs and forelimbs. Haemodynamic

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1}
\caption{\textit{In vivo} experimental protocol in pentobarbital anaesthetized dogs receiving different remifentanil regimens.}
\end{figure}
parameters were monitored using a polygraph system (model RM-6000, Nihon Kohden). An indwelling catheter (Cathalon®, Critikon, Tampa, FL) was inserted in the left external jugular vein to allow venous blood sampling.

Protein binding. Before starting remifentanil infusion, 8 ml of blood were collected in a tube containing 800 μl of sodium citrate 0.129 M. After centrifugation, blank plasma was kept frozen at −70°C until protein binding determination. Remifentanil was spiked, extemporaneously, in thawed blank plasma of each dog to provide a final concentration of 1000 ng ml⁻¹. Ultrafiltration was performed in duplicate using 30 000-μ membrane (Amicon Centrífree Micropartition System, Beverly, MA). Ultrafiltration devices containing 1 ml of plasma were centrifuged at 1900 g for 5 min at room temperature. An aliquot (50 μl) of the ultrafiltrate was then diluted in 450 ml of acidified plasma and processed as plasma samples. Total (Cₚ) and unbound concentrations (C₀) were determined the same day by high-pressure liquid chromatography. The free fraction (fₚ) was calculated from the ultrafiltrate/total concentration ratio.

PK-PD period
Each dog received two consecutive 5-min infusions of Ultiva® 1 mg kg⁻¹ min⁻¹ and 10 ng kg⁻¹ min⁻¹. This infusion scheme was designed to obtain a slow onset of action (infusion #1) and a robust characterization of the terminal elimination half-life (infusion #2). EEG was recorded continuously from drug administration until 40 min later. Blood samples (3 ml) were collected in chilled heparinized tubes at 2, 4, 6, 8, 10, 11, 12, 14, and 20 min after the first infusion started.

Washout period
Cerebrospinal fluid puncture. Before the steady-state infusion started the animal was placed in lateral recumbency with the nose flexed and ears pulled ventrally. A spinal needle was slowly introduced and cerebrospinal fluid was punctured from the cisterna magna, between the occipital crest and the most prominent points of the wings of the atlas. When cerebrospinal fluid started flowing out of the needle, the stylet was replaced until cerebrospinal fluid sampling.

Steady-state period
Approximately 2 h after the second infusion stopped, each dog received a bolus dose of remifentanil Ultiva® 50 μg kg⁻¹, immediately followed by a 60-min infusion of 40 μg kg⁻¹ min⁻¹ (infusion #3).

Arteriovenous gradient. At steady-state, arterial (femoral) and venous (jugular) blood samples (3 ml) were simultaneously collected 30, 40, and 50 min after remifentanil infusion #3 started. Blood samples were collected into heparinized tubes maintained on ice and centrifuged within 30 min. Plasma was stored at −70°C until HPLC analysis.

Plasma samples were assayed in duplicate and the mean of the two values was used.

Cerebrospinal fluid samples. At steady-state, cerebrospinal fluid (0.7 ml) was collected into acidified tubes (7 μl of citric acid 50%) at times corresponding to blood sampling. A volume of cerebrospinal fluid equivalent to the calculated dead volume of the needle was discarded before each sample collection. Cerebrospinal fluid samples were frozen at −70°C until HPLC analysis.

Pharmacokinetic period
At the end of remifentanil infusion, arterial blood samples (3 ml) were collected 1 min before the infusion ended, then every 2 min for 15 min and finally every 5 min for 15 additional min. At the end of the experiment, dogs were killed using a mixture of KCl and pentobarbital.

Red blood cell/plasma partition coefficient
Distribution in red blood cell was investigated ex vivo in a separate set of dogs as described previously. Remifentanil (1000 ng ml⁻¹) was incubated in blood at 37°C with gentle agitation (horizontal Eberbach). Samples (6 ml) were collected at 5, 60, 90, and 120 min. An aliquot (4 ml) was centrifuged. Remifentanil concentrations in whole blood (Cb) and plasma (Cp) were determined in triplicate by HPLC after liquid–liquid extraction. Using the haematocrit value, it was possible to calculate red blood cell/plasma partition coefficient as follows:

\[
\frac{C_{\text{RBC}}}{C_p} = \frac{Cb/Cp-(1-Hct)}{Hct}
\]

Sample analysis
A selective and specific HPLC method developed previously for the quantification of remifentanil in dog plasma was used. Briefly, plasma and cerebrospinal fluid samples (0.5 ml) were extracted on C-phenyl solid phase cartridges (Phenomenex, Torrance, CA). Separation was performed on a 5 μm reversed-phase Spherisorb C₁ column (150×4.6 mm ID) purchased from Phenomenex (Torrance, CA). The mobile phase contained 28% organic solvent in 2.4 mM potassium phosphate buffer and was delivered at a flow rate of 1.5 ml min⁻¹. UV detection was set at 210. Peak integration was performed using Star Chromatography Varian Software version 4.51 (Walnut Creek, CA).

This method allowed quantification of remifentanil levels as low as 7.81 ng ml⁻¹. The coefficients of variation for intra- and inter-day precision were less than 11% over the concentration range of 7.81–2000 ng ml⁻¹.

Data analysis
Pharmacodynamic analysis. The EEG signal was digitized in the analogue-to-digital converter and processed by the...
computer. A quantitative analysis of 30-s epochs was made continuously from 5 min before drug injection until the EEG waveform returned to baseline. A fast Fourier transform was used to calculate the power spectrum of the selected epoch (30 000 sample points) to obtain power spectral density vs frequency histograms (amplitude² Hz⁻¹). The power spectral density was computed for the following four predefined frequency bands: 0–4 Hz (delta waves), 4–8 Hz (theta waves), 8–12 Hz (alpha waves), and 12–30 Hz (beta waves). For each epoch the absolute power in the theta band was selected for the analysis of remifentanil pharmacodynamics. In the first two dogs, the signal was too noisy (interferences caused by the surgical lights and the heating pad) and the median value for six epochs over a 3-min period was used instead. Variability in the baseline signal ranged between 10 and 16% and theta activity did not change significantly upon administration of a pentobarbital bolus.

Pharmacokinetic analysis. The pharmacokinetics of remifentanil were evaluated for individual dogs using a traditional two-compartment model with two consecutive zero order input rates followed 2 h later by an i.v. bolus and a zero order input rate, with elimination from the central compartment. Goodness of fit was evaluated according to the Akaike’s information criterion. Pharmacokinetic analysis was based on iterative linear least square regression analysis using WinNonlin software (WinNonlin, Professional Edition version 1.5, Pharsight, Mountain View, CA). A weighting function 1/(predicted Y) was applied. Descriptive curve parameters were determined: the distribution (a) and elimination (b) rate constants and their corresponding A and B coefficients. The following parameters were derived: the first-order rate constant of elimination from the central compartment (k10), the first-order rate constants associated with drug transfer from compartment 1 to compartment 2 (k12), and from compartment 2 to compartment 1 (k21). The volume of the central compartment (Vc), the steady-state volume of distribution (Vss) and the total clearance (Cl) were then calculated.

PK-PD modelling. For each dog, the pharmacokinetic parameters derived for remifentanil were fixed during two-step PK-PD modelling. A parametric link model was used to derive the elimination rate constant from the hypothetical effect compartment (κa) using WinNonlin software. The sigmoid Emax model was used to determine the remifentanil concentration that produces 50% of the maximal observed effect (EC50) and the slope factor γ. The baseline value E₀ was measured before remifentanil administration and was fixed as an initial parameter for the model:

\[ E = \frac{E_{max}C^\gamma}{EC_{50}^\gamma + C^\gamma} + E_0 \]

A weighting function of 1 was applied. Goodness of fit was assessed by the Akaike information criterion.

Arteriovenous gradient. For each dog, the arterial concentrations (C_{Pss,a}) measured at the three collection times were compared using the Friedman Repeated Measures Analysis of Variance on Ranks. After statistical confirmation of steady-state conditions, the three concentrations were averaged. Venous concentrations (C_{Pss,v}) were treated similarly and remifentanil arteriovenous gradient (E_{brain}) across the brain was calculated as follows:

\[ E_{brain}(\%) = \frac{C_{Pss,a} - C_{Pss,v}}{C_{Pss,a}} \times 100 \]  

Effect compartment concentration. For each dog, the pharmacokinetic and PK-PD parameters derived were then used to predict the pharmacological effect in the effect compartment (Ce). In a two-compartment model, drug concentration in the effect compartment after i.v. infusion can be expressed as follows:

\[ Ce = \frac{k_{00}k_0}{V_c} \left[ \frac{(E_2-\alpha)}{\alpha(\beta-\alpha)(k_0-\alpha)}(1-e^{-\alpha T})e^{-\alpha t'} + \frac{(E_2-\beta)}{\beta(\alpha-\beta)(k_{00}-\beta)}(1-e^{-\beta T})e^{-\beta t'} + \frac{(E_2-k_0)}{k_0(\alpha-k_{00})(\beta-k_{00})}(1-e^{-k_0 T})e^{-k_0 t'} \right] \]

Where k₀ is the infusion rate, E₂, the sum of the rate constants exiting the peripheral compartment (i.e. k₂₁+k₂₀), T, the time duration of each infusion, and t’, the time after stopping the infusion.

The effect compartment concentrations derived were then corrected (C_{e,corr.}) for the unbound plasma fraction, brain extraction and cellular partition coefficient (approximated by red blood cell/plasma partition coefficient) as follows:

\[ C_{e,corr.} = C_e \times f_u \times (1-E_{brain}) \times C_{RBC}/C_p \]

Results

Arterial pressure and heart rate were monitored continuously during remifentanil administration. Haemodynamic parameters returned to their baseline value 15–30 min after infusion #2 ended. Baseline and steady-state values (infusion #3) are reported in Table 1. Remifentanil induced a rapid significant upon administration of a pentobarbital bolus. Between 10 and 16% and theta activity did not change significantly upon administration of a pentobarbital bolus.

The pharmacokinetics of remifentanil were evaluated for individual dogs using a traditional two-compartment model with two consecutive zero order input rates followed 2 h later by an i.v. bolus and a zero order input rate, with elimination from the central compartmennt. Goodness of fit was evaluated according to the Akaike’s information criterion. Pharmacokinetic analysis was based on iterative linear least square regression analysis using WinNonlin software (WinNonlin, Professional Edition version 1.5, Pharsight, Mountain View, CA). A weighting function 1/(predicted Y) was applied. Descriptive curve parameters were determined: the distribution (a) and elimination (b) rate constants and their corresponding A and B coefficients. The following parameters were derived: the first-order rate constant of elimination from the central compartment (k₁₀), the first-order rate constants associated with drug transfer from compartment 1 to compartment 2 (k₁₂), and from compartment 2 to compartment 1 (k₂₁). The volume of the central compartment (Vc), the steady-state volume of distribution (Vss) and the total clearance (Cl) were then calculated.

PK-PD modelling. For each dog, the pharmacokinetic parameters derived for remifentanil were fixed during two-step PK-PD modelling. A parametric link model was used to derive the elimination rate constant from the hypothetical effect compartment (kₐ) using WinNonlin software. The sigmoid Emax model was used to determine the remifentanil concentration that produces 50% of the maximal observed effect (EC₅₀) and the slope factor γ. The baseline value E₀ was measured before remifentanil administration and was fixed as an initial parameter for the model:

\[ E = \frac{E_{max}C^\gamma}{EC_{50}^\gamma + C^\gamma} + E_0 \]  

A weighting function of 1 was applied. Goodness of fit was assessed by the Akaike information criterion.
anaesthesia before remifentanil administration, the raw baseline EEG signal varied between dogs. This is consistent with the high coefficient of variation observed for $E_0$ (Table 2). Accordingly, for graphical illustration purposes, the data represented in Figure 2A are displayed as a percentage of maximal observed effect. The profiles showed a rapid onset and termination of effect. The mean estimate of $\gamma$ representing the sigmoidal relationship between effect site concentrations and EEG changes was 1.79 (0.51) (Table 2).

Mean equilibration half-life between remifentanil effect and central compartment concentrations ($t_{1/2\ k_{e0}}$) was 1.44 min (Table 2). Using the absolute power between 4–8 Hz, the mean EC$_{50}$ value of remifentanil in the presence of pentobarbital was found to be 21 ng ml$^{-1}$ (Table 2, Fig. 3).

Plasma concentration–time profile of remifentanil, was adequately described by a two compartment pharmacokinetic model (Fig. 4). The pharmacokinetic parameter estimates for remifentanil in anaesthetized dogs [mean (SD)] are listed in Table 3. The mean terminal elimination half-life of remifentanil in dogs was estimated to be 7.71 min.

Mean remifentanil red blood cell/plasma partition coefficient obtained by ex vivo incubation was 0.89 (0.01) and the mean unbound plasma fraction was 71% (Table 4). Based on statistical analysis, unbound arterial plasma concentrations ($C_{p\text{ss}u,a}$) were stable in each dog during the three sampling periods ($P=0.564$) confirming that steady-state was reached. Unbound venous concentrations ($C_{p\text{ss}u,v}$) were found to be consistently lower than unbound arterial concentrations (Table 4). Mean jugular venous

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**Table 1** Physiological parameters at baseline and during remifentanil steady-state conditions in pentobarbital anaesthetized dogs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Weight (kg)</th>
<th>Haematocrit (%)</th>
<th>Arterial pressure (mm Hg)</th>
<th>Heart rate (beats min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Steady-state</td>
<td>Baseline</td>
<td>Steady-state</td>
</tr>
<tr>
<td>1</td>
<td>11.0</td>
<td>31</td>
<td>190/170</td>
<td>120/100</td>
</tr>
<tr>
<td>2</td>
<td>20.0</td>
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<td>210/170</td>
<td>140/90</td>
</tr>
<tr>
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<td>19.8</td>
<td>36</td>
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<td>130/70</td>
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<td>200/180</td>
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<td>36</td>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>SD</td>
<td>4.4</td>
<td>3</td>
<td>27/21</td>
<td>13/14</td>
</tr>
</tbody>
</table>

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**Fig 2** Raw data representing (A) the percentage of the maximal observed absolute power calculated in the theta band (% $E_{\text{max}}$) and (B) the plasma concentration time profile of remifentanil in pentobarbital anaesthetized dogs.

**Fig 3** Observed and predicted absolute power in the theta band during two consecutive 5-min infusions of remifentanil in pentobarbital anaesthetized dog #4.
Table 2 Remifentanil PK-PD parameters in pentobarbital anaesthetized dogs. $k_{e0}$, effect compartment equilibration rate constant; EC50, effect compartment concentration at 50% of maximal observed effect; gamma, slope factor

<table>
<thead>
<tr>
<th>Dog</th>
<th>$E_0$ (V² Hz⁻¹)</th>
<th>$E_{max}$ (V² Hz⁻¹)</th>
<th>$k_{e0}$ min⁻¹</th>
<th>EC50 ng ml⁻¹</th>
<th>$\gamma$</th>
<th>AIC</th>
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<tbody>
<tr>
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<td>0.44</td>
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<tr>
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<tr>
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<td>82</td>
<td>50</td>
<td>63</td>
<td>28</td>
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</tr>
</tbody>
</table>

Table 3 Remifentanil pharmacokinetic parameters in pentobarbital anaesthetized dogs. A, $a$, coefficient and initial distribution rate constant; B, $b$, coefficient and terminal elimination rate constant; $k_{e0}$, elimination rate constant from compartment 1; $k_{12}$, transfer rate constant from the first to the second compartment; $k_{21}$, transfer rate constant from the second to the first compartment; $V_1$, apparent volume of distribution in the central compartment; Cl, total body clearance; $V_{ss}$, apparent volume of distribution at steady state

<table>
<thead>
<tr>
<th>Dog</th>
<th>Descriptive curve parameters</th>
<th>AIC Microconstants</th>
<th>PK parameters</th>
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<tbody>
<tr>
<td></td>
<td>A (ng ml⁻¹ kg⁻¹)</td>
<td>B (ng ml⁻¹ kg⁻¹)</td>
<td>$\alpha$ (min⁻¹)</td>
</tr>
<tr>
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<tr>
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<tr>
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<tr>
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<tr>
<td>CV</td>
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![Fig 4](https://academic.oup.com/bja/article-abstract/94/3/357/265432/1000)

**Fig 4** Plasma concentration–time profile of remifentanil in a pentobarbital anaesthetized dog (#4). Markers represent the unbound arterial, venous, and cerebrospinal fluid concentrations. Lines represent the predicted unbound arterial concentrations and effect compartment concentrations corrected for the unbound fraction, brain extraction and tissue partition coefficient.
is stopped Ce, corr is similar to or slightly exceeds arterial theta band (4–8 Hz). This may explain why delta activity frequency bands (0.5–9.5 Hz) increases but the increase in frequency and increase in amplitude. Power in the low dependent suppression of the EEG with an overall decrease in concentration amounted to 70% of mean arterial concentration (P=0.005). The mean individual arteriovenous gradient across the brain averaged 34% (Table 4).

During steady-state, the mean concentration of remifentanil in the cerebrospinal fluid was 236 ng ml⁻¹ and represented 77 and 54% of the unbound concentrations in the jugular vein and femoral artery, respectively (Table 4). The mean predicted effect compartment concentration at steady-state was 641 ng ml⁻¹ and approximated total arterial concentrations. When a correction factor was applied to Ceₐs accounting for brain extraction, unbound fraction and cellular partition coefficient, the resulting mean concentration (Ceₐcorr.) of 292 ng ml⁻¹ (Table 4) was close to that measured in the cerebrospinal fluid under steady-state conditions (236 ng ml⁻¹). Simulations indicated that when the infusion is stopped Ceₐcorr is similar to or slightly exceeds arterial concentrations (Fig. 4).

Discussion
Under steady-state conditions, a large gradient between the arterial and cerebrospinal fluid concentrations of remifentanil was observed in anaesthetized dogs. This gradient was less pronounced with the venous concentrations. When a correction factor taking into account plasma protein binding, tissue extraction and cell partition coefficient was applied to the arterial concentrations, the effect compartment concentrations derived for plasma protein binding, brain extraction, and cell partition coefficient were representative of those measured in the biophase.

Like other opioids, remifentanil produces a dose-dependent suppression of the EEG with an overall decrease in frequency and increase in amplitude. Power in the low frequency bands (0.5–9.5 Hz) increases but the increase in the delta band (0–4 Hz) is generally greater than that in the theta band (4–8 Hz). This may explain why delta activity in anaesthetized rats and dogs and spectral edge in conscious rats and humans have been the most widely used parameters to quantify the changes induced on the raw EEG signal during remifentanil PK-PD studies. In our study, dogs were maintained under pentobarbital anaesthesia before the administration of remifentanil; consequently, 95% of the total power spectrum was located in the delta band at baseline. This explains why spectral edge was not chosen to measure remifentanil-induced EEG changes.

When the absolute power in each frequency band was computed over time, we observed that delta activity remained stable after remifentanil administration while that in the theta band changed according to remifentanil blood concentrations. Using the absolute theta power instead of the relative theta ratio for the monitoring of remifentanil effect in pentobarbital-anaesthetized animals yielded the best correlation with the traditional sigmoid Emax model. This may be explained by the interdependence of the different frequency bands that results from both the calculation of the relative power itself and the low contribution of the theta band in the total power spectrum.

Remifentanil showed a rapid onset and a short duration of action in our canine study, a finding similar to that reported in isoflurane-anaesthetized dogs. Based on changes in the theta band, mean equilibration rate half-life between remifentanil plasma and effect compartment concentrations of the relative theta ratio for the monitoring of remifentanil effect in pentobarbital-anaesthetized animals yielded the best correlation with the traditional sigmoid Emax model. This may be explained by the interdependence of the different frequency bands that results from both the calculation of the relative power itself and the low contribution of the theta band in the total power spectrum.

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Table 4 Comparison of arterial, venous, and cerebrospinal fluid concentrations of remifentanil with those derived for the effect compartment under steady-state conditions in anaesthetized dogs. fₐwp free fraction in plasma; Eₐbrain brain extraction; Pₐwp, steady-state unbound arterial concentrations; Cₐwp, steady-state arterial concentrations; Cₐwp, steady-state venous concentrations; Cₛₛₐ, steady-state arterial cerebrospinal fluid concentrations; Cₛₛₐ, steady-state cerebrospinal fluid concentrations; Cₛₛₐ, steady-state effect compartment concentrations; Cₛₛₐ,corr., steady-state effect compartment concentrations corrected for plasma protein binding, brain extraction, and cell partition coefficient.

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<th>Dog</th>
<th>fₐwp (%)</th>
<th>Eₐbrain (%)</th>
<th>Cₛₛₐ (ng ml⁻¹)</th>
<th>Cₛₛₐ (ng ml⁻¹)</th>
<th>Cₛₛₐ (ng ml⁻¹)</th>
<th>Cₛₛₐ (ng ml⁻¹)</th>
<th>Cₛₛₐ (ng ml⁻¹)</th>
<th>Cₛₛₐ,corr. (ng ml⁻¹)</th>
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being that arterial levels are more representative of the concentration delivered to the effect site during the onset period. It is unclear whether this is valid for drugs undergoing elimination in the biophase. Hermann and colleagues found a clockwise hysteresis between EEG activity and remifentanil venous blood concentration while a counter-clockwise hysteresis was observed with arterial data. Such a finding was not interpreted as the development of acute tolerance, but as a faster equilibrium between the effect site and arterial concentrations compared with that between arterial and venous levels. However, drug elimination in the biophase may also contribute to this effect.

Sampling site is also crucial for determining the ‘true’ clearance of a drug. From a pharmacokinetic point of view, dosing and sampling should take place from the same site to avoid any bias. Since intra-arterial drug administration is not acceptable, this complicates the interpretation of PK parameters obtained from arterial concentrations after i.v. administration of a drug undergoing peripheral elimination. In our study, steady-state arterial concentrations of remifentanil were 1.4 times higher than those in the jugular vein. This brain extraction ratio of 34% is higher but consistent with that observed in two pentobarbital-anaesthetized dogs (24%) (Table 4). Similarly, following the administration of a bolus dose of remifentanil to healthy female subjects, a 40% overestimation in the systemic clearance was observed when calculations were based on data obtained from a peripheral vein. Of interest, the systemic clearance of remifentanil in pentobarbital/barbital anaesthetized dogs was reported to be three to four times higher (133 ml min⁻¹ kg⁻¹) than that observed in conscious dogs (41 ml min⁻¹ kg⁻¹). It was suggested that the higher clearance was a result of a higher arteriovenous gradient in the femoral compared with the saphenous vein. This indicates that the site of placement of the venous catheter (or regional tissue uptake) will also have a major impact on the estimation of systemic clearance of a drug exhibiting peripheral elimination.

Compared with alfentanil, remifentanil is more hydrophilic (octanol/water partition coefficient of 18 and 129, respectively) and the non-ionized fraction in plasma is lower (67 vs 89%) which could in part explain a smaller Vdss for remifentanil. Nevertheless, this value remains too small to account for the intracellular distribution of remifentanil. Thus, a compartmental model accounting for both central and peripheral elimination could have been applied to estimate remifentanil Vdss, an exit-site-dependent parameter but this would have no impact on the estimation of kss and EC50 values. For this reason, a pharmacokinetic analysis assuming elimination from the central compartment only was used in this study.

Previous estimates of the pharmacokinetic parameters of remifentanil based on whole blood samples were similar to those derived here using plasma concentrations. This implies that remifentanil red blood cell/plasma partition coefficient should be close to one. Accordingly, remifentanil mean red blood cell/plasma partition coefficient was shown to be 0.9.

Using this approach, the unbound concentration of drug measured in plasma and that predicted for the effect compartment concentrations are assumed to be equal at distribution equilibrium. This premise allows the derivation of Ceα without needing to know the volume of the effect site compartment. Remifentanil plasma concentration was corrected for the unbound fraction (0.7) and the concentration in CSF considered as 100 % unbound in view of the low concentration in proteins. As remifentanil undergoes elimination in the biophase, the arteriovenous gradient across the brain tissue was also included in the correction factor. Finally, because of the tight junctions present between the endothelial cells in the brain and between the epithelial cells in the choroids plexus we took into account the cellular partition coefficient in the correction factor.

The concentration of remifentanil was measured in the CSF to verify if the concentration derived for the effect compartment is anatomically and/or physiologically sound. In view of the limitations of the compartmental pharmacokinetic analysis, this type of comparison is often perceived as incorrect. However, attempts have been made in the past years to render the interpretation of compartmental analysis more physiological. By monitoring the EEG, we evaluated the supraspinal action of the opioid that causes sedation and not the analgesic action. It would have been impossible to carry out brain microdialysis and EEG monitoring simultaneously without introducing a bias. Although the brain extracellular fluid and the CSF are produced independently, they are in direct communication with one another so that changes in the composition of one are reflected in the composition of the other. In addition, the partition coefficient of remifentanil is 17 times that of morphine, we assumed that the rate of drug transfer across the blood–brain barrier and blood–CSF barrier via the choroid plexus would not be rate-limiting.

In our opinion, the CSF is part of the effect compartment but not necessarily the sole component. Our results indicate that the concentration of remifentanil measured in the CSF and that predicted for the effect compartment (after appropriate correction) are very similar under steady-state conditions. Therefore, it would be logical to infer that this concentration is representative of the extracellular concentration of remifentanil in the biophase. For remifentanil, a correction factor should therefore be applied to the EC50 based on arterial levels. In doing so, comparison of its potency with other opioids would be more appropriate.

**Conclusion**

Our study re-emphasizes the importance of sampling site when performing PK-PD modelling for an accurate estimation of total clearance and potency of drugs.
References