The Comparative Pharmacodynamics of Remifentanil and Its Metabolite, GR90291, in a Rat Electroencephalographic Model

Eugène H. Cox, Ph.D.,* Mariska W. E. Langemeijer, B.Sc.,† Josy M. Gubbens-Stibbe, B.Sc.,‡ Keith T. Muir, Ph.D.§, Meindert Danhof, Ph.D.¶

Background: The purpose of this study was to investigate the in vivo pharmacodynamics and the pharmacodynamic interactions of remifentanil and its major metabolite, GR90291, in a rat electroencephalographic model.

Methods: Remifentanil and GR90291 were administered according to a stepwise infusion scheme. The time course of the electroencephalographic effect (0.5–4.5 Hz) was determined in conjunction with concentrations of the parent drug and the metabolite in blood.

Results: Administration of remifentanil resulted in concentrations of remifentanil and GR90291 in the ranges 0–120 ng/ml and 0–850 ng/ml, respectively. When the metabolite was administered, concentrations of the metabolite in the range 0–220 μg/ml and no measurable concentrations of remifentanil were observed. The mean ± SE values of the pharmacokinetic parameters clearance and volume of distribution at steady state were 920 ± 110 ml·min⁻¹·kg⁻¹ and 1.00 ± 0.93 l/kg for remifentanil and 15 ± 2 ml·min⁻¹·kg⁻¹ and 0.56 ± 0.08 l/kg for GR90291. The relative free concentrations in the brain, as determined on the basis of the cerebrospinal fluid/total blood concentration ratio at steady state, were 25 ± 5% and 0.30 ± 0.11% for remifentanil and GR90291, respectively. Concentration–electroencephalographic effect relations were characterized on the basis of the sigmoidal E₅₀ pharmacodynamic model. The mean ± SE values for the maximal effect (E₅₀max), the concentration at which 50% of the maximal effect is obtained (E₅₀), and Hill factor for remifentanil were 109 ± 12 μV, 9.4 ± 0.9 ng/ml, and 2.2 ± 0.3, respectively (n = 8). For GR90291, the mean ± SE values for E₅₀ and the Hill factor were 103,000 ± 9,000 μg/ml and 2.5 ± 0.4, respectively (n = 6).

Conclusions: Analysis of the data on the basis of a previously postulated, mechanism-based pharmacokinetic–pharmacodynamic model for synthetic opioids revealed that the low in vivo potency of GR90291 can be explained by a low affinity to the μ-opioid receptor in combination with a poor brain penetration. (Key words: Cerebrospinal fluid; pharmacokinetics; pharmacokinetic–pharmacodynamic model.)

REMIFENTANIL is a new ultra-short-acting phenylpiperidine opioid analgesic agent.¹ Because of the presence of an ester group, remifentanil is extremely susceptible to metabolism by esterases. On administration of remifentanil, significant concentrations of the major metabolite GR90291 have been observed.² An important question is whether this metabolite affects the pharmacodynamics of the parent compound. Previous animal studies have shown that the potency of GR90291 is ¹/₅₀₀ to ¹/₁₀₀₀ less than that of remifentanil (GLAXO Wellcome Inc., unpublished data). This information, however, is based on dose–response data for which the difference in pharmacokinetics between the two compounds has not been taken into account. It has been demonstrated that there are considerable differences in the pharmacokinetic parameters between remifentanil and GR90291.² In addition, the potential difference in unbound concentration in the brain has not been taken into consideration. A final issue is that the relative potency has been determined at low subanesthetic dose levels. It cannot be

* Research Assistant, Division of Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University.
† Animal Technician, Division of Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University.
‡ Laboratory Technician, Division of Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University.
§ Associate Director of Clinical Pharmacokinetics, Glaxo-Wellcome, Inc., Glaxo Research Institute.
¶ Professor of Pharmacology, Division of Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University.

Received from the Division of Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University, The Netherlands, and Glaxo Research Institute, Research Triangle Park, North Carolina. Submitted for publication February 23, 1998. Accepted for publication October 16, 1998. Supported in part by Glaxo-Wellcome, Inc.

Address reprint requests to Dr. Danhof. Leiden/Amsterdam Center for Drug Research, Division of Pharmacology, Leiden University, Sylvius Laboratory, P. O. Box 9504, 2300 RA Leiden, The Netherlands. Address electronic mail to: m.danhof@lacdr.leidenuniv.nl

Anesthesiology, V 90, No 2, Feb 1999

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excluded that the pharmacodynamics and pharmacodynamic interactions of the opiates are quantitatively different at anesthetic doses. The objectives of the current investigation were to determine the pharmacodynamics and pharmacodynamic interactions of remifentanil and GR90291 at anesthetic dose levels and to determine the mechanism of the difference in potency in terms of pharmacokinetics (blood-brain equilibration) and pharmacodynamics (receptor binding). As GR90291 cannot be administered to humans, the study was conducted in rats, using an integrated pharmacokinetic-pharmacodynamic approach. Recently, considerable progress has been made in characterizing the pharmacodynamics of synthetic opiates in a rat electroencephalographic model using amplitude in the 0.5-4.5 Hz frequency band as a pharmacodynamic end point. An important issue in this respect is that, particularly in rats, opioid-induced overexcitation may be a complicating factor. It has been demonstrated that opioid-induced seizure activity can be suppressed effectively by administration of a constant rate infusion of midazolam. It cannot be excluded, however, that the pharmacodynamic parameter estimates obtained in the presence of a constant rate infusion of midazolam are influenced by a pharmacodynamic interaction between the opioid under investigation and midazolam. By varying the rate of administration, it has been demonstrated that acute functional adaptation is not a complicating factor when determining the in vivo potency and intrinsic activity by pharmacokinetic/pharmacodynamic modeling. Using this mode the pharmacodynamics of fentanyl, alfentanil, and sufentanil have been determined successfully in conjunction with receptor binding characteristics. By mechanism-based modeling, a high correlation between in vitro receptor binding characteristics and in vivo pharmacodynamics was obtained. In the current investigation, the same approach was applied to characterize the pharmacodynamics of remifentanil and its metabolite GR90291.

Methods

Chemicals

Remifentanil hydrochloride was obtained from Glaxo-Wellcome, Inc. (Research Triangle Park, NC). GR90291 was obtained as its trifluoroacetate from Glaxo-Wellcome Ltd. (Ware, UK). Freshly prepared solutions of remifentanil and GR90291 (312.5 μg/ml and 25 mg/ml, respectively, for a rat of 250 g) in physiologic saline were used in all experiments. Midazolam was donated by Hoffmann-LaRoche (Basel, Switzerland). A freshly prepared solution of ~2 mg/ml in physiologic saline was used. Vecuronium bromide was obtained from Organon Technika BV (Boxtel, The Netherlands). A freshly prepared solution of 2 mg/ml in physiologic saline was used.

Pharmacokinetic-Pharmacodynamic Experiment

The protocol of this study was approved by the Committee on Animal Experimentation of Leiden University. In the study, three groups of six to eight male Wistar rats, 5-7 weeks old with an average body weight of 267 g (range, 230-294 g), were used (Sylvius Laboratory Breeding Facility, Leiden, The Netherlands). The rats had seven electroencephalographic electrodes implanted in the skull over the cortex area 1 week before the start of the experiment. One day before the experiment, four indwelling polythene cannulas were implanted, one in the right femoral artery, two in the right jugular vein, and one in the right femoral vein. The cannulas in the right jugular vein were used for administration of the opioids and midazolam, whereas cannula in the femoral vein was used for the administration of vecuronium bromide. The cannula in the femoral artery was used for the serial collection of blood samples for determination of the concentration of remifentanil and GR90291.

In all experiments, midazolam was infused at a rate of 5.5 mg · kg⁻¹ · h⁻¹ to prevent opioid-induced seizure activity. To reach steady state rapidly, midazolam was infused according to a Wagner infusion scheme with an initial infusion rate three times the steady-state infusion rate, during 16 min.

Thirty minutes after the start of the infusion of midazolam, the rats received either remifentanil, GR90291, or physiologic saline (placebo) according to a stepwise decreasing infusion scheme with maximum infusion rates of 50 μg · kg⁻¹ · min⁻¹ and 4 mg · kg⁻¹ · min for remifentanil and GR90291, respectively (fig. 1). In each experiment, the first infusion rate was maintained for 30 min, and each subsequent lowering step was maintained for 10 min. This infusion scheme was used because the elimination half-lives of remifentanil and GR90291 are too short to obtain a sufficient number of electroencephalographic effect measurements after administration of a bolus infusion to obtain a complete description of the concentration-effect relationship. The stepwise zero-order administration of the opioids was performed using a computer-controlled infusion device with a Harvard 22 syringe pump (Harvard Apparatus, Inc., South...
PHARMACODYNAMICS OF REMIFENTANIL AND GR90291

![Graphs showing concentration-time profile of remifentanil and GR90291](image)

Fig. 1. Concentration-time profile of remifentanil (filled circles) and GR90291 (open circles) on administration of remifentanil (top) or GR90291 (bottom) in two representative rats. The solid lines represent the infusion rate of the opioids.

Natick, MA) connected to an Epson PC personal computer through a RS232 interface.

Two arterial blood samples of 50 μl each were collected at the following time points (t) after the start of the opioid infusion: 2, 5, 10, 20, 28, 38, 48, 58, 68, 78, 88, 98, 108, 118, 128, 138, 148, 158, 168, and 220 min. Electroencephalographic recording and subsequent analysis were performed as described previously. Two bipolar electroencephalographic leads (F3-C3 and C1-O1) were recorded continuously using a Nihon-Kohden AB-621G Bioelectric Amplifier (Hockloos, Amsterdam, The Netherlands) and concurrently digitized at a rate of 210 Hz using a CED 1401+ interface (CED, Cambridge, UK). The digitized signal was fed into a 80486 computer (Inteb BV, Sassenheim, The Netherlands) and stored on hard disk for off-line analysis. For each 5 s epoch, quantitative electroencephalographic parameters were obtained off-line by fast Fourier analysis with a user-defined program in the data analysis software package Spike 2 (version 4.60; CED). Electroencephalographic effect data points were obtained by averaging spectral parameter values over 4-min time intervals at the time of arterial blood sample collection. At the samples taken at 2, 5, and 10 min after the start of the opioid infusion, 1-, 2-, and 2-min time intervals, respectively, were used for the averaging of the electroencephalographic signal. The amplitude in the 0.5- to 4.5-Hz (Δ) frequency band was used, as the effect of opioids on this frequency range was most robust. This parameter has been used successfully in previous animal investigations with synthetic opioids.

During the study, rats showed extensive respiratory depression and muscle rigidity at the highest infusion rates of remifentanil and GR90291. All rats were ventilated artificially with air using an Amsterdam Infant Ventilator (model MK3; Hockloos BV) and a custom-made ventilation mask. Ventilation settings were as follows: ventilation frequency = 110 breaths/min; inspiration: expiration ratio = 1:2; and air supply flow rate > 2 l/min. In a previous investigation, it was demonstrated that ventilation according to this regimen is adequate to maintain the pH, arterial carbon dioxide tension, and arterial oxygen tension within the normal physiologic range. Ventilation was started when muscle rigidity appeared. At that moment, the rats received a bolus dose of 0.15 mg vecuronium bromide and were connected to the ventilation system through the rodent face mask. Administration of the muscle relaxant was then maintained every time the muscle rigidity returned (usually after ~5 min) by administration of 0.10 mg vecuronium bromide. This procedure was continued until spontaneous respiratory activity of the rat returned and muscle rigidity did not reappear.

During and after artificial ventilation, the respiratory status of the animal was monitored by measuring arterial pH, arterial carbon dioxide tension, and arterial oxygen tension levels using a Corning 178 pH/Blood Gas Analyzer (Corning, Medfield, MA). During the experiment, the body temperature of the rats was monitored using a YSI Tele-thermometer (Yellow Springs Instrument Corporation, Inc., Yellow Springs, OH) and kept constant between 37.5 and 38.5°C.

**Determination of the Cerebrospinal Fluid-to-Total Blood Concentration Ratio**

The relative free fraction of the opioids in the brain was determined on the basis of the cerebrospinal fluid
(CSF): total blood concentration ratio at steady state. For this purpose, two additional groups of seven or eight male Wistar rats were used. The vascular cannulation procedure was as described earlier. Instantaneous pseudo-steady-state concentrations of the opioids were obtained using a computer-controlled infusion device. The STANPUMP program was used to clamp concentrations of opioid in blood at the value for the respective concentration at which 50% of the maximal effect is obtained (EC<sub>50</sub>), as determined by the pharmacokinetic-pharmacodynamic experiment. The pharmacokinetic parameters for controlling the infusion pump were obtained from a pilot study. Before the start of the infusion of opioid, an infusion of midazolam was initiated as described earlier. During the infusion of opioid, rats were artificially ventilated, and muscle rigidity was managed as described earlier. Thirty minutes after the start of the infusion of opioid, two arterial blood samples of 100 μl were drawn. Simultaneously, a CSF sample was obtained by cisternal puncture. In previous investigations, it has been shown that the CSF:plasma concentration ratio closely reflects the concentration of free drug in the brain. Data Analysis

The pharmacokinetic parameter estimates for remifentanil and GR90291 were calculated by noncompartmental methods. Clearance (Cl) was calculated according to the following equation:

\[ Cl = \frac{D}{AUC} \]

where D is the total dose of the administered drug and AUC is the area under the concentration–time curve using the linear trapezoidal method. The real mean residence time (MRT<sub>R</sub>) of the opioids was calculated according to the following equation:

\[ MRT_R = MRT_{APP} - MIT = \frac{AUMC}{AUC} - MIT \]

where AUMC is the area under the first moment of the concentration–time curve using the linear trapezoidal method, MRT<sub>APP</sub> is the apparent mean residence time, and MIT is the mean input time for the opioids according to the infusion scheme used. Volume of distribution at steady state (V<sub>d,ss</sub>) was determined using the following equation:

\[ V_{d,ss} = Cl \cdot MRT_R \]

The concentration-electroencephalographic effect relations of the opioid compounds were investigated using the data analysis software package SIPIAR (version 3.3; Simed, Creteil, France) and user-defined subroutines. The concentration-effect relation was described on the basis of the sigmoidal maximal effect (E<sub>max</sub>) model:

\[ E_C = E_0 + \frac{E_{max} \cdot C^n}{C^n + EC_{50}} \]

where EC<sub>50</sub> is the effect at the measured blood concentration C, E<sub>0</sub> is the baseline effect, and n is the Hill factor reflecting the sigmoidicity of the curve.

The concentration-electroencephalographic effect relations of the different opioids also were simulated according to the operational model of agonism.
\[
\frac{E_s}{E_m} = \frac{\tau^* \cdot [C]^n}{(K_A + [C]^n + \tau^* \cdot [C]^n}
\]

where \(E_s\) is the effect at the ligand concentration \(C\); \(E_m\) is the maximum effect in the system; \(\tau\) is the efficacy parameter, defined as the ratio of \([R_u]\) (the total concentration of available receptors) to \(K_E\) (the concentration of occupied receptors that elicits half-maximal effect); \(n\) is the slope factor of the transduction function; and \(K_A\) is the agonist equilibrium/dissociation constant.

This model is based on classical receptor theory and combines two independent parts to describe drug action: (1) an agonist-dependent part, incorporating agonist affinity and intrinsic efficacy; and (2) a tissue-dependent part, determined by receptor concentration and the nature of the stimulus-effect relation. The operational model of agonism thereby explicitly recognizes the central role of the stimulus-effect relation.

The receptor binding characteristics of the radioligand \(^3\)H-naloxone were determined by fitting the following equation to the data from the saturation experiment:

\[
B = \frac{B_{max} \cdot C_t}{K_d + C_t}
\]

where \(B\) is the number of receptors occupied, \(B_{max}\) is the total number of specific binding sites, \(K_d\) is the ligand concentration at which 50% of the receptors is occupied, and \(C_t\) is the free ligand concentration. The values for the opioid concentration that displaces 50% of \(^3\)H-naloxone (IC\(_{50}\)) for the three opioids were determined by fitting the following equation to the data from the displacement study:

\[
B = \frac{B_0 \cdot IC_{50}}{IC_{50} + C_d}
\]

where \(B_0\) is the specific binding of the radioligand in the absence of displacer and \(C_d\) is the concentration of displacer added. The \(K_t\) values for the opioids were calculated from the IC\(_{50}\) values according to the Cheng-Prusoff equation\(^15\):

\[
K_t = \frac{IC_{50}}{L^* + K_t^*}
\]

where \(L^*\) is the concentration of \(^3\)H-naloxone and \(K_t^*\) is the equilibrium dissociation constant of \(^3\)H-naloxone as determined in the saturation experiment. The receptor binding data were analyzed using the software package

GRAPH PRISM\(^{®}\) (version 2.0; GraphPad\(^{®}\), San Diego, CA).

Statistical evaluation of the pharmacokinetic and pharmacodynamic parameter estimates between the different treatment groups was performed by the Mann-Whitney U test, using the statistical module in the SIPHAR package.

Results

Figure 1 shows the concentration-time profiles of remifentanil and GR90291 during and after the stepwise infusion of remifentanil in a representative rat. Concentrations of remifentanil in the range 0–120 ng/ml were observed. Considerable concentrations of the metabolite of remifentanil, GR90291, were observed (0–850 ng/ml).

Figure 1B shows the concentration-time profiles of GR90291 during and after the stepwise infusion of GR90291 in a representative rat. Concentrations of the GR90291 were in the range 0–220 \(\mu\)g/ml. Remifentanil was cleared extremely rapidly from the blood (CI = 920 ± 110 ml/min/kg), whereas the CI of the metabolite GR90291 was significantly lower (15 ± 2 ml/min/kg, \(P < 0.01\)). In addition, a significantly lower V\(_{d,ss}\) value for GR90291 was observed (0.56 ± 0.08 vs. 1.0 ± 0.9 l/kg for remifentanil, respectively, \(P < 0.05\)). The CSF-blood concentration ratio was determined on the basis of concentrations of the drugs in blood and CSF during steady-state conditions. In this experiment, the mean ± SE concentrations in blood 30 min after the start of the STANPUMP infusion were 3.2 ± 0.6 ng/ml and 119 ± 7 \(\mu\)g/ml for remifentanil and GR90291, respectively, which is in agreement with the target concentration (EC\(_{50}\)) as shown in table 1. The mean ± SE relative free fractions of remifentanil and GR90291 were 25 ± 5% and 0.30 ± 0.11%, respectively (\(P < 0.05\)). Figure 2 shows the time course of the electroencephalographic effect before, during, and after the infusion of remifentanil (top), GR90291 (middle), or physiologic saline (placebo, bottom) in three representative rats. Midazolam increased the baseline electroencephalographic effect only slightly (fig 2). In the remifentanil and the GR90291 groups, a profound increase in the \(\delta\) frequency band (0.5–4.5 Hz) of the power spectrum was observed compared with placebo.

Figure 3 shows the concentration–electroencephalographic effect relations of remifentanil and GR90291 for all rats. The electroencephalographic effect of the
Table 1. Pharmacodynamic Parameter Estimate Baseline EEG Effect (E₀), Concentration at Half maximal EEG Effect (ECₑ₀), Maximum EEG Effect (Eₘₐₓ), and Hill Factor of Remifentanil and GR90291 on the Basis of Whole Blood Concentrations

<table>
<thead>
<tr>
<th>Rat</th>
<th>E₀ (μV)</th>
<th>ECₑ₀ (ng/ml)</th>
<th>Eₘₐₓ (μV)</th>
<th>Hill Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remifentanil</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>12.2</td>
<td>77</td>
<td>1.8</td>
</tr>
<tr>
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<td>67</td>
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<tr>
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<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>47</td>
<td>7.4</td>
<td>136</td>
<td>1.6</td>
</tr>
<tr>
<td>8</td>
<td>77</td>
<td>10.6</td>
<td>71</td>
<td>4.1</td>
</tr>
<tr>
<td>Mean</td>
<td>62</td>
<td>9.4</td>
<td>109</td>
<td>2.2</td>
</tr>
<tr>
<td>SEM</td>
<td>13</td>
<td>2.6</td>
<td>34</td>
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<tr>
<td>GR 90291</td>
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</tr>
<tr>
<td>9</td>
<td>76</td>
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<td>3.9</td>
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<tr>
<td>10</td>
<td>77</td>
<td>129,000</td>
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<tr>
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<tr>
<td>SEM</td>
<td>5</td>
<td>9,000</td>
<td>—</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*The difference in the values of the ECₑ₀ of remifentanil and GR90291 is statistically significant at P < 0.05.

remifentanil and GR90291 groups could be related directly to the concentrations in blood and could be characterized satisfactorily on the basis of the sigmoidal Eₘₐₓ pharmacodynamic model. For GR90291, the data did not cover the full concentration range from E₀ to Eₘₐₓ. Therefore, Eₘₐₓ was fixed to the value obtained for remifentanil. This is reasonable, because the results from the in vitro investigations showed that both compounds are full agonists at the μ-opioid receptor, as is discussed later. The pharmacodynamic parameter estimates for both compounds are summarized in table 1. Based on total concentrations in blood, an 11,000-fold difference in potency was observed between remifentanil and GR90291.

The results of the displacement studies using ³H-naltrexone in rat brain homogenates are represented in table 2. A 1,000-fold difference in Kᵢ values was observed between remifentanil and GR90291, and in the presence of 100 mw NaCl a considerable increase in the Kᵢ value (sodium shift) was observed for both compounds. Table 2 also presents the values of the potency based on concentrations of free drug (ECₑ₀,ₚ) for comparison with the receptor binding data. The free fraction was determined in vitro on the basis of the CSF-plasma concentration ratio. When calculated for concentrations of free drug, the potency ratio between remifentanil and GR90291 is 140-fold, which is considerably lower than the ratio of 11,000 when using total concentrations in whole blood. The concentration-electroencephalographic effect relation of the opioids was further explored on the basis of an operational model of pharmacologic agonism. For this purpose, the concentration-electroencephalographic effect relation of remifentanil and GR90291, and those previously obtained for alfentanil, fentanyl, and sufentanil, were simulated according to the equation of the operational model of agonism (Eq. 5).

As is seen in figure 4, the model can describe the concentration-effect curves of the five opioids by keeping the agonist-independent parameters (E₀ and n) constant and assuming that the Kᵢ value for each agonist is identical to the Kᵢ value estimated in vitro and that the τ values for each agonist are identical to the sodium shift measured for each agonist multiplied by a constant, agonist-independent factor (4.7).

Discussion

The pharmacodynamics of the metabolite GR90291 are of interest, because high concentrations of this metabolite are observed after administration of remifentanil in patients undergoing elective inpatient surgery. A potential problem is that this metabolite cannot be administered to patients to determine its relative potency and intrinsic activity. In the current study, therefore, the pharmacodynamics of remifentanil and its metabolite GR90291 were determined in the rat in vivo using an integrated pharmacokinetic/pharmacodynamic approach, using quantitative electroencephalographic parameters (0.5–4.5 Hz) as a pharmacodynamic end point. This method has been used previously to characterize the in vitro pharmacodynamics of three other synthetic opioids, alfentanil, fentanyl, and sufentanil. In that study, the concentration-electroencephalographic effect relations were analyzed by a mechanism-based pharmacodynamic model. It was shown that on basis of in vitro receptor binding characteristics of an opioid (Kᵢ and sodium shift), as determined by displacement of ³H-naltrexone in washed rat brain homogenates, it is possible to predict the in vivo pharmacodynamic parameters (potency and intrinsic activity). Further, it was shown that opioids with a sodium shift larger than one all behave as full agonists. It is noteworthy that estimates of the relative potency and intrinsic activity of drugs ob-
tained in animal experiments may have a good predictive value for the situation in humans. Particularly for synthetic opiates, the prediction of the potency and intrinsic activity in humans has been shown to be good. Further, it has been demonstrated that for synthetic opiates administered alone, quantitative electroencephalographic parameters match with depth of anesthesia and thus can be used for initial dose finding.

Egan et al. showed hysteresis in the concentration-electroencephalographic effect relation of remifentanil in humans, but no hysteresis was observed in the current study, because of the stepwise administration scheme whereby electroencephalographic measurements were obtained at the end of each infusion step. In addition, proteresis (i.e., clockwise hysteresis) was not observed, indicating that no tolerance to the electroencephalographic effect occurred within the time frame of the experiments. This is in agreement with earlier findings for alfentanil. The blood concentration-electroencephalographic effect relations of remifentanil and GR90291 were described on the basis of the sigmoidal $E_{\text{max}}$ pharmacodynamic model (Eq. 4). This resulted in estimates of $E_{\text{max}}$, $E_{0}$, and the slope factor of the curve. For the GR90291 group, the data did not cover the full concentration range from $E_{0}$ to $E_{\text{max}}$. Therefore, $E_{\text{max}}$ was fixed to the value found for remifentanil, i.e., 109 $\mu$V. The intrinsic efficacies of the compounds were determined also in vitro on basis of the sodium shift in the $^3$H-Naloxone displacement assay using washed rat brain homogenates. As is seen in table 2, GR90291 shows a considerable sodium shift (4.5) and can therefore be considered as a full agonist in the electroencephalographic model. Identical intrinsic activity for remifentanil and GR90291 has been observed in the guinea pig ileum assay and recently also in a dog electroencephalographic model.

A competitive interaction is expected between remifentanil and GR90291, because both compounds bind to the $\mu$-opioid receptor (table 2), albeit with different affinity. On administration of remifentanil, concentrations of GR90291 in blood were observed in the concentration range 0–850 ng/ml. At these concentrations, no effect of GR90291 on the electroencephalogram is observed (fig. 3). The pharmacodynamic parameters for remifentanil are therefore not biased by the presence of GR90291. Comparison of the pharmacodynamic parameter estimates of GR90291 with the values obtained for remifentanil then yielded a significant (11,000-fold) difference in $E_{0}$. The value of the intrinsic activity ($E_{\text{max}}$) of remifentanil is similar to that
of fentanyl, alfentanil, and sufentanil. The \textit{in vitro} potency of remifentanil, as reflected in the concentration of opioid resulting in 50% of the maximum effect (9.4 ng/ml), was similar to that for fentanyl (10.1 ng/ml).

Remifentanil was more potent (1.41 ng/ml) whereas alfentanil (289 ng/ml) was less potent than remifentanil. GR90291 was the least potent opioid investigated, with a 350-fold lower potency than alfentanil. An important question is to what extent pharmacokinetic-pharmacodynamic data obtained in animal studies can be used to predict quantitatively the (relative) potency and intrinsic activity in humans. For a large number of drugs, it has been demonstrated that effective concentrations are similar in animals and in humans. The concentration-electroencephalographic effect relations of opioids also have been determined in humans. It is noteworthy that the EC\(_{50}\) values of the different opioids in rats are within the same concentration ranges as those obtained in the human electroencephalographic model (table 3). Further, all opioids portrayed similar (full) intrinsic activity (E\(_{max}\)) in both species. These similarities demonstrate the usefulness of the electroencephalographic animal model to estimate the (relative) \textit{in vivo} pharmacodynamic profile of new synthetic opioids in humans. The relation between \textit{in vitro} receptor binding characteristics and \textit{in vivo} pharmacodynamics was in-

Fig. 3. Concentration–electroencephalographic effect relation of remifentanil (solid line) and GR90291 (dashed line) in representative rats. The solid line represents the best fit of the sigmoidal E\(_{max}\) model (Eq. 4) to the actual data. For GR90291, E\(_{max}\) has been fixed to 109 \(\mu\)V.

Table 2. \textit{In Vitro} Receptor Affinity in the Absence (K\(_{i,nA}\)) and the Presence (K\(_{i,nNa}\)) of 100 mm NaCl, \textit{In Vitro} Efficacy (Na-shift) and \textit{In Vitro} Potency (Free Concentration at Half-maximal EEG Effect, EC\(_{50,w}\)) of Remifentanil and GR90291

<table>
<thead>
<tr>
<th></th>
<th>EC(_{50,w}) (nM)</th>
<th>K(_{i,nA}) (nM)</th>
<th>K(_{i,nNa}) (nM)</th>
<th>Na-shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remifentanil</td>
<td>5.69 ± 1.26</td>
<td>12.4 ± 2.8</td>
<td>117 ± 9</td>
<td>9.4</td>
</tr>
<tr>
<td>GR90291</td>
<td>780 ± 268</td>
<td>11,000 ± 2,000</td>
<td>50,000 ± 4,800</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Fig. 4. (A) Operational model of agonism simulations of the self-normalized concentration–electroencephalographic effect relations of five opioids. The solid lines were simulated using Eq. 5 with the values for E\(_{max}\) (100%) and n (2.3) held constant for all five agonists. The affinity (K\(_i\)) values were set to the K\(_i\) values estimated in the presence of NaCl (table 2), and the efficacy parameter (\(\tau\)) of each agonist was expressed as a constant (4.7) times the sodium shift (table 2). The dashed lines were simulated with the E\(_{max}\) model (Eq. 4) using the parameter values estimated from the experimental data (table 2). The lines were simulated using Eq. 5 with the following parameter values: E\(_{max}\) = 100%, n = 2.5, K\(_A\) = 20 nM, and \(\tau\) = 4.7 × sodium shift.

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Table 3. Estimates of the Pharmacodynamic Parameter Concentration at Half-maximal EEG Effect (EC50) and Maximal EEG Effect (Emax) of Four Synthetic Opioids in Rats and Human

<table>
<thead>
<tr>
<th></th>
<th>EC50 (ng/ml)</th>
<th>Emax (μV)</th>
<th>Human (mg/ml)</th>
<th>Human (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufentanil</td>
<td>1.4</td>
<td>104</td>
<td>0.7</td>
<td>16.5</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>10.1</td>
<td>89</td>
<td>7.5</td>
<td>15.2</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>289</td>
<td>111</td>
<td>520</td>
<td>14.8</td>
</tr>
<tr>
<td>Remifentanil</td>
<td>9.4</td>
<td>109</td>
<td>19.5</td>
<td>15.0</td>
</tr>
<tr>
<td>GR90291</td>
<td>105,000</td>
<td>109</td>
<td>—</td>
<td>13.8</td>
</tr>
</tbody>
</table>

* Determined by the amplitude in the 0.5–4.5 Hz frequency band.
† Determined by the spectral edge.

vestigated for remifentanil and GR90291 in combination with the data previously obtained for alfentanil, fentanyl, and sufentanil. As is seen in figure 4, the concentration-electroencephalographic effect relation of the five opioids can be adequately described on the basis of the operational model of pharmacologic agonism15 (Eq. 5), which demonstrates its general applicability for the mechanistic description of the in vivo pharmacodynamics of opioids. There is excellent agreement between the predicted and the observed concentration–effect relations for alfentanil, fentanyl, and sufentanil. For remifentanil and GR90291, the observed concentration–effect relations are less close to the predicted. Experimental factors are the most likely explanation for this difference. Remifentanil in particular is extremely unstable in biologic fluids. This complicates the accurate determination of the actual concentration–time profile and in particular the free fraction. In addition, estimation of the relative free fraction of GR90291 in the brain is also difficult, because the concentrations in the CSF are low.

To describe the pharmacodynamics of opioids in vivo, it is essential that information is obtained on the free concentration of the drug in the brain. In the current investigation, the free concentrations of the opioids in the brain were estimated on the basis of the CSF:total blood concentration ratio of the drug at steady state. This method has been shown to be a good reflection of the relative free concentration of the drug in the brain. There is a remarkable difference in the relative free concentration in the brain between remifentanil (25.0%) and GR90291 (0.3%). The question is what is the reason for this difference. A difference in binding to blood components, such as plasma proteins and blood cells, may be a possible explanation. Another factor may be that the transport rate of GR90291 across the blood-

brain barrier into the brain is limited as a result of its higher hydrophilic character, compared with remifentanil. Further, it cannot be excluded that the transport of GR90291 across the blood–brain barrier may be even more complicated than just a passive diffusion process.21

On administration of remifentanil, considerable accumulation of GR90291 was observed. Based on the pharmacodynamics of GR90291 obtained in the current study, the maximum concentrations of GR90291 (850 ng/ml) were too low to expect a pharmacodynamic interaction with remifentanil. Regarding a pharmacodynamic interaction between the two compounds, however, it is important to consider clinical/pathologic situations in which the pharmacokinetics and the distribution of GR90291 may be altered. In particular, the low CI of GR90291 (relative to the CI of remifentanil) and its low relative concentration in the brain are of interest. Both factors in theory may be affected in disease. For instance, it has been shown that, in patients with renal disease, elimination of GR90291 from the body is strongly reduced, which is reflected in a reduction in CI of 96%.22 Thus, in renal failure, a larger degree of accumulation of GR90291 may be expected. Further, the binding of GR90291 to blood components may be altered in different states of disease. In this respect, it is important that the relative free fraction of GR90291 is extremely low (0.3%) and, further, that this metabolite has a relatively small volume of distribution (0.56 l/kg). The most important proteins in the plasma regarding binding of drugs are albumin and α1-acid glycoprotein, and the concentrations of these proteins in plasma may be differently affected in several disease states.23 Finally, transport of drug across the blood–brain barrier may be altered in pathologic conditions.24 Thus, in disease states, these factors or a combination of these factors may contribute to an increased free concentration of GR90291 in the brain that may result in a more profound contribution in the pharmacologic effects.

It can be concluded that, after administration of remifentanil, considerable concentrations of the metabolite GR90291 are measured. A similar intrinsic activity was observed for both compounds, whereas a 11,000-fold lower potency was observed for GR90291. This difference appears to be the result of a difference in affinity to the μ-opioid receptor and a difference in brain penetration.

The authors thank Prof. Dr. Douwe D. Breimer for critical reading of the manuscript and Hofmann-LaRoche for the generous donation of midazolam. The STANPUMP program was kindly provided by Dr. S. L.
Shafer, Department of Anesthesia, Stanford University, Palo Alto, California.

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