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

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

Methods: Remifentanyl 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 remifentanyl resulted in concentrations of remifentanyl 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 remifentanyl were observed. The mean ± SE values of the pharmacokinetic parameters clearance and volume of distribution at steady state were $920 \pm 110 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ and $1.00 \pm 0.93 \text{ l/kg}$ for remifentanyl

and $15 \pm 2 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ and $0.56 \pm 0.08 \text{ l/kg}$ 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 $25 \pm 5\%$ and $0.30 \pm 0.11\%$ for remifentanyl and GR90291, respectively. Concentration–electroencephalographic effect relations were characterized on the basis of the sigmoidal E_{max} pharmacodynamic model. The mean ± SE values for the maximal effect (E_{max}), the concentration at which 50% of the maximal effect is obtained (EC_{50}), and Hill factor for remifentanyl were $109 \pm 12 \mu\text{V}$, $9.4 \pm 0.9 \text{ ng/ml}$, and 2.2 ± 0.3 , respectively ($n = 8$). For GR90291, the mean ± SE values for EC_{50} and the Hill factor were $103,000 \pm 9,000 \mu\text{g/ml}$ 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.)

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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.³ An important issue in this respect is that, particularly in rats, opioid-induced over-excitation 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.³ 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.⁴ Using this mode the pharmacodynamics of fentanyl, alfentanil, and sufentanil have been determined successfully in conjunction with receptor binding characteristics.⁵ By mechanism-based modeling, a high correlation between *in vitro* receptor binding characteristics and *in vivo* pharmacodynamics was obtained.⁵ 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 $\mu\text{g}/\text{ml}$ 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.⁶ 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 $\text{mg} \cdot \text{kg} \cdot \text{h}$ to prevent opioid-induced seizure activity.³ To reach steady state rapidly, midazolam was infused according to a Wagner infusion scheme⁷ 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 $\mu\text{g} \cdot \text{kg} \cdot \text{min}$ and 4 $\text{mg} \cdot \text{kg} \cdot \text{min}$ 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

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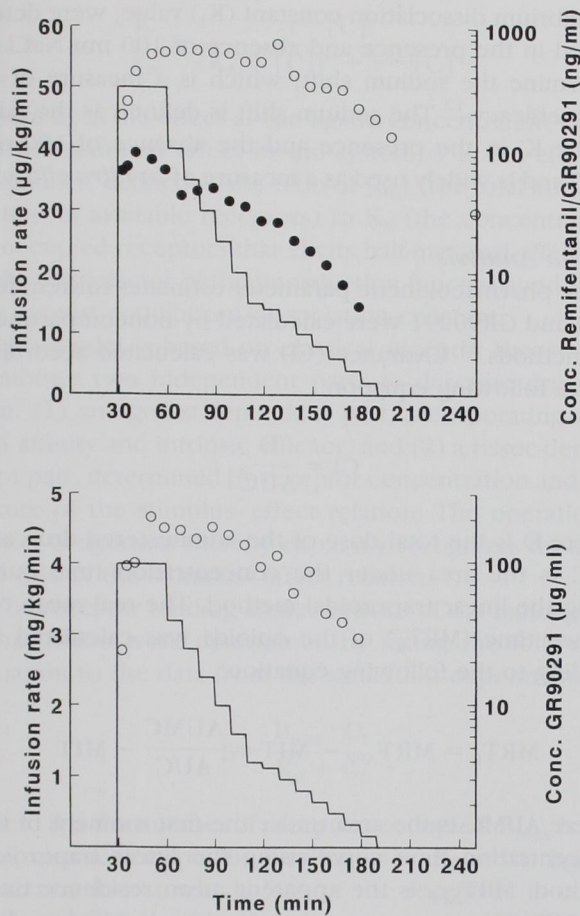


Fig. 1. Concentration-time profile of remifentanil (filled circles) and GR90291 (open circles) on administration of remifentanil (top) or GR90291 (bottom) in two representative rats. The solid lines represent the infusion rate of the opioids.

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.³ Two bipolar electroencephalographic leads (F₁-C₁ and C₁-O₁) were recorded continuously using a Nihon-Kohden AB-621G Bioelectric Amplifier (Hoekloos, Amsterdam, The Netherlands) and concurrently digitized at a rate of 210 Hz using a CED 1401_{plus} interface (CED, Cambridge, UK). The digitized signal was fed into a 80436 computer (Inteb BV, Sassenheim, The Netherlands) and stored on hard disk for off-line analysis. For each 5-s epoch, quantitative electroencephalographic parameters were ob-

tained 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.³⁻⁵

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; Hoekloos 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.³ 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 \approx 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 program⁸ 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_{50}), 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 μ l 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.⁹

Drug Assay

The blood and CSF samples drawn were inactivated immediately by mixing them with 0.2 ml acetonitrile. Subsequently, 200 μ l internal standard solution was added, containing 10 ng D4-GR87084 or 100 ng D4-GR87084 for the remifentanyl or the GR90291 samples, respectively. An extra 2 ml acetonitrile was then added, followed by brief vortexing. Next, the samples were extracted with 4 ml methylene chloride, after which either the organic phase (remifentanyl samples) or the aqueous phase (GR90291 samples) were stored at -80°C until analysis. The concentrations of remifentanyl and GR90291 in the blood samples were determined by gas chromatography-mass spectrometry,^{10,11} respectively, and slightly modified. The lower limits of quantification for remifentanyl and GR90291 for 100- μ l blood samples were 1 and 10 ng/ml, respectively, and the interday coefficients of variation for remifentanyl (1-1,000 ng/ml) and GR90291 (0.01-1,000 μ g/ml) were <11.5% and 13.2%, respectively.

Receptor Binding

In vitro receptor binding characteristics of remifentanyl and GR90291 were determined by displacement of ³H-naloxone using rat brain homogenates.⁵ Receptor binding affinities, as expressed in the *in vitro* estimated

equilibrium dissociation constant (K_i) value, were determined in the presence and absence of 100 mM NaCl to determine the sodium shift, which is a measure of *in vitro* efficacy.¹² The sodium shift is defined as the ratio of the K_i in the presence and the absence of 100 mM NaCl and is widely used as a measure of *in vitro* efficacy.

Data Analysis

The pharmacokinetic parameter estimates for remifentanyl and GR90291 were calculated by noncompartmental methods.¹³ 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_R) 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_{APP} 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 ($V_{d,ss}$) 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 SIPHAR (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_{max}) model:

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

where E_C is the effect at the measured blood concentration C, E_0 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 agonism¹⁴:

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$$\frac{E_c}{E_m} = \frac{\tau^n \cdot [C]^n}{(K_A + [C])^n + \tau^n \cdot [C]^n}$$

where E_c is the effect at the ligand concentration C ; E_m is the maximum effect in the system; τ is the efficacy parameter, defined as the ratio of $[R_0]$ (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 ^3H -naloxone were determined by fitting the following equation to the data from the saturation experiment:

$$B = \frac{B_{\max} \cdot C_f}{K_d + C_f}$$

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_f is the free ligand concentration. The values for the opioid concentration that displaces 50% of ^3H -naloxone (IC_{50}) for the three opioids were determined by fitting the following equation to the data from the displacement study:

$$B = \frac{B_0 \cdot \text{IC}_{50}}{\text{IC}_{50} + C_d}$$

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_I values for the opioids were calculated from the IC_{50} values according to the Cheng-Prusoff equation¹⁵:

$$K_I = \frac{\text{IC}_{50}}{L^* \left(1 + \frac{K_d^*}{L^*} \right)}$$

where L^* is the concentration of ^3H -naloxone and K_d^* is the equilibrium dissociation constant of ^3H -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 remifentanyl and GR90291 during and after the stepwise infusion of remifentanyl in a representative rat. Concentrations of remifentanyl in the range 0–120 ng/ml were observed. Considerable concentrations of the metabolite of remifentanyl, 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 $\mu\text{g/ml}$. Remifentanyl was cleared extremely rapidly from the blood ($\text{Cl} = 920 \pm 110 \text{ ml} \cdot \text{min} \cdot \text{kg}$), whereas the Cl of the metabolite GR90291 was significantly lower ($15 \pm 2 \text{ ml} \cdot \text{min} \cdot \text{kg}$, $P < 0.01$). In addition, a significantly lower $V_{d,ss}$ value for GR90291 was observed (0.56 ± 0.08 vs. $1.0 \pm 0.9 \text{ l/kg}$ for remifentanyl, 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 \pm SE concentrations in blood 30 min after the start of the STANPUMP infusion were $3.2 \pm 0.6 \text{ ng/ml}$ and $119 \pm 7 \mu\text{g/ml}$ for remifentanyl and GR90291, respectively, which is in agreement with the target concentration (EC_{50}) as shown in table 1. The mean \pm SE relative free fractions of remifentanyl and GR90291 were $25 \pm 5\%$ and $0.30 \pm 0.11\%$, respectively ($P < 0.05$). Figure 2 shows the time course of the electroencephalographic effect before, during, and after the infusion of remifentanyl (top), GR90291 (middle), or physiologic saline (placebo, bottom) in three representative rats. Midazolam increased the baseline electroencephalographic effect only slightly (fig. 2). In the remifentanyl and the GR90291 groups, a profound increase in the δ 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 remifentanyl and GR90291 for all rats. The electroencephalographic effect of the

Table 1. Pharmacodynamic Parameter Estimate Baseline EEG Effect (E_0), Concentration at Half-maximal EEG Effect (EC_{50}), Maximum EEG Effect (E_{max}), and Hill Factor of Remifentanyl and GR90291 on the Basis of Whole Blood Concentrations

Rat	E_0 (μV)	EC_{50}^* (ng/ml)	E_{max} (μV)	Hill Factor
Remifentanyl				
1	45	12.2	77	1.8
2	67	13.8	174	1.4
3	82	8.2	130	2.1
4	53	7.9	120	1.6
5	60	10.0	83	2.3
6	65	5.4	84	2.5
7	47	7.4	136	1.6
8	77	10.6	71	4.1
Mean	62	9.4	109	2.2
SEM	13	2.6	34	0.3
GR 90291				
9	76	76,000	—	3.9
10	77	129,000	—	2.5
11	45	101,000	—	1.1
12	74	73,000	—	3.0
13	76	81,000	—	2.9
14	49	128,000	—	2.0
Mean	62	103,000	—	2.5
SEM	5	9,000	—	0.4

* The difference in the values of the EC_{50} of remifentanyl and GR90291 is statistically significant at $P < 0.05$.

remifentanyl and GR90291 groups could be related directly to the concentrations in blood and could be characterized satisfactorily on the basis of the sigmoidal E_{max} pharmacodynamic model. For GR90291, the data did not cover the full concentration range from E_0 to E_{max} . Therefore, E_{max} was fixed to the value obtained for remifentanyl. 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 remifentanyl and GR90291.

The results of the displacement studies using 3H -naloxone in rat brain homogenates are represented in table 2. A 1,000-fold difference in K_1 values was observed between remifentanyl and GR90291, and in the presence of 100 mM NaCl a considerable increase in the K_1 value (sodium shift) was observed for both compounds. Table 2 also presents the values of the potency based on concentrations of free drug ($EC_{50,u}$) for comparison with the receptor binding data. The free fraction was determined *in vivo* on the basis of the CSF:plasma concentration ratio. When calculated for concentrations of free

drug, the potency ratio between remifentanyl 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 remifentanyl and GR90291, and those previously obtained for alfentanil, fentanyl, and sufentanil⁵, 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 (E_m and n) constant and assuming that the K_A value for each agonist is identical to the K_1 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 remifentanyl in patients undergoing elective inpatient surgery.² 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 remifentanyl 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 vivo* pharmacodynamics of three other synthetic opioids, alfentanil, fentanyl, and sufentanil.⁵ 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 (K_1 and sodium shift), as determined by displacement of 3H -naloxone 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-

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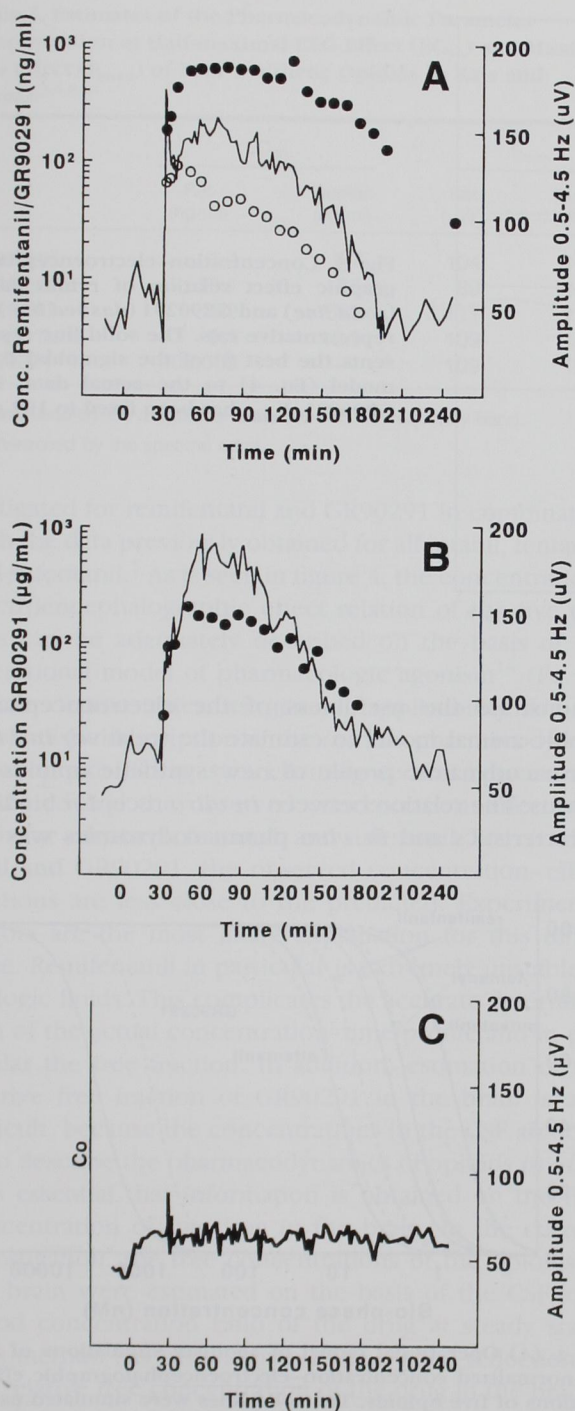


Fig. 2. Effect-time profile of the electroencephalographic effect (0.5–4.5 Hz) during and after a stepwise infusion of remifentanil (top), GR90291 (middle), or physiologic saline (bottom) in representative rats. Open circles = measured concentrations of remifentanil in the blood; filled circles = measured concentrations of GR90291 in the blood. (Note that the concentrations of remifentanil and GR90291, in the top and middle, differ by three orders of magnitude as they are given in ng/ml and $\mu\text{g/ml}$, respectively.)

tained in animal experiments may have a good predictive value for the situation in humans.¹⁶ Particularly for synthetic opiates, the prediction of the potency and intrinsic activity in humans has been shown to be good.⁵ Further, it has been demonstrated that for synthetic opiates administered alone, quantitative electroencephalographic parameters match with depth of anesthesia¹⁷ and thus can be used for initial dose finding.⁹

Egan *et al.*¹⁷ 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.⁴ The blood concentration-electroencephalographic effect relations of remifentanil and GR90291 were described on the basis of the sigmoidal E_{max} pharmacodynamic model (Eq. 4). This resulted in estimates of E_{max} , EC_{50} , 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_{max} . Therefore, E_{max} was fixed to the value found for remifentanil, *i.e.*, 109 μV . The intrinsic efficacies of the compounds were determined also *in vitro* on basis of the sodium shift in the ³H-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 assay¹ and recently also in a dog electroencephalographic model.¹⁸

A competitive interaction is expected between remifentanil and GR90291, because both compounds bind to the μ -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 parameter estimates 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 EC_{50} . The value of the intrinsic activity (E_{max}) of remifentanil is similar to that

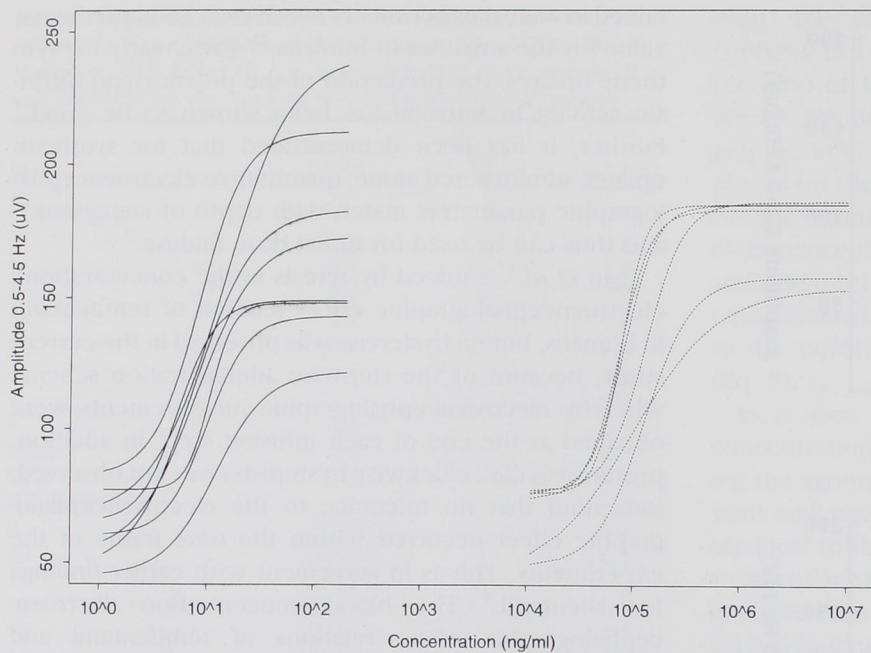


Fig. 3. Concentration–electroencephalographic effect relation of remifentanyl (solid line) and GR90291 (dashed line) in representative rats. The solid line represents the best fit of the sigmoidal E_{max} model (Eq. 4) to the actual data. For GR90291, E_{max} has been fixed to 109 μ V.

of fentanyl, alfentanil, and sufentanil.⁵ The *in vivo* potency of remifentanyl, 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).

Sufentanil was more potent (1.41 ng/ml) whereas alfentanil (289 ng/ml) was less potent than remifentanyl. 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.¹⁶ The concentration–electroencephalographic effect relations of opioids also have been determined in humans.^{17,19,20} 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) *in vivo* pharmacodynamic profile of new synthetic opioids in humans. The relation between *in vitro* receptor binding characteristics and *in vivo* pharmacodynamics was in-

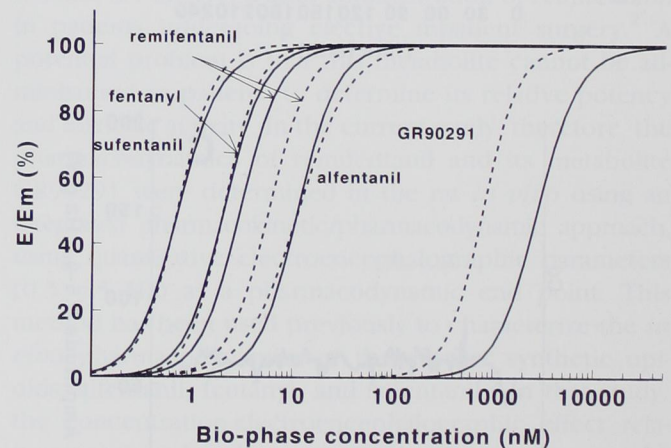


Fig. 4. (A) Operational model of agonism simulations of the self-normalized concentration–electroencephalographic effect relations of five opioids. The solid lines were simulated using Eq. 5 with the values for E_m (100%) and n (2.3) held constant for all five agonists. The affinity (K_A) values were set to the K_i values estimated in the presence of NaCl (table 2), and the efficacy parameter (τ) 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: $E_m = 100\%$, $n = 2.3$, $K_A = 20$ nM, and $\tau = 4.7 \times$ sodium shift.

Table 2. *In Vitro* Receptor Affinity in the Absence ($K_{i,-Na}$) and the Presence ($K_{i,+Na}$) of 100 mM NaCl, *In Vitro* Efficacy (Na-shift) and *In Vivo* Potency (Free Concentration at Half-maximal EEG Effect, $EC_{50,u}$) of Remifentanyl and GR90291

	$EC_{50,u}$ (nM)	$K_{i,-Na}$ (nM)	$K_{i,+Na}$ (nM)	Na-shift
Remifentanyl	5.69 ± 1.26	12.4 ± 2.8	117 ± 9	9.4
GR90291	780 ± 268	$11,000 \pm 2,000$	$50,000 \pm 4,800$	4.5

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Table 3. Estimates of the Pharmacodynamic Parameter Concentration at Half-maximal EEG Effect (EC_{50}) and Maximal EEG Effect (E_{max}) of Four Synthetic Opioids in Rats and Human^{3,4,6,14}

	EC_{50}		E_{max}	
	Rat (ng/ml)	Human (ng/ml)	Rat (μV)*	Human (Hz)†
Sufentanil	1.4	0.7	104	16.5
Fentanyl	10.1	7.5	89	15.2
Alfentanil	289	520	111	14.5
Remifentanil	9.4	19.5	109	15.0
GR90291	105,000	—	109	13.8

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

† Determined by the spectral edge.

investigated for remifentanil and GR90291 in combination with the data previously obtained for alfentanil, fentanyl, and sufentanil.⁵ 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 agonism¹⁴ (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.⁹ 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 Cl of GR90291 (relative to the Cl 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 Cl 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 α_1 -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.

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