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Pharmacokinetic-pharmacodynamic modelling of the EEG effect of alfentanil in rats: assessment of rapid functional adaptation

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1 The purpose of the present investigation was to quantify rapid functional adaptation in the concentration-pharmacological effect relationship of alfentanil in rats using quantitative EEG parameters as a pharmacodynamic endpoint. Three groups of 6–7 rats received in a randomized fashion two consecutive infusions of 2.00, 3.14, or 4.24 mg/kg^{-1} of alfentanil in 20, 40 or 60 min, respectively. The EEG was continuously recorded and frequent arterial blood samples were collected for determination of the alfentanil concentration by gas chromatography.

2 The pharmacokinetics of alfentanil were most adequately described by a bi-exponential function. The values (mean ± s.e., $n=20$) of clearance, volume of distribution at steady-state and terminal half-life were 45 ± 3 ml.min^{-1}.kg^{-1}, 0.91 ± 0.09 l/kg^{-1} and 23 ± 1 min, respectively, and independent of the administered dose.

3 Increase in power in the 0.5–4.5 Hz (delta) frequency band of the EEG was used as the measure of the pharmacological response. By pharmacokinetic-pharmacodynamic modeling the individual concentration-EEG effect relationships of alfentanil were derived which were successfully quantified by the sigmoidal E_{max} pharmacodynamic model. When the results of the first of the two consecutive infusions were compared, no systematic differences in the pharmacodynamic parameters were observed for the different infusion rates. The averaged values of the pharmacodynamic parameters of alfentanil were (mean ± s.e., $n=20$): $E_0 = 56 \pm 3$ μV, $E_{max} = 93 \pm 8$ μV, $EC_{50} = 235 \pm 27$ ng.ml^{-1} and Hill factor = 1.6 ± 0.1, respectively. For the second of the two consecutive infusions a significantly higher value of the EC_{50} of 404 ± 56 ng.ml^{-1} was observed ($P<0.05$), while the values of the other pharmacodynamic parameters were unchanged. Simulations according to a mechanism-based model indicated that the observed change in concentration effect relationship can be explained by a 40% loss of functional μ-opioid receptors.

4 The results of the present study show that upon the administration of a single intravenous dose, acute functional adaptation does not interfere with the assessment of the concentration-EEG effect relationship of alfentanil. Upon repeated administration however functional adaptation may be a complicating factor.

Keywords: Opiates; alfentanil; EEG; pharmacokinetic-pharmacodynamic modelling; tolerance

Introduction

Since the development of fentanyl in the 1960s, synthetic opiates have been used widely in anaesthesia. Their excellent analgesic properties, combined with the cardiovascular stability, make them particularly useful in cardiac anesthesia. A limitation of many existing synthetic opiates is prolonged respiratory depression at the termination of surgery. Current research efforts focus therefore on the synthesis of opioids with pharmacokinetic properties which allow a more precise control of the intensity and the duration of the pharmacological response (James, 1994; Rosow, 1993). Integrated pharmacokinetic/pharmacodynamic modeling may be a useful tool to optimize the dosing of these compounds (Peck *et al*., 1992; Lemmens *et al*., 1994).

In the past decade considerable progress has been made in the development of chronically instrumented rat models for (PK/PD) modeling studies with CNS active drugs such as barbiturates (Ebling *et al*., 1991; Mandema and Danhof, 1990), benzodiazepines (Mandema *et al*., 1991a,b) and baclofen (Mandema *et al*., 1992). In these models realistic estimates of potency and intrinsic activity can be obtained. Interestingly, values of the relative potency and intrinsic activity in animals are often very similar to the values observed in humans (Levy, 1993). Preclinical PK/PD modeling studies may therefore be of great value to link pharmacological investigations with the subsequent clinical development of CNS active drugs (Danhof *et al*., 1993).

Recently, a chronically instrumented rat model has been developed in which the time course of the effect on the electroencephalogram (EEG, amplitude in the 0.5–4.5 Hz frequency band) following an anaesthetic dose of a synthetic opiate can be determined in conjunction with blood concentrations. In the model, respiratory depression is managed by artificial ventilation with air as to maintain arterial blood gasses within physiological limits, body temperature is stabilized with isothermal pads and opiate induced seizures are prevented by a steady-state infusion of midazolam (Cox *et al*., 1997). An important question is to what extent acute functional tolerance development is a complicating factor in the estimation of pharmacodynamic parameters in this model. In previous investigations with morphine, using the anti-nociceptive response as a pharmacodynamic endpoint, significant acute functional tolerance development has been demonstrated by administering the drug in infusions of different duration (Gårdmark *et al*., 1993).

The aim of the present investigation was to study acute functional tolerance development to the EEG effect of alfentanil. For that purpose three groups of 6–7 rats received,

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in a randomized fashion, two consecutive infusions of different doses of alfentanil over different infusion lengths. EEG was recorded continuously in conjunction with determination of the blood concentrations. The data were analysed by simultaneous PK/PD modeling to derive the concentration-EEG effect relationship in individual rats.

Methods

Chemicals

Alfentanil hydrochloride and the internal standard R38527 were donated by Janssen Pharmaceutica BV (Beersse, Belgium). In the animal experiments alfentanil was administered as a solution of approximately 1 $mg.ml^{-1}$ in physiological saline. Midazolam was donated by Hoffmann-LaRoche (Basel, Switzerland). A solution of approximately 2 $mg.ml^{-1}$ was prepared with an equimolar quantity of hydrochloric acid. Vecuronium bromide was obtained from Organon Technika BV (Boxtel, The Netherlands). A solution of 2 $mg.ml^{-1}$ was used.

Animals and Surgery

Male SPF rats of Wistar descent with a body weight between 250 and 300 g were used in the experiments (Broekman BV, Someren, The Netherlands). The rats were housed individually in plastic cages at constant temperature of 21°C and a controlled light-dark cycle (lights on: 0700 h to 1900 h). Food (Standard Laboratory Rat Mouse and Hamster Diets, RMH-TM, Hope Farms, Woerden, The Netherlands) and tap water were available *ad libitum*. One week before the experiment the rats had seven cortical EEG electrodes implanted under fentanyl/fluanisone anaesthesia (HYPNORM^{R}, Janssen Pharmaceutica BV, Beerse, Belgium) as described before (Mandema & Danhof, 1990). One day before the experiment, four permanent cannulas were implanted: one in the femoral artery, two in the left jugular vein and one in the femoral vein. The cannulas in the left jugular vein and the femoral vein were used for the administration of alfentanil, midazolam and vecuronium, respectively. The cannula in the femoral artery was used for the collection of blood samples. The protocol of the study was approved by the Committee on Animal Experimentation of Leiden University.

Pharmacokinetic-pharmacodynamic experiments

Three groups of 6–7 rats were randomly assigned to different drug treatments. The experimental protocol of the study is summarized in Figure 1. Each rat received two consecutive intravenous infusions of alfentanil. The two subsequent alfentanil doses given to the three treatment groups were 2.00 and 4.24, 3.14 and 3.14 and 4.24 and 2.00 $mg.kg^{-1}$, respectively. The doses of 2.00, 3.14 and 4.24 $mg.kg^{-1}$ were administered in 20, 40 and 60 min, respectively. These administration schemes were designed as to result in approximately similar maximum blood concentrations. In all treatment groups the second dose was given 130 min after the termination of the first infusion. The experiments were always started between 0900 h and 0930 h. To determine the pharmacokinetics of alfentanil a total of 36 blood samples (50 or 100 μl) was collected at fixed intervals over the time course of the experiment. Blood samples were immediately haemolyzed with 0.5 ml of deionized water and stored at −20°C until analysis.

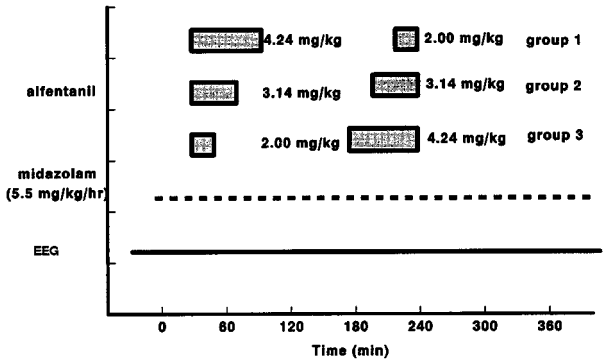

Figure 1 Schematic presentation of the experimental protocol of the pharmacokinetic-pharmacodynamic experiment for the three treatment groups.

Two bipolar EEG leads (C_l-O_l and C_r-O_r) were continuously recorded using a Nihon-Kohden AB-621G Bioelectric Amplifier (Hoekloos BV, Amsterdam, The Netherlands) and concurrently digitized at a rate of 210 Hz using a CED 1401_{plus} interface (CED, Cambridge, U.K.). 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 EEG parameters were obtained off-line by fast Fourier analysis with a user-defined program within the data analysis software package Spike 2, version 4.60 (CED, Cambridge, U.K.). The change in amplitude in the 0.5–4.5 Hz frequency band of the EEG power spectrum was used as pharmacological endpoint. Reduction of EEG data was performed by averaging amplitude values over predetermined time intervals.

In order to prevent alfentanil-induced seizures, rats received a continuous infusion of midazolam at a rate of 5.5 $mg.kg^{-1}.h^{-1}$. To reach steady-state rapidly, midazolam was adminstered according to a Wagner infusion scheme, with an initial infusion rate at three times the steady-state infusion rate for 16 min (Wagner, 1974). The midazolam infusion was started 30 min before the first administration of alfentanil. Three blood samples of 50 μl per rat were drawn randomly to monitor the midazolam concentration over the time course of the experiment.

During and after the infusions of alfentanil severe respiratory depression and muscle rigidity occurred. Rats were artificially ventilated with air using an Amsterdam Infant Ventilator, model MK3 (Hoekloos, Amsterdam, The Netherlands) through a custom made ventilation mask. The ventilation settings were: ventilation frequency 62 beats min^{-1}, I-E ratio 1 : 2 and air supply flow rate 0.7–1.0 liter min^{-1}. When muscle rigidity appeared, the rat received an intravenous bolus injection of 0.15 mg vecuronium bromide and artificial ventilation was started. Administration of vecuronium in a dose of 0.10 mg was repeated each time muscle rigidity reappeared (usually every 5 min) until spontaneous respiratory activity returned and muscle rigidity did not reappear. In order to ascertain adequate artificial ventilation arterial pH, pCO_2 and pO_2 values were obtained using a Corning 178 Blood Gas Analyzer (CIBA Corning, Houten, The Netherlands) and were carefully maintained within physiologically accepted ranges. A maximum total blood volume of 2.3 ml per individual rat was drawn.

During the experiments, body temperature was stabilized between 37.5 and 38.5°C with the aid of Delta Phase Isothermal Heating Pads (Braintree, Braintree, MA, U.S.A.)

and ventilation with air preheated to 32°C. Body temperature was monitored using a YSI Tele-Thermometer (Yellow Springs Instrument Corporation Inc., Yellow Springs, OH, U.S.A.).

Chemical assays

Concentrations of alfentanil in blood were determined by gas chromatography (GC) with nitrogen-phosphorus detection according to Woestenborghs *et al*. (1981) with slight modifications. Briefly, 80 ng of R38527 (internal standard) in 40 μl ethanol was added to a haemolyzed blood sample. The sample was alkalized by the addition of 1.5 ml of 0.5 M sodium triphosphate solution and subsequently extracted with 5 ml of pentane on a whirlmixer for 30 s. After centrifugation the organic layer was separated and evaporated to dryness under reduced pressure. The residue was reconstituted in 40 μl of ethanol and 1–2 μl were injected into the GC system. The GC system was a Hewlett-Packard 5890a gas chromatograph equipped with a nitrogen-phosphorus detector and a split/splitless capillary inlet port (Hewlett-Packard, Amsterdam, The Netherlands). The column was a fused silica capillary column (10 m * 0.31 mm ID) with a 100% methyl-silicone stationary phase (Chrompack Nederland BV, Bergen op Zoom, The Netherlands). The carrier gas was helium (flow rate: 4 ml.min^{-1}). The temperature of the inlet port was 300°C, of the column 220°C and of the detector 320°C. Data processing was performed using a Chromatopack C-3RA integrator (Shimadzu, Kyoto, Japan). Linear calibration curves were obtained in the concentration range of 25–2000 ng.ml^{-1}. In a 100 μl blood sample the detection limit was 10 ng.ml^{-1}. Intra-assay variability was 3% over the entire concentration range. The inter-assay variability at the concentrations 50, 300 and 1500 ng/ml was 15, 4 and 4%, respectively.

The blood concentrations of midazolam were determined by HPLC with u.v. detection as previously described (Mandema *et al*., 1991b). The intra- and inter-assay variation was less than 6%.

Data analysis

The pharmacokinetics and pharmacodynamics of alfentanil were quantified for the individual rats and for the separate infusions. The blood concentration-time profiles during and after infusion were characterized by a poly-exponential equation:

$$C(t) = \sum_{i=1}^{n} \frac{C_i}{\lambda_i \cdot T} \left(1 - e^{-\lambda_i \cdot t}\right) \qquad (t < T) \qquad (1A)$$

$$C(t) = \sum_{i=1}^{n} \frac{C_i}{\lambda_i \cdot T} \left(1 - e^{-\lambda_i \cdot T}\right) \cdot e^{\lambda_i \cdot (t-T)} \qquad (t \geq T) \qquad (1B)$$

where C(t) is the blood concentration of alfentanil at time t, T is the duration of the infusion and C_i and λ_i are the coefficients and the exponents of the equation, respectively. Different models were investigated and tested according to the Akaike Information Criterion (Akaike, 1974). Various pharmacokinetic parameters were calculated for the first infusion data from the coefficients and exponents of the fitted functions by standard methods (Gibaldi & Perrier, 1982).

For each individual rat and for each separate infusion, the coefficients and exponents of the equation were used to calculate the alfentanil concentrations at the time points of the EEG measurements.

The sigmoidal E_{max} pharmacodynamic model was used to describe the relationship between alfentanil concentration and EEG effect:

$$E_C = E_0 + \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n} \qquad (2)$$

in which E_C is the EEG effect at alfentanil blood concentration C, E_0 is the no-drug effect, E_{max} is the maximum effect, EC_{50} is the alfentanil concentration at 50% of the maximum effect and n is the slope of the curve.

The concentration-EEG effect relationship of alfentanil was also simulated according to the operational model of agonism as proposed by Black & Leff (1983):

$$\frac{E_C}{E_m} = \frac{\tau^n \cdot [C]^n}{(K_A + [C])^n + \tau^n \cdot [C]^n} \qquad (3)$$

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 and K_E, the concentration of occupied receptors that elicits half-maximal effect, n the slope factor of the transduction function and K_A is the agonist equilibrium/dissociation constant. The pharmacokinetic- and pharmacodynamic data were analysed using the data analysis program Siphar, version 3.0 (Simed, Creteil, France).

Statistics

The pharmacokinetic and pharmacodynamic parameter estimates of alfentanil in the different treatment groups were statistically compared by a parametric one-way analysis of variance (ANOVA). When parameter estimates were compared between the first and the second infusion, a paired Student's t-test was used. In case of non-homogeneity, as determined by Bartlet's test, the non-parametric Kruskal-Wallis test was used. A probability of less than 0.05 was considered statistically significant.

Results

Figure 2 shows the blood concentration and the EEG effect *versus* time profiles of alfentanil in a typical rat. Upon the first alfentanil infusion a maximum effect on the 0.5–4.5 Hz frequency band of the EEG was attained rapidly. After termination of the infusion the EEG effect gradually returned to pre-infusion values. Upon the second alfentanil infusion a very similar EEG effect *versus* time profile was observed. The blood concentration *versus* time profile in all treatment groups and for both infusions per individual was most adequately described by a bi-exponential function. The pharmacokinetic parameter estimates are presented in Table 1. Upon the first infusion no significant differences in the estimated parameters were observed for the different infusion rates. Comparison of the elimination half-life of alfentanil between the two infusions yielded a small but statistically significant increase upon the second infusion for the 20 min–60 min group ($P<0.05$). This difference however, was not considered relevant for this study.

No time delay (hysteresis or proteresis) between blood concentration and EEG effect was observed in any of the treatment groups treatment groups and for both infusions, and the two were correlated directly to each other by the sigmoidal E_{max} model. Figure 3 shows the derived concentration-EEG effect relationship. The pharmacodynamic parameter estimates upon the first infusion are

summarized in Table 2. Upon the first infusion no significant effect of infusion rate on the pharmacodynamic parameter estimates was observed.

Comparison of pharmacodynamic estimates between the first and the second infusion yielded a significant shift in EC_{50} from 235 ± 27 ng.ml^{-1} for the first infusion to 404 ± 56 ng.ml^{-1} for the second infusion ($P < 0.05$, Table 2). No differences were observed for the other pharmacodynamic parameters.

During the experiments the midazolam plasma concentration was constant at a value of 1020 ± 300 ng.ml^{-1} (mean $\pm$ s.d., $n = 53$). No differences in midazolam concentrations were observed between the treatment groups and between the two consecutive infusions.

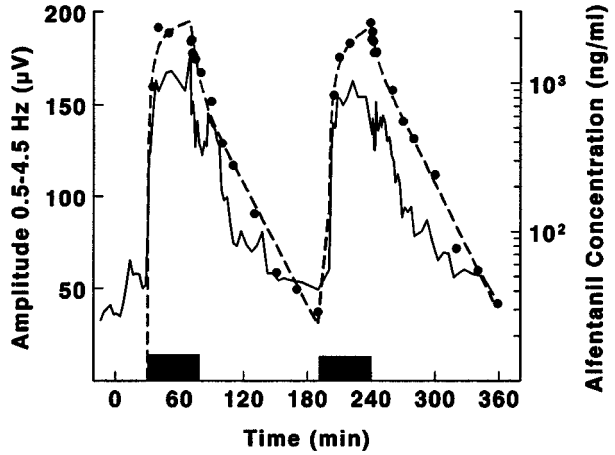

Figure 2 Blood concentration (closed circles) and EEG effect *versus* time profile in a typical rat after administration of two consecutive infusions of 3.14 mg.kg^{-1} alfentanil in 40 min. The dashed line represents the best fit to the blood concentrations according to the pharmacokinetic model. The solid bars represent the duration of the alfentanil infusions.

Table 1 Influence of infusion rate on pharmacokinetic parameter estimates of alfentanil obtained from the first infusion and the values of steady-state midazolam concentration in the three treatment groups (mean $\pm$ s.e.)

	n	Cl (ml·min^{-1}·kg^{-1})	$V_{d,ss}$ (l·kg^{-1})	$t_{½,\alpha,n}$ (min)	$C_{ss,midazolam}$ (ng·ml^{-1})
20 min	7	53 ± 6	1.19 ± 0.2	24 ± 2	880 ± 62
40 min	7	37 ± 4	0.75 ± 0.09	25 ± 2	1080 ± 58
60 min	6	44 ± 3	0.76 ± 0.04	18 ± 1	1104 ± 74

Discussion

In the present investigation the pharmacokinetic/pharmacodynamic relationship of alfentanil was determined in the rat using the effect on the electroencephalogram (amplitude in the 0.5–4.5 Hz frequency band) as a pharmacodynamic endpoint. In a previous investigation it has been demonstrated that upon the intravenous administration of a single dose of 2000 μg/kg of alfentanil the blood concentration-EEG effect relationship can be described by the sigmoidal E_{max} pharmacodynamic model (Cox *et al.*, 1997). However, the development of acute tolerance may affect the observed *in-vivo* concentration-effect relationship of opioids (Gårdmark *et al.*,1993; Veng-Pedersen & Modi, 1993; Mandema & Wada, 1995). In the present investigation, the development of acute tolerance was examined by administering alfentanil as infusions of different duration. A similar approach has previously been applied to examine acute tolerance development to the EEG effect of midazolam (Mandema *et al.*, 1991b) and for the antinociceptive effect of morphine (Gårdmark *et al.*, 1993). Furthermore, each rat received two consecutive infusions of alfentanil, an approach which has also been used to

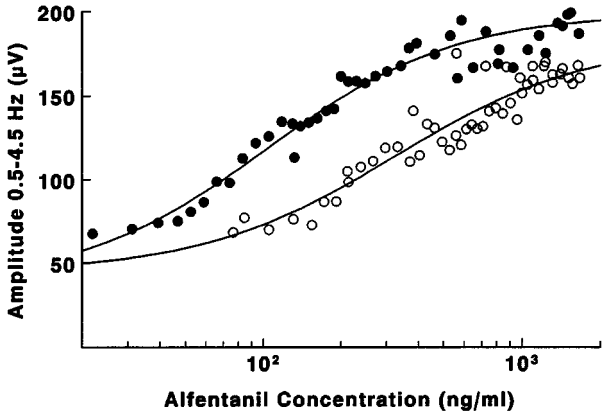

Figure 3 Concentration-EEG effect relationship of alfentanil upon repeated administration in a typical rat. The closed circles represent measured EEG effect data from the first infusion and the open circles EEG effect data from the second infusion. The solid lines represent the best fits to the actual data points according to the sigmoidal E_{max} model for each infusion.

Table 2 Influence of infusion rate and repeated administration on pharmacodynamic parametert estimates of alfentanil (mean $\pm$ s.e.)

Infusion scheme	*20/60 min*	*40/40 min*	*60/20 min*	*Total*
Number of animals	7	7	6	20
Infusion I				
E_0, μV	55 ± 4	53 ± 4	59 ± 6	55 ± 3
E_{max}, μV	95 ± 17	111 ± 10	69 ± 9	93 ± 8
EC_{50}, ng·ml^{-1}	202 ± 51	289 ± 41	210 ± 39	235 ± 27
Slope-factor	1.5 ± 0.2	1.7 ± 0.2	1.6 ± 0.2	1.6 ± 0.1
Infusion II				
E_0, μV	47 ± 9	57 ± 4	59 ± 6	54 ± 4
E_{max}, μV	104 ± 18	97 ± 14	67 ± 14	90 ± 10
EC_{50}, ng·ml^{-1}	332 ± 120	481 ± 88	396 ± 78[a]	404 ± 56[a]
Slope-factor	1.6 ± 0.3	2.2 ± 0.3	2.4 ± 0.5	2.0 ± 0.2

[a]Significantly higher than for infusion I ($P < 0.05$).

characterize functional tolerance to the haemodynamic effects of nicotine (Porchet *et al*., 1988).

For none of the infusion rates, hysteresis or proteresis was observed between the blood concentration and the EEG effect. The two could be related to each other on the basis of the sigmoidal E_{max} pharmacodynamic model, resulting in estimates of the maximum effect (E_{max}), the concentration at 50% of the maximum effect (EC_{50}) and the slope factor of the curve. Identical values of these pharmacodynamic parameters were obtained for each of the different infusion rates. These findings indicate that within the time frame of the first infusion no functional tolerance development occurs to the EEG effect of alfentanil. This is in contrast with observations in previous investigations in dogs by Wauquier *et al*. (1988) and in rats by Mandema & Wada (1995) where proteresis was observed in the plasma concentration-EEG effect relationship after intravenous infusion. An important question is to what extent opioid induced CNS over-excitation may explain this apparent discrepancy. It is well established that administration of (synthetic) opioids may result in epileptiform activity (Urca *et al*., 1977; Carlsson *et al*., 1982; Maekawa *et al*., 1984; Tommasino *et al*., 1984; Young *et al*., 1984; Keykhah *et al*., 1985; Kofke *et al*., 1993; Cox *et al*., 1997). Seizure activity may be a confounding factor in the assessment of spectral EEG parameters of opioids. Convulsive patterns in the EEG contribute considerably to the amplitude in the 0.5–4.5 Hz frequency band. These convulsions do not persist continuously, but often damp down to a nearly iso-electric EEG pattern (Cox *et al*., 1997). It may be that the apparent tolerance observed by Wauquier *et al*. (1988) and by Mandema & Wada (1995) is actually a peak effect in the EEG induced by overexcitation of the CNS, followed by a decrease as a result of a neurological feed-back regulatory process. It has been shown that alfentanil-induced convulsions can be successfully suppressed by the co-administration of the anticonvulsant midazolam. The present study shows that in a situation where midazolam is co-administered, no hysteresis or proteresis is observed. The value of the EC_{50} of 235 $ng.ml^{-1}$ in the present study is substantially smaller than the value of 845 $ng.ml^{-1}$ reported by Mandema & Wada (1995). Likewise, the value of the maximal effect is much smaller (200% *versus* 700% of the baseline effect, respectively). An important question is what may explain these differences. In this respect it is important to realize that there is a methodological difference between the present study and the study by Mandema & Wada (1995). In the present study midazolam has been co-administered to prevent alfentanil-induced activity, whereas in the study by Mandema & Wada this has not been the case. It cannot be excluded that the observed differences in the pharmacodynamic parameter estimates result from a pharmacodynamic interaction between alfentanil and midazolam. At present very little quantitative information is available on the pharmacodynamic interactions between alfentanil and midazolam. Clinical data seem to suggest that there is a synergistic interaction between synthetic opiates and benzodiazepines (Rosow, 1997). Furthermore, experiments *in vitro* have provided evidence for a synergistic interaction at the level of the spinal cord (Feng & Kendig, 1996) and these findings have been confirmed in an *in vivo* study (Luger *et al*., 1995). Interestingly however in the latter study an antagonistic rather than a synergistic interaction was observed at the supraspinal level. At present it is therefore very difficult to draw final conclusions with regard to the pharmacodynamic interaction between alfentanil and midazolam when using quantitative EEG parameters as a pharmacodynamic endpoint. It should be realized however that in a addition to the potential pharmacodynamic interaction between alfentanil and midazolam, there may also be other explanations for the observed differences in the pharmacodynamic parameter estimates. One such explanation may be the pharmacodynamic model that was used to derive the pharmacodynamic parameter estimates. In their study, Mandema & Wada (1995) used a physiological tolerance model to obtain the pharmacodynamic parameter estimates of alfentanil in the absence of tolerance development. An important question is whether on basis of the proposed tolerance model indeed realistic estimates of potency and intrinsic activity have been obtained. In this respect it is important that in the study by Mandema & Wada (1995) the highest values of the pharmacological response observed in the experiment, account for less than 40% of the estimated value of E_{max}. This seriously complicates the estimation of both the values of E_{max} and EC_{50}. Others have indicated that in the situation of tolerance development, use of the sigmoidal E_{max} model may not be appropriate, since it is not possible to differentiate between ceiling of the pharmacological effect and the development of acute tolerance (Porchet *et al*., 1988).

The values of the pharmacokinetic parameters of alfentanil were independent of the duration of the infusion (Table 1). The values are however slightly different from those reported in studies by Björkman *et al*. (1994) and Mandema & Wada (1995). This can possibly be explained by the differences in strains of rats that were used. Another factor is that non-linearities in the pharmacokinetics of alfentanil in rats have been demonstrated (Mandema & Wada, 1995).

In the present study, no functional tolerance development was observed during the first infusion. When the pharmacodynamic parameter estimates obtained from the second infusion data were compared with the estimates obtained from the first infusion data, the value for EC_{50} was increased significantly (Table 2), whereas the estimates of the other pharmacodynamic parameters were unchanged. This cannot be explained by a difference in the concentration of midazolam as these were constant throughout the experiment. A circadian rhythm in the CNS sensitivity for the effects of opioids may be responsible for this shift in EC_{50} values. Circadian variation in morphine-induced analgesia has been reported by Morris & Lutsch (1967) and Frederickson *et al*. (1977). In both studies minimum analgesic activity was observed towards the end of the light phase when compared to the end of the dark phase. This is similar to the findings in the present study, where there is also a decrease in potency over the time course of the light phase. An alternative explanation is however that the first alfentanil infusion may have induced desensitization of central μ-opioid receptor system. This explanation would be in line with numerous *in-vivo* and *in-vitro* studies which have shown that upon exposure to opioid agonists the efficacy of the μ-opioid receptor system may be attenuated by several molecular- and cellular mechanisms (for reviews see Johnson & Fleming, 1989; Collin & Cesselin, 1991). More recently there have been attempts to characterize quantitatively the rate and extent of functional tolerance development to opiates using more traditional measures of analgesic response (Ekblom *et al*., 1993; Gardmark *et al*., 1993; Ouellet & Pollack, 1995). In all these studies however morphine has been used as a model drug. The advantage of alfentanil is that, in contrast to morphine, this drug is not metabolized into (inter)active metabolites. Formation of (inter)active metabolites may be a complicating factor in studies on functional tolerance development (Cox *et al*., 1997).

In a separate publication we have proposed to characterize the pharmacodynamics of synthetic opioids on basis of the operational model of agonism (Cox *et al*., 1998). In terms of

this model, that was originally proposed by Black & Leff (1983), the observed reduction in potency without an associated change in intrinsic activity or slope of the concentration-effect relationship can only be explained by a reduction in the operational affinity and/or efficacy of alfentanil. The most simple explanation would be a decreased apparent affinity (K_A) of alfentanil for the μ-opioid receptors, since K_A affects only EC_{50} and not E_{max} or the Hill factor. Alternatively, the loss of potency may reflect a reduced efficacy (τ) of the system. This explanation however, implies the assumption of the presence of a considerable receptor reserve, since in the case of low efficacy agonists a reduction in τ not only affects EC_{50}, but also E_{max} and Hill factor. Interestingly, there is indeed evidence for a large receptor reserve for synthetic opioids with respect to their EEG effect in the rat (Cox *et al*., 1998). To illustrate this, the effect of different values for τ given a constant value of K_A for alfentanil was simulated and is presented in Figure 4. Also shown is the mean concentration-EEG effect relationship of alfentanil from the two consecutive infusions in the present study. It can be seen that the increase in EC_{50} in the sigmoidal E_{max} model upon the second infusion is similar to a decrease in efficacy in the operational model. The decrease in the value of τ, for instance expressed as the ratio of the two values, is an indication of the loss of functional receptors. In this model a decrease of τ from 100 to 60 was observed upon the second infusion. This would be, for example, similar to the effect of a 40% reduction of functional opioid receptors in the brain. Also it can be seen that a decrease in τ will not result in a decrease of intrinsic activity (E_m) until τ decreases to values lower than four.

In order to further characterize the functional tolerance development quantitatively, attempts were made to quantify both the rate and extent of the development of tolerance by fitting the data of the two infusions to an integrated pharmacokinetic-pharmacodynamic model that included the development of tolerance. Over the past 15 years several integrated pharmacokinetic-pharmacodynamic models have been proposed for the quantification of functional tolerance development. Between those models differences exist regarding the actual factor that causes tolerance development. The responsible stimulus may be either time (Chow *et al*., 1985; Ambre *et al*., 1988), drug concentration (Hammarlund *et al*., 1985; Porchet *et al*., 1988; Ekblom *et al*., 1993; Gårdmark *et al*., 1993) or drug effect (Bauer & Fung, 1990; Veng-Pedersen & Modi, 1993). The data obtained in the presented EEG study were analysed according to a number of these models. Neither of these models could adequately describe the tolerance development observed in this study. The reason for this may be that each of these models assumes tolerance development as a continuous dynamic process. In case of a single (or first) drug administration three different situations may occur in which this process is reflected in a different way. First, in the situation of a gradual increase and decrease of the effect, proteresis should be observed in the blood concentration-effect relationship. The second situation arises when the maximum effect is rapidly obtained after the start of the infusion, i.e. there is no increasing limb in the blood concentration-effect plot. In this situation the EC_{50} value is primarily estimated from the effect data obtained after termination of the infusion. In this situation, the extent of tolerance development, will be reflected in an increase of EC_{50}, which is related to the duration of the infusion. In the third situation a rebound effect is observed upon termination of drug exposure, regardless whether the initial maximum effect is rapidly obtained or not. In the present study, the maximum EEG effect was rapidly obtained in all treatment groups (see Figure 2). However, no effect of duration of infusion on the value of the EC_{50} was observed. The data obtained form the first infusion do therefore not provide evidence of a continuous process of tolerance development. An intriguing question is whether tolerance development is always a continuous process or whether, alternatively, it can also be a quantal response to a previous drug exposure or drug effect. It has been suggested that a drug effect may be primed in a memory system, and may become manifest at the second exposure to the drug. In fact such a phenomenon has been observed for tolerance development to the analgesic effect of morphine in rats (Siegel, 1975; Dafters *et al*., 1988).

On basis of the observations in the present investigation it can be concluded that, in contrast to several studies reported previously, upon a single intravenous infusion reliable estimates of the *in-vivo* pharmacodynamics of alfentanil can be obtained that are not confounded by acute functional tolerance development.The model therefore provides a suitable basis for the investigation of the *in-vivo* pharmacodyamics of synthetic opioids. Differences in pharmacodynamics upon repeated administration however may indicate a functional adaptation process, that requires further investigation.

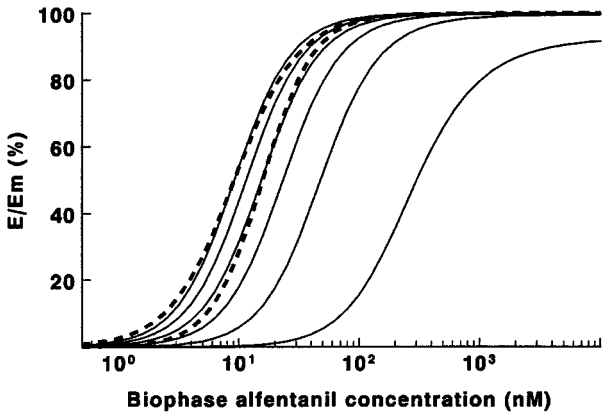

Figure 4 Simulation of the concentration-effect relationship of alfentanil based on an operational model of pharmacological agonism (Equation 3). From the left to the right the solid lines represent values for τ of 100, 80, 60, 40, 20 and 4, respectively. The receptor binding constant for alfentanil, K_A, has been set to 906 nM, as has been determined *in-vitro* by Cox *et al*., 1998. The operational slope factor, *n*, has been set to the average value of the slope factor of the two consecutive infusions in all treatment groups as obtained from the EEG data and the maximum effect (E_m) was set to 100% for both infusions. The dashed lines represent the mean concentration-EEG relationship of alfentanil observed upon the first (left) and the second (right) infusion. These lines coincide with values for τ of 100 and 60 for the first and the second infusion, respectively. The same model has recently also been used to correlate *in-vitro* receptor binding characteristics with *in-vivo* pharmacodynamics for a number of synthetic opioids (Cox *et al*., 1998).

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