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Relationship between gamma-hydroxybutyrate plasma concentrations and its electroencephalographic effects in the rat

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Abstract

In view of the potential interest in an objective parameter for the depth of coma in intoxications with the recreational drug gamma-hydroxybutyrate (GHB), we have studied the relationship between the plasma concentrations and the electroencephalographic (EEG) changes induced by GHB in the rat. Fifteen rats randomly received either 150 (n = 3), 200 (n = 6) or 300 mg kg^{-1} (n = 6) GHB over 5 min, followed by a supramaximal dose of 450 mg kg^{-1} over 5 min at the end of the experiment. Plasma concentrations were determined with HPLC. The EEG was continuously recorded and the amplitude in the 15.5–30 Hz frequency band was quantified using aperiodic analysis. The plasma concentration–time profiles were fitted to a two-compartment model with Michaelis–Menten elimination. The pharmacokinetic parameters V_{max}, K_m and the apparent volume of distribution (Vd) proved to be independent of the dose and the mean pooled values were V_{max} 2068 ± 140 μg min^{-1} kg^{-1}, K_m 58 ± 16 μg mL^{-1} and Vd 476 ± 12 mL kg^{-1}. The EEG amplitude in the 15.5–30 Hz frequency band displayed a monophasic inhibition and the effect–plasma concentration curve showed hysteresis. This hysteresis between EEG effect and plasma concentrations was minimized by simultaneous calculation of hypothetical effect-site concentrations and fitting the effect vs effect-site concentration curve to a sigmoid inhibitory E_{max} model. The descriptors of this E_{max} model (E_{max}, EC50, $k_{e,0}$, γ and E_0) were independent of the dose with an equilibration half-life $t_{\frac{1}{2}}k_{e,0}$ of 5.6 ± 0.3 min (mean value of the pooled results of the 5-min treatment groups). To investigate the origin of this hysteresis, a dose of 600 mg kg^{-1} GHB was infused over either 45 or 60 min each in three animals. The hysteresis was much less pronounced with 45 min than with 5 min and was absent with 60-min infusions. This indicated that the hysteresis was due to a distribution delay between the central compartment and the effect site. This study showed that the concentration–effect relationship of GHB could be characterized in individual rats using aperiodic analysis in the 15.5–30 Hz frequency band.

Introduction

Gamma-hydroxybutyrate (GHB) is a naturally occurring substance with neuromodulating properties (Maitre 1997). It crosses the blood–brain barrier after peripheral administration and in high doses induces behavioural responses including sedation and anaesthesia (Cash 1994). GHB has been used in anaesthesia, particularly for cases involving hypovolaemic, septic or cardiogenic shock (Kleinschmidt et al 1998) but is nowadays only sporadically used because of the

long induction time, unpredictable awakening time (Holmes & Henderson 1978) and incomplete analgesia (Kleinschmidt et al 1997). However, there is renewed interest in the substance as it is increasingly misused as a recreational drug with high doses leading to deep coma and even death (Chin et al 1998; Zvosec et al 2001). Clinical assessment of the depth of coma in these patients is difficult. Remarkable hyperreactivity to external stimuli like intubation (Li et al 1998) and an unexpected fast recovery from coma have been reported (Louagie et al 1997). The relation between hypnotic effect and the plasma concentration of GHB has been studied by Helrich et al (1964) in 16 volunteers. Their study observed a broad concentration range between GHB plasma concentrations and subjective parameters of consciousness. An objective parameter for the depth of coma such as changes in the electroencephalograph (EEG) which has been used as a surrogate parameter for the depth of anaesthesia might be useful (Dingemanse et al 1997). Due to the obvious ethical problems with the study of GHB in man we undertook experiments in the rat with the aim to assess the relationship between GHB plasma concentrations and the EEG effect. The monophasic inhibition in the high frequency band of the EEG was chosen as a parameter and studied for different doses and infusion schemes to test the reliability of the observed pharmacokinetic–pharmacodynamic relationship.

Materials and Methods

Animal instrumentation

The study protocol was approved by the Ethics Committee for Animal Research of the Faculty of Medicine of the University of Ghent. Male Wistar rats (280–420 g) were purchased from Iffa Credo and kept at 21°C with 12-h light–dark cycle. Surgery for the instrumentation was carried out under pentobarbital anaesthesia (60 mg kg^{-1}, i.p.) as described by De Paepe et al (1999). Briefly, one week before the experiment, epidural EEG electrodes were implanted in frontal, central and occipital positions on both sides of the skull. A reference electrode was placed on lambda. Two days before the experiment, polyethylene catheters (PE 10) filled with heparin solution (100 IU mL^{-1}) were inserted into the femoral artery and vein, and exteriorized at the nape of the neck. To minimize restraining stress during the experiment the animals were put in a restraining cage on several occasions before the actual experiment. The arterial line was used for the acquisition of blood samples while the venous line served for the infusion of GHB. The core temperature was measured every hour with a flexible thermistor probe inserted rectally to a depth of 5 cm and when the temperature fell below 37°C the animal was externally warmed by a heating lamp. The EEG was measured and recorded using a D/EEG Lite digital EEG recorder (Telefactor, Zwolle, The Netherlands) at a sampling rate of 200 Hz. The low-pass and high-pass filter was set at 1 and 70 Hz, respectively.

After overnight fasting, the rat was loosely restrained in a cage. All experiments were started between 0800 and 0900 h.

Experimental protocol

First series of experiments

In a first series of experiments the pharmacokinetics and the EEG effect of different doses of GHB were studied. Fifteen rats were randomized into three treatments groups and after 30 min of baseline EEG recording, received a total dose of GHB (Sigma Chemical Corporation, Bornem, Belgium) of either 150 (n = 3), 200 (n = 6), or 300 mg kg^{-1} (n = 6) over a 5-min infusion time. At the end of the experiment, i.e. 3 h after the start of the first infusion of GHB, each rat received 450 mg kg^{-1} GHB over 5 min to assess the maximum EEG effect in each rat. Three control animals received a sodium chloride solution containing a similar amount of sodium as used in the 450 mg kg^{-1} GHB dose.

The GHB was dissolved in water and, for each dose, the GHB concentration of the injection solution was calculated to administer a total volume of 0.3 mL per 100 g body weight. Arterial blood samples of 100 μL were taken for determination of GHB plasma concentrations at regular time intervals. Sampled blood was replaced with the same amount of isotonic saline solution. The EEG was assessed continuously during the whole experiment and the return of righting reflex was measured every 5 min.

Second series of experiments

In a second series of experiments, a total intravenous dose of 600 mg kg^{-1} was administered during 45 (n = 3) or 60 min (n = 3). EEG was continuously recorded and arterial blood samples of 100 μL were taken for determination of GHB plasma concentrations at regular time intervals. The return of righting reflex was measured every 5 min starting 15 min after the start of the infusion and the EEG was recorded continuously.

Drug assay

GHB was determined in rat plasma (20 μL) by a validated high-pressure liquid chromatography method (De

Vriendt et al 2001). The calibration curve ranged from 10 to 750 μg mL^{-1} GHB. Quality control samples at low (20 μg mL^{-1}), medium (300 μg mL^{-1}) and high (700 μg mL^{-1}) concentrations were analysed in duplicate together with the samples. The coefficient of variation was $< 10\%$ ($n = 12$) and the accuracy was between 98% and 105% ($n = 12$). The lower limit of quantitation was 10 μg mL^{-1}.

Analysis of data

The pharmacokinetics of GHB were quantified for each individual rat. The plasma concentration–time profiles during and after infusion were fitted to various one- and two-compartmental models using Winnonlin version 1.5 (Scientific Consulting, Inc). The best fitting model was chosen based on the Akaike Information Criterion (Akaike 1974) and the visual inspection of the curve. In all animals, the data were most adequately fitted to a two-compartment open model with Michaelis–Menten elimination kinetics (Gabrielsson & Weiner 1997) and a weight factor of y^{-2}:

$$dC_1/dt = R/V_C - Cl_d C_1/V_C + Cl_d C_2/V_C - V_{max} C_1/(K_m + C_1)V_C \quad (1)$$

$$dC_2/dt = Cl_d C_1/V_C - Cl_d C_2/V_C \quad (2)$$

$$\text{with } Cl_d = k_{1,2} V_C = k_{2,1} V_C \quad (3)$$

where dC_1/dt was the rate of decline of drug concentration at time t, V_C the distribution volume of the central compartment, R the infusion rate, Cl_d the intercompartmental clearance, C_1 the concentration in the central compartment, C_2 the concentration in the peripheral compartment, V_{max} the theoretical maximum rate of the elimination, K_m the Michaelis–Menten constant, $k_{1,2}$ the transfer rate constant from the central to the peripheral compartment, and $k_{2,1}$ the transfer rate constant from the peripheral to the central compartment.

The effect of GHB in each individual rat was determined by means of the electroencephalogram. Bipolar EEG was recorded at the fronto-central (F_1C_1, F_2C_2) and fronto-occipital (F_1O_1, F_2O_2) leads. Due to the amplitude in the fronto-central leads being greater than in the fronto-occipital leads with no difference between the left and right fronto-central leads, the F_1C_1 lead was used to assess the GHB effects from the EEG signal. The EEG data were averaged over predetermined intervals. The interval duration (5–30 s) depended on the rate of change of the signals. In preliminary experiments, the aperiodic analysis algorithm (Gregory & Pettus 1986) and fast Fourier analysis (Bührer et al 1990) were used for the quantification of the raw EEG signal. The resulting parameters were then assessed with the criteria according to Bührer et al (1990): the numerical parameter had to be stable at baseline; show both onset and recovery of the EEG effect in parallel to drug concentrations, with tolerance of some delay; and the duration of the maximum effect had to be proportional to the dose administered. This assessment showed that the amplitude per second parameter of the 15.5 Hz to 30 Hz band, calculated with aperiodic analysis, fulfilled these criteria.

The pharmacokinetic parameters were used to calculate the GHB concentrations at the time points of the EEG measurements. These concentrations were correlated with the measured EEG effects for each individual rat. Hysteresis in the EEG effect vs the plasma concentration relationship was observed and was minimized in a parametric way (Della Paschoa et al 1998) using Winnonlin version 1.5 (Scientific Consulting, Inc). The effect-site concentration of GHB was calculated using the following link model:

$$dC_e/dt = k_{e,0} C_1 - k_{e,0} C_e \quad (4)$$

where C_e was the effect-site concentration, $k_{e,0}$ was the first-order rate constant for the distribution from the central compartment to the effect site and C_1 was the concentration in the central compartment.

A sigmoid inhibitory E_{max} model was used to describe the relationship between the effect-site concentration and the effect:

$$E = E_0 - E_{max} C_e^n/(EC50^n + C_e^n) \quad (5)$$

where E_0 was the baseline effect, E_{max} the maximal inhibition of the EEG effect measured after the infusion of 450 mg kg^{-1} GHB, EC50 the concentration required to obtain 50% depression of the baseline effect, and n a constant expressing the shape of the concentration–effect relationship. Finally, the effect-site concentration of GHB at the return of righting reflex was determined from the effect vs time curve and the effect vs effect site concentration curve.

Statistical analysis

The results were expressed as mean $\pm$ s.e.m. Individual maximal observed effect values were compared with E_{max} values within each dose group with Student's *t*-test for paired values. Pharmacokinetic parameters and pharmacodynamic descriptors were compared between

dose groups with one-way analysis of variance followed by a Newman–Keuls test (Statistica version '99, Statsoft Inc., Tulsa, OK). $P < 0.05$ was considered as a significant difference.

Results

First series of experiments

The mean plasma concentrations of GHB are shown as a function of time for the three dose groups in Figure 1. Plasma concentrations rose rapidly during infusion and reached a maximum at the end of the infusion. This maximum was followed by a first phase of rapid decline followed by a slower second phase. The plasma concentration–time profiles were adequately fitted in all rats using a two-compartment open model with Michaelis–Menten elimination kinetics with the pharmacokinetic parameters shown in Table 1. Pharmacokinetic parameters obtained with the three doses were not significantly different. The average pooled values were for V_{max} 2068 ± 1400 μg min^{-1} kg^{-1}, K_m 58 ± 16 μg mL^{-1} and Vd 476 ± 12 mL kg^{-1}.

The EEG amplitude (15.5–30 Hz) vs time curve is shown for a typical rat in each dose group in Figure 2A. It displays the EEG effect of the first infusion (150 or 300 mg kg^{-1}) over 5 min starting at time zero, followed by a second infusion after 180 min with the fixed dose of 450 mg kg^{-1} over 5 min. In the 200 and 300 mg kg^{-1} dose group, the maximal EEG depression observed after the first infusion (E1) was not significantly different from the maximal attainable depression (E_{max}) of the second infusion (450 mg kg^{-1}), whereas in the 150 mg kg^{-1} dose group the maximal effect after the first infusion (E1) was significantly smaller than the maximal observed effect (E_{max}) after the second infusion (450 mg kg^{-1}) ($P < 0.05$). In three control rats, the administration of a sodium chloride solution, with the same amount of sodium as in the 450 mg kg^{-1} GHB dose, did not induce any changes in the EEG.

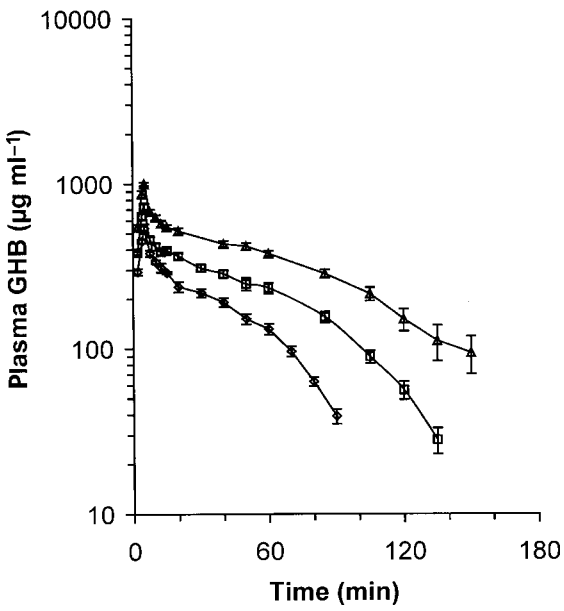

Figure 1 Average plasma concentration–time curves for GHB after an intravenous infusion of 300 mg kg^{-1} (n = 6; △), 200 mg kg^{-1} (n = 6; □) and 150 mg kg^{-1} (n = 3; ○) over 5 min. Each value represents the mean ± s.e.m. (bar).

The EEG effect vs plasma concentration curve for an individual rat in each dose group is shown in Figure 2B. For each dose, hysteresis was observed which was more pronounced with the higher doses. The hysteresis was minimized by calculation of the apparent effect-site concentrations using a first-order link model and by fitting the observed EEG effects to an inhibitory E_{max} model (Figure 2C). The EC50, E_0, E_{max}, $k_{e,0}$ and $t_{\frac{1}{2}}k_{e,0}$, the half-life for distribution between the central compartment and the effect site, showed no significant differences between the three dosing groups (Table 2).

The infusion of GHB caused sedation in each dose group. The return of the righting reflex could only be assessed systematically in the 300 mg kg^{-1} group. The reflex returned at 51 ± 5 min and the calculated effect-site concentration at this time was 452 ± 35 μg mL^{-1}. In the 200 mg kg^{-1} group the animals were deeply sedated but a return of the righting reflex could only be measured in three animals (27 ± 6 min) because frequent sampling immediately after the discontinuation of the infusion rendered a proper assessment of the reflex impossible. The three rats in the 150 mg kg^{-1} group were only slightly sedated and did not lose their righting reflex.

Second series of experiments

In this series, GHB (600 mg kg^{-1}) was infused over a longer period of time, 45 and 60 min. A dose of 600 mg kg^{-1} was chosen as it induced a maximal EEG effect as observed after the 450 mg kg^{-1} dose in the first series of experiments. The plasma concentration–time curves were most adequately fitted with a two-compartment model with Michaelis–Menten elimination kinetics (Figure 3). The pharmacokinetic parameters are listed in Table 1 and are in the same range as in the first series of experiments. However, it should be mentioned that the determination of the V_{max} and K_m in the 600 mg kg^{-1} over 45 min group was less accurate due to a short sampling period (420 min) during which the

Table 1 Pharmacokinetic parameters after the intravenous infusion of GHB for different dose regimens in the rat.

Dose	V_{max} ($\mu g\ min^{-1}\ kg^{-1}$)	K_m ($\mu g\ mL^{-1}$)	Vd ($mL\ kg^{-1}$)
First series			
150 mg kg^{-1}/5 min (n = 3)	1889 ± 244	49 ± 29	439 ± 21
200 mg kg^{-1}/5 min (n = 6)	2032 ± 323	35 ± 11	482 ± 23
300 mg kg^{-1}/5 min (n = 6)	2195 ± 118	85 ± 36	488 ± 12
Second series			
600 mg kg^{-1}/45 min (n = 3)	2703 ± 214	370 ± 130*	526 ± 43
600 mg kg^{-1}/60 min (n = 3)	2586 ± 539	70 ± 24	565 ± 20

V_{max} is the theoretical maximum rate of the elimination, K_m the Michaelis–Menten constant and Vd the volume of distribution. Each value represents mean ± s.e.m. *$P < 0.05$, compared with other dose regimens (one-way analysis of variance followed by Newman–Keuls test).

first-order elimination phase of GHB had not yet been reached.

The EEG amplitude (15.5–30 Hz) vs time curves for a typical rat of each of the two infusion groups were similar to those of the first series of experiments but the maximal depression was sustained (Figure 4A). The hysteresis was still present with the infusion during 45 min albeit less pronounced compared with the infusion regimens over 5 min. It was absent during the infusion over 60 min (Figure 4B). Figure 4C shows the effect vs effect-site concentration curves. The minimization of the observed hysteresis in the 45-min group yielded pharmacodynamic descriptors comparable with the descriptors in the 5-min infusion groups. The E_{max}, EC50, and E_0 in the 60-min infusion group were not statistically different from the values in the other groups (Table 2). However, a large difference was observed in the $k_{e,0}$ and the $t_{\frac{1}{2}}k_{e,0}$ reflecting the disappearance of the hysteresis (Table 2). The return of the righting reflex could be assessed in all animals and occurred at 146 ± 25 and 166 ± 11 min in the 45-min and the 60-min infusion group, respectively; the effect-site concentrations were 636 ± 44 and 519 ± 29 $\mu g\ mL^{-1}$, respectively.

Discussion

GHB is an old anaesthetic that is the subject of growing concern because of misuse as a recreational drug. Modelling the pharmacokinetic/pharmacodynamic (PK/PD) relationship of GHB by the introduction of a continuous and quantitative parameter such as EEG changes, as a surrogate measure of the depth of coma, may be of help. A validated PK/PD model implies that the pharmacokinetic parameters and the pharmacodynamic descriptors are independent of the dose and time (Derendorf & Meibohm 1999) and this was tested by the administration of different dose regimens of GHB.

Plasma concentration–time profiles of different doses of GHB (150, 200 and 300 mg kg^{-1}) infused over 5 min were measured and could be fitted to a two-compartmental model with Michaelis–Menten elimination kinetics. GHB has a small apparent volume of distribution and a relatively fast distribution phase. The calculated V_{max}, K_m and Vd were comparable with the values reported by Lettieri & Fung (1979) who used a one-compartment model with Michaelis–Menten elimination kinetics. However, in contrast with our findings, those authors did not observe a capacity limited elimination at low doses (200 mg kg^{-1}), which was probably due to the limited number of samples in their study. Michaelis–Menten elimination kinetics for GHB have also been reported in the dog (Shumate & Snead 1979), the cat (Snead 1976) and in man at non-anaesthetic doses used in the treatment of alcohol dependence (Ferrara et al 1992). The V_{max} in rats was approximately 10-times greater than in man (70–200 $\mu g\ kg^{-1}\ min^{-1}$) (van Ginneken et al 1974). This higher metabolic rate may be one of the factors responsible for the higher dose of GHB needed to induce sleep in rats.

We investigated whether the raw EEG changes induced by GHB could be quantified and related to the plasma concentrations as has been done for other anaesthetics (Dutta et al 1997; De Paepe at al 1999). GHB-induced changes in the EEG have been described in various species in a qualitative way (Winters & Spooner 1965) but a continuous and quantitative parameter is needed to model the PK/PD relationship. Quantifi-

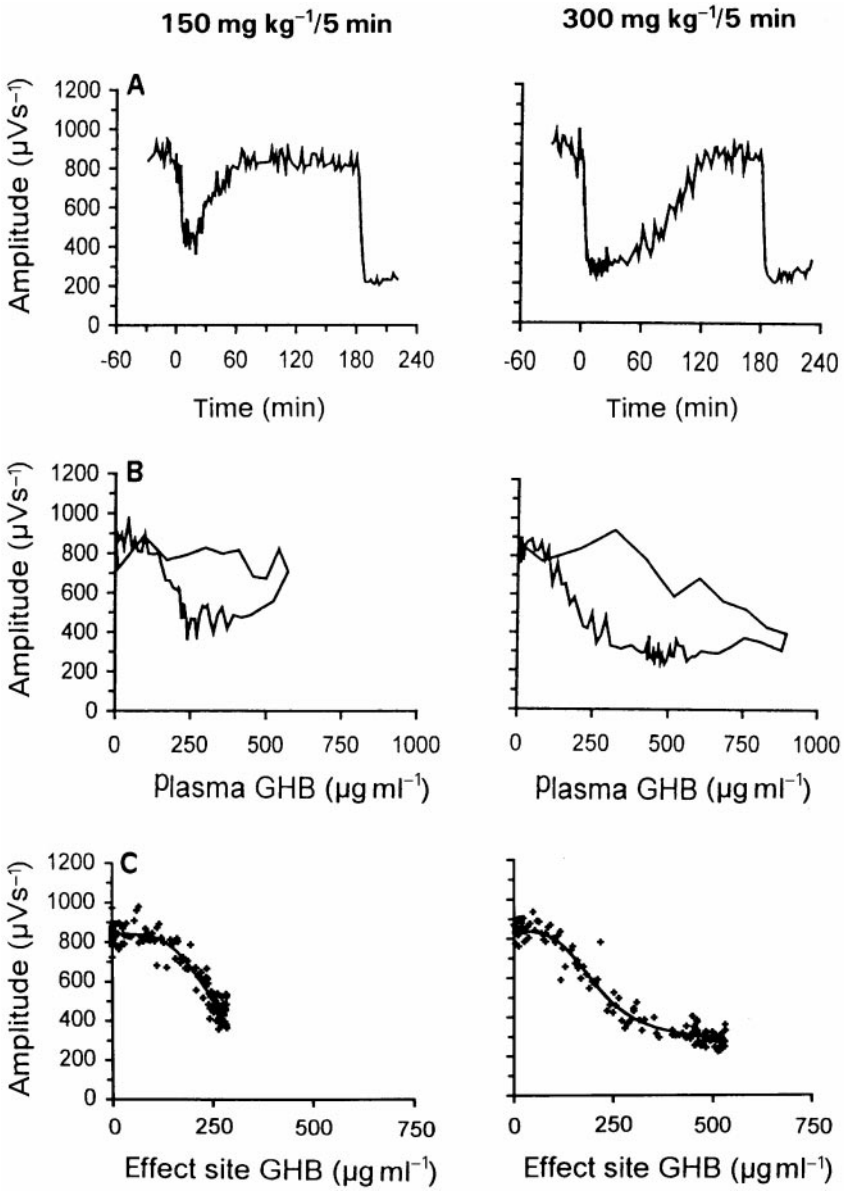

Figure 2 EEG effect in the 15.5–30 Hz frequency band after intravenous infusion of 150 or 300 mg kg^{-1} GHB over 5 min, followed by a dose of 450 mg kg^{-1} over 5 min starting at 180 min (panel A). EEG effect as a function of plasma concentration (panel B) and as a function of the calculated effect site concentration (panel C). The panels A, B and C show data for one representative rat of each dose group.

cation of the EEG changes induced by GHB was reported by Entholzner et al (1995) using fast Fourier transformation analysis of the EEG effects during the sedation of patients with GHB. Those authors made no attempt to correlate the EEG changes to plasma concentrations of GHB. Moreover, Fourier analysis may turn unstable during burst-suppression patterns. We therefore used aperiodic analysis, which has been found to be superior during this burst-suppression pattern (Mandema & Danhof 1992). With aperiodic analysis a monophasic activation of the total amplitude in the lower frequency band (< 7.5 Hz) and a monophasic inhibition in the 15.5–30 Hz band were observed. The activation in the lower frequency band reflected the changes observed in the raw EEG as described by Bearden et al (1980). However, preliminary experiments showed that at higher doses of GHB (> 300 mg kg^{-1}) this parameter turned into a biphasic pattern and this

Table 2 Pharmacodynamic parameters after the intravenous infusion of GHB for different dose regimens in the rat.

Dose	E1 ($\mu V\ s^{-1}$)	E_{max} ($\mu V\ s^{-1}$)	EC50 ($\mu g\ mL^{-1}$)	E_0 ($\mu V\ s^{-1}$)	$k_{e,0}$ (min^{-1})	$t_{\frac{1}{2}}k_{e,0}$ (min)
First series						
150 mg kg^{-1}/5 min	502 ± 32*,**	700 ± 27	268 ± 8	935 ± 69	0.12 ± 0.02	5.78 ± 0.86
200 mg kg^{-1}/5 min	587 ± 31	658 ± 31	298 ± 18	835 ± 30	0.13 ± 0.01	5.33 ± 0.35
300 mg kg^{-1}/5 min	644 ± 32	659 ± 36	325 ± 27	892 ± 42	0.13 ± 0.01	5.33 ± 0.58
Second series						
600 mg kg^{-1}/45 min		799 ± 151	468 ± 20	1066 ± 138	0.14 ± 0.03	5.00 ± 1.00
600 mg kg^{-1}/60 min		645 ± 24	350 ± 21	834 ± 58	8.01 ± 4.43	0.37 ± 0.30

E1 is the maximum depression observed of the EEG amplitude in the 15.5–30 Hz frequency band reached by the dose regimen (first infusion); E_{max} is the maximal attainable depression after the second infusion with a fixed dose of 450 mg kg^{-1}/5 min; EC50 is the effect site concentration when 50 % of the maximum is reached; E_0 is the baseline value of the EEG parameter; $k_{e,0}$ and $t_{\frac{1}{2}}k_{e,0}$ are respectively the first-order rate constant and the half-life of the distribution from the central compartment to the effect site. Results represent mean ± s.e.m. *$P < 0.05$ compared with other dose regimens using one-way analysis of variance, followed by the Newman–Keuls test; **$P < 0.05$ compared with E_{max} in this dose group using paired Student's *t*-test.

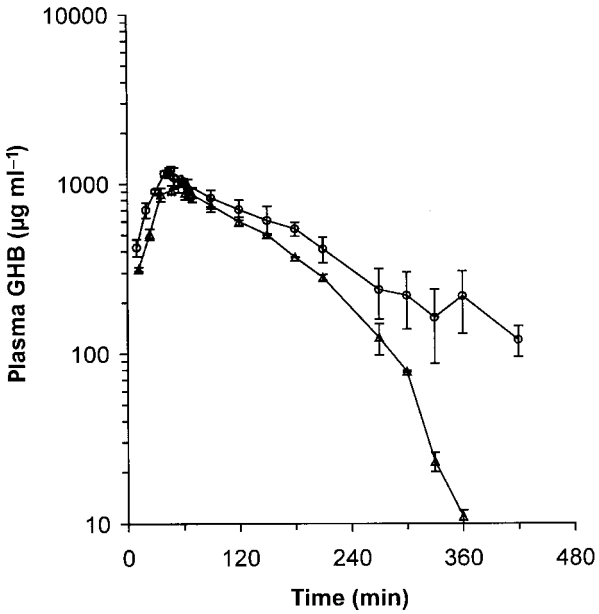

Figure 3 Average plasma concentration–time curves for GHB after an intravenous infusion of 600 mg kg^{-1} over 45 min (n = 3; ○) and 60 min (n = 3; △). Each value represents the mean ± s.e.m. (bar).

dose dependency precluded the PK/PD modelling. Therefore, we preferred to use the total amplitude in the 15.5–30 Hz band since it could be modelled independently of the dose. Interestingly, a monophasic inhibition in the 15.5–30 Hz frequency band has been described after the intravenous infusion of the $GABA_B$ agonist baclofen (Mandema et al 1992), which may suggest that the EEG changes by exogenous administered GHB may be related to $GABA_B$ receptor activation (Snead 1996; Gobaille et al 1999). This EEG parameter appeared to be stable at baseline and showed onset and recovery of the EEG effect in parallel to drug concentrations, with occurrence of some delay; preliminary experiments with the administration of different doses of GHB showed that the duration of the maximum effect was proportional to the dose administered.

Hence, using this EEG parameter, we tried to model the PK/PD relationship of GHB given over 5 min. Hysteresis between EEG effect and plasma concentration was observed with the three different doses. This may theoretically be explained by either a distribution delay between central compartment and effect site, the formation of an active metabolite, the occurrence of acute tolerance (Mandema & Danhof 1992a) or a decline in body temperature during the experiment (Cox et al 1997). The latter explanation is unlikely as we chose to warm up the rats when the body temperature fell below 37°C. It could be argued that this rewarming may have altered the EEG effects of GHB since GHB has been shown to induce hypothermia in the dose range used (Snead 1990). However, we felt that this was unlikely since only one animal (one of the 300 mg kg^{-1}/5 min group) had to be rewarmed. Moreover, there seems to be no relationship between the EEG changes and the hypothermia induced by GHB (Snead 1990). Tolerance has been suggested in experiments administering a seven-day infusion of GHB in the rat (Ratomponirina et al 1999) but to our knowledge there are no data on the occurrence of acute tolerance. The fact that the ascending portion of the loops followed the same pattern in each dose group suggested that after the effect compartment reached equilibrium with the central com-

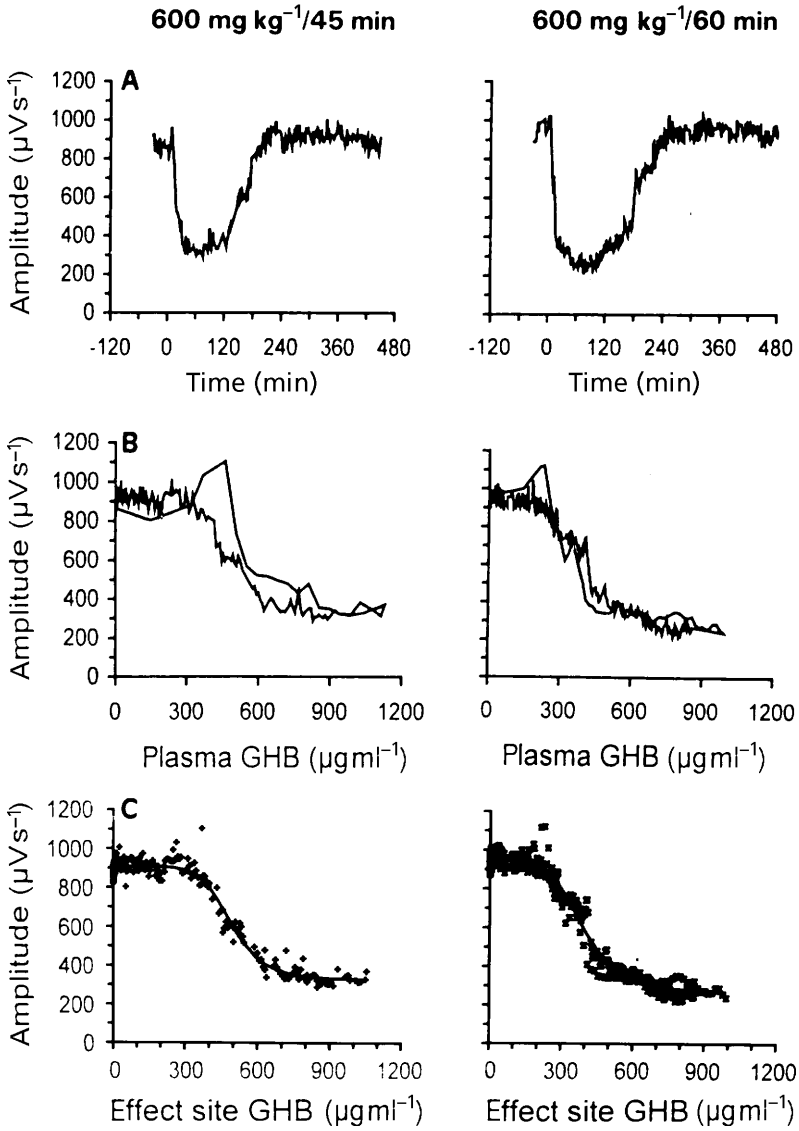

Figure 4 EEG effect in the 15.5–30 Hz frequency band after an intravenous infusion of 600 mg kg^{-1} GHB over 45 min and 60 min (panel A). EEG effect as a function of plasma concentration (panel B) and as a function of the calculated effect site concentration (panel C). The panels A, B and C show data for one representative rat of each dose group.

partment, the effect was directly proportional to the GHB plasma levels. This pleads for a distribution delay as the explanation for the observed hysteresis (Chiang & Barnett 1984). To test the hypothesis that the observed hysteresis between EEG effect and plasma concentration was due to a distribution delay we infused GHB over longer periods of time. The hysteresis was much less pronounced in the 45-min infusion than in the 5-min experiments and was no longer observed with the infusion over 60 min. This favours the hypothesis that hysteresis was due to a distribution delay and argues against the occurrence of acute tolerance or the development of active metabolites.

Minimization of this hysteresis yielded pharmacodynamic descriptors that were independent of the dose. The obtained $k_{e,0}$ corresponded to an equilibration half-life between plasma and effect site of approximately 5 min, which was higher than the values obtained in the rat for other anaesthetics such as etomidate (2.6 min; De Paepe et al (1999)) and propofol (2 min; Dutta & Ebling (1998)). This longer half-life was in agreement with the longer induction time of GHB when used as an

anaesthetic. Obviously, this $k_{e,0}$ value should be interpreted cautiously as it assumed that the observed equilibration between central compartment and effect site followed first-order kinetics. Snead (1978) reported that the brain concentrations paralleled the plasma concentrations, an argument for a first-order process.

The effect-site concentration at the return of the righting reflex in the different dose groups was larger than the EC50 value. This indicated that the EEG parameter used was associated with sedation and hypnosis rather than with deeper levels of CNS depression (Bol et al 2000). Further experiments are necessary to establish the relationship between the EEG parameter used and the neurological effects of GHB in the rat, e.g. by studying other reflexes like whisker reflex, startle reflex to noise and the cornea reflex as a function of the described EEG parameter changes.

In conclusion, the monophasic depression in the 15.5–30 Hz frequency band induced by GHB fulfilled the criteria for a suitable EEG parameter for modelling the pharmacokinetic–pharmacodynamic relationship of GHB in the rat. The hysteresis observed in the model was most likely due to a distribution delay between central compartment and effect site and not to the occurrence of acute tolerance or the formation of active metabolites within the time span and the dose range of the experiments. The effect site concentration of GHB at return of the righting reflex indicated that the EEG parameter might have been associated with sedation and hypnosis. The relationship between the described parameter and other neurological effects requires further investigation.

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