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A comparison of the concentration-effect relationships of midazolam for EEG-derived parameters and saccadic peak velocity

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1 Concentration-effect relationships of midazolam were assessed in an open study in six healthy volunteers. Saccadic eye movements and EEG parameters derived by fast Fourier transform (FFT) and aperiodic analysis (AP) were used to quantify drug effects.

2 Midazolam was infused at a rate of 0.6 mg kg^{-1} h^{-1} for a maximum of 15 min. Hypnotic effects were avoided by terminating infusions when subjects could no longer perform the eye movement test properly.

3 Wake-sleep transitions could be recognized through frequent observation of eye movements. The dose needed to reach maximum conscious sedation averaged 0.10 mg kg^{-1}, ranging from 0.06 to 0.13 mg kg^{-1}.

4 Sigmoidal concentration-effect relationships were found for EEG beta-amplitudes in five of six subjects, with average EC_{50} values (± s.d.) of 120 ± 54 ng ml^{-1} for FFT and 104 ± 40 ng ml^{-1} for AP. For the 'total number of waves' in the beta frequency range (AP) an average ($n = 6$) EC_{50} of 63 ± 37 ng ml^{-1} was found. Changes in EEG alpha-amplitudes were found in three subjects, resulting in an average EC_{50} value of 55 ± 32 ng ml^{-1}.

5 For saccadic peak velocity (PV) concentration-effect relationships were linear in five subjects and sigmoidal in one. The maximal measured decrease in PV averaged −44 ± 9%.

6 The differences in concentration-effect relationships for various effect parameters call for further studies with emphasis on the external validity and reproducibility of data. In such studies the dose needed to reach wake-sleep transition may be used as a relevant clinical end-point.

Keywords midazolam EEG saccadic eye movements concentration-effect relationship

Introduction

Concentration-effect relationships are used to study factors influencing drug effects and to compare the potency and intrinsic efficacy of drugs within a class. Ideally they should allow prediction of (changes in) relevant drug effects in individual subjects, based on a knowledge of individual concentration-effect relationships. In man, the concentration-effect relationships of benzodiazepines have been studied primarily using EEG derived parameters and saccadic peak velocity.

EEG parameters are generally derived by fast Fourier transform or aperiodic analysis of the signal, with parameters representing activity in a fixed frequency domain for a specific lead. However, the selection of EEG parameters remains arbitrary as their relationships to clinical end-points are largely undefined. Although criteria have recently been suggested for selecting an optimal parameter to evaluate the pharmacodynamics of midazolam [1], conclusions from that study were not

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supported by a statistical evaluation of the results and no attempt was made to relate the observed changes in the EEG to relevant clinical effects. Comparisons with clinical end-points and other measures of response are needed to enhance the interpretation of results obtained with EEG derived pharmacodynamic parameters.

Parameters summarizing EEG beta activity have mostly been used to evaluate concentration-effect relationships of benzodiazepines [2–4]. Decreases in alpha activity have also been used to quantify benzodiazepine effects [5], but this parameter cannot be used in subjects without a baseline alpha rhythm and 'ceiling effects' occur when alpha activity is fully suppressed. Such ceiling effects may not be a problem when the parameter represents a relevant endpoint. However, for EEG derived parameters this assumption may not be justified.

Concentration-effect relationships of benzodiazepines have mostly been described with a sigmoid-E_{max} model for EEG parameters. In animal models EC_{50} values for EEG effects of different benzodiazepines have been shown to correlate with receptor binding affinity and with EC_{50} values for anticonvulsant activity [6]. EEG effects in rats were also consistent with differences in intrinsic efficacy of drugs acting at the benzodiazepine receptor [4]. However, the EC_{50} values for a given compound depend on the choice of EEG effect-parameters and may be affected by physiological changes with the onset of sleep [7]. The change from waking- to sleep-EEG should preferably be avoided when evaluating drug effects on EEG. As individual sensitivities to benzodiazepines differ, dose adjustments are needed to study concentration-effect relationships over a wide range of plasma drug concentrations while avoiding hypnotic effects.

Saccadic peak velocity is highly sensitive to benzodiazepine effects [8] and appears to relate consistently to changes in arousal induced by drugs or by sleep deprivation [9–11]. Relationships between plasma concentrations of benzodiazepines and changes in saccadic peak velocity have been described in individual subjects with linear [10] and sigmoidal [12] models. Observations of eye movements may also be used to detect wake-sleep transitions, as these are characterised by a loss of visual fixation and the occurrence of slow 'drifting' eye movements [13]. Wake-sleep transition occurs during stage-one sleep or drowsiness, prior to the occurrence of sleep-spindles in the electroencephalogram which mark the onset of stage-two sleep [7,14]. Compared to EEG registrations, which are typically unstable during the period of drowsiness [7], wake-sleep transition is sharper and more clearly visible in the pattern of eye movements [13]. The amount of drug needed to reach wake-sleep transition has obvious clinical relevance and may be used as a reference parameter for sedative drug effects.

In this study the duration of infusions of midazolam was adapted to individual responses in order to reach 'maximal conscious sedation'. In this way major interactions between effect measures and physiological changes in EEG contents caused by sleep were avoided. After review of the available literature the infusion rate was set at 0.15 mg kg^{-1} for a maximum of 15 min. Concentration-effect relationships were evaluated for saccadic eye movements and for EEG parameters derived by fast-Fourier- and aperiodic analysis.

Methods

Subjects

Six healthy non-smoking volunteers (three female, three male, aged 19 to 29 years, weights 59 to 91 kg) were recruited after formal approval of the study protocol by the Ethics Review Board of the Leiden University Hospital. All volunteers received a full medical examination and gave written informed consent before entry to the study.

Trial design

The study was of an open, single dose design. Each subject received an infusion of midazolam at 0.6 mg kg^{-1} h^{-1}, for a maximum of 15 min or until sedative effects were too strong to perform the eye movement test properly. Performance was considered inadequate when subjects were unable to track the 0.3 Hz sinus used for calibration or when slow drifting eye movements occurred, indicating the absence of visual fixation.

Pharmacodynamic assessments were made in three baseline sessions and at 5, 10, 15, 20, 25, 35, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 360, 420 and 480 min after the start of infusion. Pharmacodynamic testing was performed in a quiet room with dimmed lights. Blood samples were taken with each effect measurement except at 75, 105 and 210 min. Each pharmacodynamic session started with a 1 min EEG registration, followed by registration of saccadic eye movements, measurement of blood pressure and heart rate and another one-minute EEG registration. For the purpose of concentration-effect modelling, actual delta times were calculated separately for the 48 measures of EEG and the 24 measures of saccadic eye movements.

Pharmacodynamics

EEG registrations were made using silver-silverchloride electrodes, fixed with collodion at Fz, Cz, Pz and Oz, with a common ground electrode at Fpz (international 10/20 system). The electrode resistances were kept below 5 kOhm. During recordings the subjects were sitting with their eyes closed. A cushioned head support was used to avoid artifacts caused by contractions of neck musculature. EEG signals were obtained from leads Fz-Cz and Pz-Oz. The signals were amplified by use of a Nihon Kohden AB-621G bioelectric amplifier (Nihon Kohden Corporation, Tokyo, Japan) with a time constant of 0.3 s and a low pass filter at 100 Hz. For the fast Fourier analysis, data collection and analysis were performed using customized CED software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of 8 s were recorded. The signal was AD-converted using a CED 1401 laboratory interface (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artifacts were identified by visual inspection and these

were excluded from analysis. Fast Fourier analysis was performed to obtain the sum of amplitudes in the delta- (0.5–3.5 Hz), alpha- (7.5–11.5 Hz) and beta- (11.5–30 Hz) frequency ranges. For aperiodic analysis, analogue recordings of EEG signals were made simultaneously (Instrumentation recorder HP-3964A, Hewlett Packard, San Diego, California, US). These signals were analyzed using a Lifescan EEG monitor (Neurometrics Inc., San Diego, California, US) with automatic artifact rejection. The sum of amplitudes and the 'total number of waves' (TNW) for the beta frequency range were calculated as effect parameters.

Saccadic eye movements were recorded as described previously [8]. Stimuli consisted of 14 stepwise horizontal displacements of 30° (±15°), at random intervals ranging from 3 to 6 s. The equipment used for stimulus display, signal collection and amplification was from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan). Subjects were instructed to track the stimulus as fast as possible at all times. When subjects failed to respond to the stimulus they received verbal stimulation from the investigator to obtain recordings over the largest possible range of plasma drug concentrations. The average peak velocity, inaccuracy and latency of all artifact-free saccades in a session were calculated as pharmacodynamic parameters. Saccadic inaccuracy was defined as the absolute value of the difference between the stimulus size and the corresponding saccade, expressed as a percentage of the stimulus size.

Blood pressure and heart rate were measured with subjects seated, using an automatic oscillometric blood pressure monitor (MPV 7201, Nihon Kohden Corporation, Tokyo, Japan).

Blood sampling, drug analysis and pharmacokinetics

Blood samples (8 ml) were drawn into a heparinized syringe (Safety-Monovette, Sarstedt, Nümbrecht, Germany) from a cannula inserted into a forearm vein. Samples were centrifuged at 5000 *g* for 6 min and plasma was stored at −40° C until analysis. Plasma concentrations of midazolam were measured by gas-liquid chromatography with electron capture detection according to the methods described by Mandema *et al* [12]. The detection limit of the assay was 1 ng ml^{-1}. Pharmacokinetic analysis was performed using the software package SIPHAR (Cimed, Creteil, France). Computational algorithms and weighting factors were adapted for each individual to obtain an optimal interpolation of the actual data points.

Concentration-effect modelling

Concentration-effect relationships were assessed for total plasma concentrations of midazolam using the software package SIPHAR (Cimed, Creteil, France). The α-hydroxy metabolite of midazolam was assumed not to contribute significantly to the effects as plasma concentrations of the metabolite are low after intravenous administration of midazolam [12]. After visual inspection of the plotted data, a linear model or a sigmoid-E_{max} model was chosen to describe the concentration-effect curve. In choosing between a linear or E_{max} model for a given effect parameter, preference was given to the latter providing that realistic parameter estimates could be obtained for the majority of subjects. The calculations were performed using a weighted least squares algorithm and the weighting factor w = 1. Hypothetical effect-site concentrations were calculated by use of a link model when concentration-effect plots showed clear hysteresis loops with at least two effect measures on the ascending limb of the curve. In these cases non-parametric k_{eo} values were calculated, based on methods described by Fuseau & Sheiner [15].

Statistical analysis

Paired *t*-tests comparing measurements at each time point with average baseline values were performed to provide an estimate the duration of drug effects. The duration of effect was defined as the time between the start of infusion and the first time point where no difference from average baseline values was found at the $P < 0.05$ level. Associations between parameters were sought by calculating Pearson's correlation coefficients. For EC_{50} values and slopes of concentration-effect plots approximate 95% confidence intervals were calculated using the standard errors for the parameter estimates.

Results

In all subjects the infusion of midazolam had to be discontinued before 15 min because of strong sedative effects. The durations of infusion ranged from 5 to 13 min, with total administered doses averaging 0.10 mg kg^{-1} (range; 0.06–0.13 mg kg^{-1}, Table 1). Three subjects failed to perform eye movement tests in up to four sessions after discontinuation of the infusion. At the time of maximal effect disturbances of eye movement control occurred in all subjects, as evidenced by dysmetria of saccades, poor fixation of the target and the occurrence of slow drifting eye movements. For these sessions best estimates of the effect-parameters were obtained from the remaining saccades.

Following midazolam infusion the maximum plasma drug concentration averaged (± s.d.) 277 ± 143 ng ml^{-1} (range; 65–452 ng ml^{-1}). The average elimination half-life 84 ± 23 min. The individual values for C_{max} and $t_{½}$ are shown in Table 1.

FFT-analysis of EEG registrations revealed changes in alpha-, beta- and delta- amplitudes. A clear decrease

Table 1 Administered doses, maximum plasma concentrations and elimination half-lives of midazolam

Subject (sex)	*Dose (mg)*	*Dose (mg kg^{-1})*	C_{max} *(ng ml^{-1})*	$t_{½}$ *(min)*
1 (M)	4.1	0.06	65	127
2 (F)	6.8	0.12	325	88
3 (F)	8.5	0.13	340	80
4 (M)	4.6	0.06	145	66
5 (M)	9.6	0.11	452	79
6 (F)	6.4	0.10	336	66
Mean	6.7	0.10	277	84
s.d.	2.1	0.03	143	23

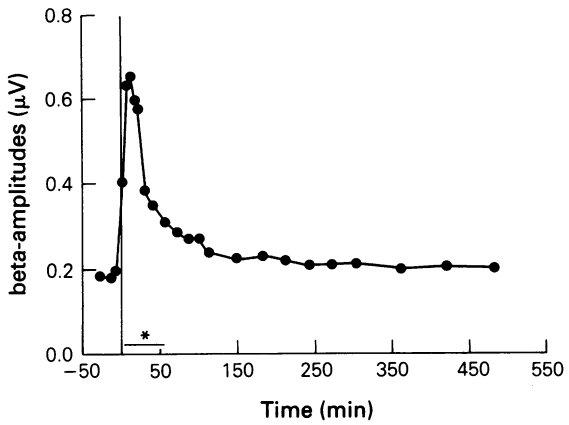

Figure 1 Average change in EEG beta-amplitudes at Fz-Cz. * = time period with EEG beta-amplitudes significantly changed from baseline ($P < 0.05$).

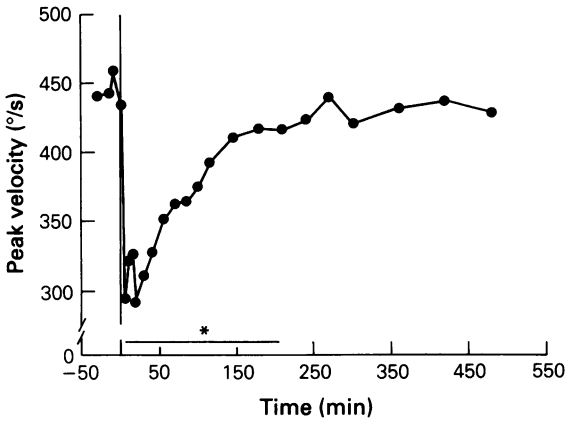

Figure 2 Average change in saccadic peak velocity. * = time period with saccadic peak velocity significantly changed from baseline ($P < 0.05$).

of alpha-amplitudes occurred at Pz-Oz in three subjects with a pronounced baseline alpha rhythm. Increased beta-amplitudes were found in all subjects, mainly at Fz-Cz. The average effect curve is shown in Figure 1. Delta amplitudes increased in both leads but changes were more variable across subjects. Sigmoidal concentration-effect relationships were found for occipital alpha-amplitudes in three subjects. For FFT beta-amplitudes sigmoidal concentration-effect relationships were found in five subjects with an average EC_{50} of 120 ± 54 ng ml^{-1}. In one subject the relationship was linear with no evident E_{max}. The changes in EEG beta-amplitudes assessed by aperiodic analysis were similar to those obtained by FFT, with an average EC_{50} of 104 ± 40 ng ml^{-1}. Changes in beta-TNW showed a similar time course to EEG beta-amplitudes. However, lower EC_{50} values were found for this parameter, with an average of 63 ± 37 ng ml^{-1}. There were positive correlations between EC_{50} values obtained by FFT- and AP analysis ($r = 0.99$ ($P < 0.01$, $n = 5$) for FFT beta-amplitudes *vs* AP beta-amplitudes and $r = 0.87$ ($P = 0.05$, $n = 5$) for FFT beta-amplitudes *vs* beta-TNW). A significant change from baseline was found up to 55 min for FFT beta-amplitudes, up to 85 min for AP beta-amplitudes and up to 110 min for TNW-beta. Individual parameter estimates for EEG concentration-effect relationships, including confidence intervals for EC_{50} values, are summarized in Table 2.

The concentration-effect curves for EEG parameters were steep in most cases, with slope factors averaging 3.3 ± 1.8 for FFT beta-amplitudes, 3.0 ± 1.3 for aperiodic beta-amplitudes and 3.8 ± 2.9 for beta-TNW. For FFT alpha-amplitudes a slope factor of 56.9 was obtained in one subject, indicating a virtual all or none response. Hysteresis loops in concentration effect plots were not consistently found for any subject or any EEG parameter. The calculated equilibration half-lives ($t_{½eo}$) were generally short (Table 2). However, in one subject a $t_{½eo}$ of 12.8 min was found for FFT alpha-amplitudes. For this subject the EC_{50} value calculated without the use of a link model was 26 ng ml^{-1} as compared with 34 ng ml^{-1} when the link model was applied.

Following infusion of midazolam the maximum decrease of saccadic peak velocity averaged 44 ± 9% (Figure 2). Saccadic peak velocity remained significantly below baseline values for 210 min. In addition, large increases in saccadic latency and inaccuracy were observed during the first hour. Concentration-effect relationships for saccadic peak velocity were linear in five subjects but followed a sigmoid-E_{max} curve in one. However, in the latter case the apparent E_{max} may have been an artefact caused by verbal stimulation or by an increase in the proportion of relatively fast saccades where many could not be analyzed as a result of poor coordination of eye movements. For this subject a linear fit of concentration-effect data was also calculated for

Table 2 Pharmacodynamic parameters; EEG (95% confidence intervals for EC_{50} values in parentheses)

	alpha-amplitudes (FFT)			*beta-amplitudes (FFT)*			*beta-amplitudes (aperiodic analysis)*			*beta-total number of waves (aperiodic analysis)*		
Subject	EC_{50} ($ng\ ml^{-1}$)	*N*	$t_{½eo}$ (*min*)	EC_{50} ($ng\ ml^{-1}$)	*N*	$t_{½eo}$ (*min*)	EC_{50} ($ng\ ml^{-1}$)	*N*	$t_{½eo}$ (*min*)	EC_{50} ($ng\ ml^{-1}$)	*N*	$t_{½eo}$ (*min*)
1	38 (37–39)	56.9	—	41 (37–45)	6.1	—	46 (32–60)	3.2	—	29 (20–38)	2.4	—
2	92 (87–97)	5.8	—	118 (113–123)	4.1	—	108 (103–114)	3.8	—	72 (69–76)	9.0	—
3	(change from baseline too small)			142 (123–161)	2.2	1.1	118 (101–135)	2.2	1.2	63 (56–69)	3.2	1.2
4	(change from baseline too small)			(linear ce-relationship)			(linear ce-relationship)			34 (27–40)[1]	0.9	—
5	(change from baseline too small			190 (103–278)	1.4	1.0	155 (148–161)	4.5	—	120 (114–126)	5.2	—
6	34 (25–43)[2]	2.8	12.8	110 (99–122)	2.8	0.8	91 (48–134)	1.1	3.2	33 (27–39)	1.9	3.3
Avg	55	21.8	—	120	3.3	—	104	3.0	—	59	3.8	—
SD	32	30.4	—	120	1.8	—	40	1.3	—	35	2.9	—

[1]Sigmoidal model obtained using fixed values for E_0 and E_{max}.
[2]Without use of a link model a value of 26 ng ml^{-1} was obtained.

Table 3 Pharmacodynamic parameters; peak velocity. (95% confidence intervals for slopes of concentration-effect plots in parentheses)

	Saccadic peak velocity		
Subject	*Intercept (°/s)*	*Slope (°/s (ng ml^{-1})$^{-1}$)*	*$t_{1/2eo}$ (min)*
1	413	−2.9 (−4.1, −1.7)	–
2	407	−1.3 (−1.2, −1.4)	2.4
3[1]	533	−1.1 (−2.2, −0.0)	–
4	446	−1.1 (−1.2, −1.0)	–
5	461	−0.6 (−0.7, −0.5)	1.5
6	389	−1.8 (−2.2, −1.4)	–
Mean	442	−1.5	–
s.d.	52	0.8	–

[1]Linear relationship for first part of the curve; sigmoidal model appropriate when three highest plasma concentrations are included.

the first part of the curve. The slopes of concentration-effect plots for saccadic peak velocity (PV-slopes) averaged −1.5 ± 0.8 °/s (ng ml^{-1})$^{-1}$. The individual values and confidence intervals for these slopes are shown in Table 3. Hysteresis of the concentration-effect curve for saccadic peak velocity was found in two subjects (Table 3).

The accuracy of the estimates of PV-slopes and EC_{50} values for EEG parameters were similar with coefficients of variation between 7 and 9%. Scatter plots showed apparent correlations between EC_{50} values for EEG beta effects and the doses needed to reach wake-sleep transition. The Pearson's correlation coefficients were 0.99 ($P < 0.01$) for EC_{50} values of FFT beta-amplitudes ($n = 5$) and 0.87 ($P < 0.05$) for EC_{50} values of beta-TNW ($n = 6$). The correlation between PV-slopes and doses needed to reach wake-sleep transition did not reach statistical significance ($r = 0.73$, $P = 0.1$, $n = 6$). However, when calculated for the five subjects included in the correlation for FFT beta-amplitudes, a similar correlation coefficient was found for PV-slopes ($r = 0.97$, $P < 0.01$, $n = 5$).

Increased heart rates were observed during the first hour after the start of the infusion; paired *t*-tests indicated significant differences from baseline up to 35 min ($P < 0.01$ at 5, 10, 15 and 20 min, $P = 0.15$ at 25 min and $P < 0.05$ at 35 min). The maximum measured increase averaged 14 ± 2 beats min^{-1}. No significant changes were observed in systolic- and diastolic blood pressures.

Discussion

Despite large interindividual differences in response, the methods used in this study allowed concentration-effect relationships of midazolam to be evaluated for the full range of plasma drug concentrations associated with 'conscious sedation'. Wake-sleep transition was assessed by frequent monitoring of saccadic eye movements and infusions were terminated when this point was reached. However, changes in wakefulness were occasionally very fast and a more accurate adjustment of individual doses should be possible at lower infusion rates. (This has been demonstrated in a subsequent study where lower infusion rates were used to study the effects of midazolam and its major metabolite [12]). The doses needed to reach wake-sleep transition in our study were markedly lower than expected. However, it was difficult to estimate the degree of sedation to be expected from the available literature as infusion rates are often not clearly stated, while clinical effects are, at best, described briefly.

As might be expected the EC_{50} values for EEG beta-amplitudes obtained by fast Fourier transform and by aperiodic analysis were highly correlated. The EC_{50} values for TNW-beta were also closely correlated, but the lower EC_{50} values and longer duration of effects indicated the 'total number of waves' to be a more sensitive measure. In another study, using the same methods and equipment, lower EC_{50} values (average: 77 ng ml^{-1}) were found for FFT beta-amplitudes [12]. While such differences may reflect variability in EC_{50} values across small groups of subjects, an effect of differing infusion rates cannot be excluded. An average EC_{50} of 40 ng ml^{-1} has been reported for *relative* beta-amplitudes at F_3–C_3 [2]. However, the low EC_{50} values for relative beta-amplitudes are likely caused by concurrent decreases in alpha activity. Similar low EC_{50} values have been found for alpha-amplitudes [5]. Obviously, the choice of parameters and differences in electrode positioning may affect concentration-effect relationships. However, despite a slight difference in electrode positions the discrepancy between EC_{50} values for TNW-beta in our study and the 290 ng ml^{-1} reported for hypnotic doses of midazolam [3] remains hard to explain.

The effects of midazolam on saccadic eye movements confirmed existing data on benzodiazepines. In line with the larger clinical effects, the maximal decrease in saccadic peak velocity was larger than the 29% decrease observed after an oral dose of 20 mg temazepam [10]. The inter-individual variability in PV-slopes following midazolam was similar to the fourfold variability reported for temazepam [10], which may indicate a more general variability in response to benzodiazepines for this parameter. By contrast to the results of this study, sigmoidal concentration-effect relationships for saccadic peak velocity were found in all subjects when lower infusion rates were used [12]. This difference was apparently caused by the fact that with the more gradual approach of wake-sleep transition subjects performances could be sustained for a longer period. Verbal stimulation at high levels of sedation and a selection of relatively fast saccades may have caused the maximizing of effects on saccadic peak velocity in the study which involved lower infusion rates.

The elucidation of concentration-effect relationships can be useful in pharmacological research. However, several factors may affect the accuracy of concentration-effect parameters. *First* pharmacokinetic models may not describe the measured plasma drug concentrations accurately. Discrepancies are often largest around peak concentrations and this may affect the estimates of EC_{50} values. *Second*, data are transformed when hysteresis loops are minimized by use of a link model. The use of a link model is based on the assumption of an equilibration delay between plasma concentrations and concentrations at the effect site [15]. However, hysteresis in concentra-

tion effect plots may also result from unknown post receptor events, which may or may not be reproducible. In our study the values of $t_{½eo}$ were generallly small and not likely to affect EC_{50} estimates to a large extent. Much larger differences in $t_{½eo}$ values have occasionally been reported [5]. In such cases, the reproducibility of $t_{½eo}$ values should be demonstrated if concentration-effect parameters are to be predictive. *Third*, the translation of the pharmacological effect of interest into values of a pharmacodynamic parameter may be different at various effect levels, as in the case of saccadic peak velocity where stimulation of subjects and a selection of relatively fast saccades may cause 'maximum' effects at high levels of sedation. Comparisons of the intrinsic efficacy and potency of drugs will be confounded when the observed maximum effect results from a characteristic of the parameter rather than a maximum pharmacological effect. In man, E_{max} values for EEG parameters also tend to coincide with wake-sleep transition while increasing plasma concentrations of benzodiazepines cause further CNS depression. This may indicate that these E_{max} values do not represent maximal effects in the pharmacological sense but an interaction of pharmacological and physiological effects. While the potential effects of sleep have been addressed previously [2], interactions may also occur with the onset of drowsiness or wake-sleep transition as these are associated with changes in alpha- and beta frequencies [7,14].

Although inaccuracy of the parameter estimates in concentration-effect models may affect their predictive value, confidence intervals for parameter estimates are not generally reported. In this study the 95% confidence intervals for EC_{50} values indicate these to be reasonably precise in most subjects. However, it should be noted that these confidence intervals do not account for the inaccuracy of the underlying pharmacokinetic parameter estimates. Therefore, the accuracy of concentration-effect parameters may have been overestimated.

The apparent correlation of EC_{50} values for EEG beta amplitudes with doses needed to reach wake-sleep transition indicates that this concentration-effect parameter may reflect individual sensitivities to the sedative effects of benzodiazepines. However, it should be noted that the actual value of beta amplitudes will not reflect the level of sedation because of large interindividual differences in EEG amplitudes. Small increases in EEG beta activity have also been attributed to increased activity in aminergic arousal systems after administration of cocaine [16] while increases observed after benzodiazepines and after smoking cigarettes have been explained as anxiolytic effects [17]. Obviously, this lack of specificity for behavioural effects should be considered when using EEG derived parameters to evaluate drug interactions. Saccadic peak velocity may be more suitable as an effect parameter in interaction studies, although ceiling effects should be considered at high levels of sedation. As with EC_{50} values for EEG beta-amplitudes there was an apparent correlation between PV-slopes and doses needed to reach wake-sleep transition. However, the difference in correlation coefficients observed when using five or six subjects, illustrates the need to confirm these relationships with larger data sets.

While saccadic peak velocity and EEG parameters allow a detailed evaluation of the effects of benzodiazepines, and possibly of other drugs, attempts should be made to standardize methods and to test the predictive value of concentration-effect parameters. In addition, the external validity of parameters should be studied if concentration-effect relationships are to be meaningful. At present, this favours the use of saccadic eye movements as a sensitive test with consistent behavioural correlates. The assessment of wake-sleep transition by monitoring eye movements may provide a useful clinical end point for the evaluation of sedative drug effects.

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