Characterization of the Pharmacodynamic Interaction between Parent Drug and Active Metabolite In Vivo: Midazolam and α-OH-Midazolam

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ABSTRACT

The pharmacodynamic interaction between midazolam and its active metabolite α-OH-midazolam was investigated to evaluate whether estimates of relevant pharmacodynamic parameters are possible after administration of a mixture of the two. Rats were administered 10 mg/kg of midazolam, 15 mg/kg of α-OH-midazolam, or a combination of 3.6 mg/kg of midazolam and 35 mg/kg of α-OH-midazolam. Increase in the 11.5- to 30-Hz frequency band of the electroencephalogram was used as the pharmacodynamic endpoint. The pharmacodynamics of midazolam and α-OH-midazolam after combined administration were first analyzed according to an empirical and a competitive interaction model to evaluate each model’s capability in retrieving the pharmacodynamic estimates of both compounds. Both models failed to accurately estimate the true pharmacodynamic estimates of midazolam and α-OH-midazolam. The pharmacodynamic interaction was subsequently analyzed according to a new mechanism-based model. This approach is based on classical receptor theory and allows estimation of the in vivo estimated receptor affinity and intrinsic in vivo drug efficacy. The relationship between stimulus and effect is characterized by a monotonically increasing function f, which is assumed to be identical for midazolam and α-OH-midazolam. The pharmacodynamic interaction is characterized by the classical equation for the competition between two substrates for a common receptor site. This mechanism-based interaction model was able to estimate the pharmacodynamic parameters of both midazolam and α-OH-midazolam with high accuracy. It is concluded that pharmacodynamic parameters of single drugs can be estimated after a combined administration when a mechanistically valid interaction model is applied.

In many instances drug effects are the result of the combined action of two or more chemical entities, when two or more drugs are given in combination, when a drug is converted into an active metabolite, or in the situation where, for an enantiomeric drug, a racemate of the two enantiomers is administered or metabolic interconversion occurs. In these situations the intensity of the pharmacological response can generally not be predicted by simply adding the effects of the two compounds separately; rather complex interaction models may be required. In this respect a clear distinction must be made between competitive interactions (where the two compounds interact at the same receptor) and noncompetitive interactions (where the two compounds bind to a separate receptor; Danhof and Mandema, 1992).

Many psychotropic drugs are converted into active metabolites (Caccia and Garratini, 1990) and these metabolites may contribute significantly to the pharmacological response (Danhof and Mandema, 1995). A well known example is benzodiazepines, where active metabolites have been demonstrated to contribute significantly to the effect (Crevoisier et al., 1983; Garzone and Kroboth, 1989; Mandema et al., 1992b).

When a drug is converted into an active metabolite, generally the situation arises where two active species are present which, as result of their close structural similarity, compete for the same receptor. Therefore, typically a competitive interaction model is required to characterize the combined effect of a drug and its active metabolite. Recently the modeling of the pharmacodynamic interactions between benzodiazepines has been the subject of a number of investigations. Specifically, empirical interaction models have been proposed to characterize the interactions between a benzodiazepine full agonist on one hand and benzodiazepine partial, competitive, or inverse (ant)agonists on the other (Mandema et al., 1992a,c). So far, however, the modeling of the pharmacodynamic interactions between two full agonists (or between parent drug and its active metabolite) has not been reported.

In this paper a new mechanism-based model is proposed

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ABBREVIATIONS: EEG, electroencephalogram; CI, clearance; Vss, volume of distribution at steady-state; Emax, maximal drug effect; N, constant expressing the sigmoidicity of the concentration-effect relationship; Kapp, in vivo estimated receptor affinity; θPD, in vivo drug efficacy.
for the modeling of competitive drug-drug interactions between full agonists in vivo that can also be used to characterize the pharmacodynamic interactions between a parent drug and its active metabolite. This model is based on receptor theory and contains the pharmacodynamic parameters in vivo estimated receptor affinity ($K_{RYP}$) and intrinsic in vivo drug efficacy ($\epsilon_{DP}$). The relationship between stimulus and effect is characterized by a monotonically increasing function $f$, which is assumed to be identical for both parent drug and metabolite. The pharmacodynamic interaction is characterized by the classical equation for the competition between two ligands for a common receptor site. This new model is applied in a study on the pharmacodynamic interaction between midazolam and its active metabolite $\alpha$-OH-midazolam in rats, using quantitative electroencephalogram (EEG) parameters as a pharmacodynamic endpoint. The performance of the new model is compared to that of the previously proposed, more empirical interaction models.

**Materials and Methods**

**Animals.** Three groups of 6 to 8 male Wistar rats (Sylvius Laboratory Breeding Facility, Leiden, the Netherlands) weighing (mean ± S.E.) 261 ± 7 g were used in the study. The animals were kept individually in plastic cages with a normal 12-h light/dark cycle and were fed on a commercially available diet (Standard Laboratory Rat, Mouse and Hamster Diets, RMG-TM, Hope Farms, Woerden, the Netherlands) and water ad libitum. From the night before the experiment onward, the animals were deprived of food but had free access to water. For the measurement of EEG signals, chronic cortical EEG electrodes were implanted into the skull of the animals 1 week before the kinetic-dynamic experiments as described previously (Mandema et al., 1991b). One day before the experiment, indwelling cannulae were implanted in the right jugular vein (for drug administration) and the right femoral artery (for blood sample collection).

**Drug Dosage and Blood Sampling.** Rats received 10 mg/kg midazolam, 15 mg/kg $\alpha$-OH-midazolam, or a combined administration of 3.6 mg/kg midazolam and 35 mg/kg $\alpha$-OH-midazolam during a 15-min infusion. Drugs were dissolved in 0.9% saline with the aid of an equimolar quantity of hydrochloric acid. To determine the pharmacokinetics of midazolam and $\alpha$-OH-midazolam, blood samples of 100 or 200 $\mu$l (near the end of the experiment) were collected at fixed time intervals after drug administration over a period of 280 min. After the experiment the animals were sacrificed and a final blood sample was obtained by aortic puncture to be used for protein binding measurements. Heparinized blood samples were centrifuged and plasma was separated and stored at −35°C until the time of analysis.

**EEG Measurements.** The output from bipolar EEG leads was continuously recorded using a Nihon Kohden EEG system consisting of a bioelectric input box JB-682G (Mihon Kohden Corporation, Tokyo, Japan), bioelectric amplifier AB-621G, and bioelectric input panel PB-680G. The low pass filter was set at 100 Hz, the time constant at 0.3 s. During the course of the experiment the animals were forced to walk in a slowly rotating drum to prevent spontaneous fluctuations in the level of vigilance (Mandema et al., 1991b). EEG recordings were commenced 15 min before drug administration for baseline determination. Two EEG leads, the fronto-central and central occipital lead on the left hemisphere, were quantified on-line by aperiodic analysis (Gregory and Pettus, 1986) as described previously (Mandema et al., 1991b). The amplitudes ($\mu$V/Hz) in the 11.5- to 30-Hz frequency band of the fronto-central lead as change over baseline were used as a measure of drug effect intensity.

**Drug Analysis and Plasma Protein Binding.** Plasma concentrations of midazolam, $\alpha$-OH-midazolam, and 4-OH-midazolam were determined by a gas chromatographic assay using electron-capture detection as described previously (Mandema et al., 1992b). Detection limits were 20 ng/ml for all three compounds. The extent of plasma protein binding of midazolam and $\alpha$-OH-midazolam was determined for each individual animal by ultrafiltration at 37°C using the Amicon Micropartition System (Amicon Division, Danvers, MA) as described previously (Mandema et al., 1991b).

**Pharmacokinetics.** Data were analyzed with the NONMEM computer program as developed by Beal and Sheiner (NONMEM project group, University of California at San Francisco, San Francisco, CA). The plasma concentration-time profiles of the drugs after i.v. infusion were described by a polyexponential equation for i.v. infusion:

$$C(t) = R_0 \cdot \sum_{i=1}^{a} \frac{A_i}{\alpha_i} (e^{-\alpha_i t} - e^{-\alpha_i (t-T_i)})$$

where $(t-T_i)$ is defined by:

$$(x) = \begin{cases} x & \text{for } x > 0 \\ 0 & \text{otherwise} \end{cases}$$

In this equation, $C(t)$ is the plasma concentration of the drugs at time $t$, $T$ is the duration of infusion, and $A_i$ and $\alpha_i$ are, respectively, the coefficients and exponents of the disposition function. Interindividual error was modeled according to a log-normal distribution and a proportional error model was used to describe the residual error. Empirical Bayes estimates of the coefficients and exponents were derived for each animal. Basic pharmacokinetic parameters clearance (CI), volume of distribution at steady-state ($V_{ss}$), and terminal half-life ($T_1/2$) were calculated from the coefficients and exponents of the fitted functions according to standard procedures (Gibaldi and Perrier, 1982).

**Pharmacodynamics.** Pharmacodynamic data were analyzed with the NONMEM computer program as developed by Beal and Sheiner (NONMEM project group). Individual pharmacokinetic profiles were used to drive the pharmacodynamic fitting. Concentrations of midazolam and $\alpha$-hydroxy-midazolam were directly linked to the EEG effect and characterized according to the sigmoidal maximal drug effect ($E_{\text{max}}$) model (Holford and Sheiner, 1982):

$$E(C) = E_{\text{max}} \cdot \frac{C^N}{C^N + EC_{50}^N}$$

where $E(C)$ is the observed effect at concentration $C$, $E_{\text{max}}$ is the maximal effect, $EC_{50}$ is the concentration at half-maximal effect, and $N$ is the Hill factor, a constant expressing the sigmoidicity of the concentration-effect relationship. Residual error was described on the basis of an additive error model.

**Empirical and Competitive Interaction Modeling.** The results obtained upon the combined administration of midazolam and $\alpha$-OH-midazolam were analyzed on the basis of two different interaction models: an empirical interaction model and a pure competitive model. First, the data obtained upon administration of midazolam separately and upon administration of $\alpha$-OH-midazolam were directly linked to the $E_{\text{max}}$ effect and characterized according to the sigmoidal maximal drug effect ($E_{\text{max}}$) model (Holford and Sheiner, 1982):

$$E(C_A, C_B) = E_{\text{max}A} \cdot \frac{(C_A/EC_{50A})^{N_A}}{1 + (C_A/EC_{50A})^{N_A}} + E_{\text{max}B} \cdot \frac{(C_B/EC_{50B})^{N_B}}{1 + (C_B/EC_{50B})^{N_B}}$$

An empirical model for competitive interaction between two ligands acting at the same receptor was proposed by Holford and Sheiner (1982):

$$E(C_A, C_B) = \frac{E_{\text{max}A} \cdot (C_A/EC_{50A})^{N_A} + E_{\text{max}B} \cdot (C_B/EC_{50B})^{N_B}}{1 + (C_A/EC_{50A})^{N_A} + (C_B/EC_{50B})^{N_B}}$$
where $E(C_{A}, C_{B})$ is the combined effect of drugs A and B at concentration $C_{A}$ and $C_{B}$. $E_{\text{max}}$ is the maximal effect, $E_{C50}$ is the plasma concentration at half-maximal effect of drugs A and B when present separately, and N is the Hill factor, a constant expressing the sigmoidicity of each drug’s concentration effect relationship. Several variations to the model were included in the evaluation, either assuming the $E_{\text{max}}$ or the Hill coefficient (N) of midazolam and α-OH-midazolam to be equal. A limitation of this model is that when the Hill factor is not exactly 1, either a synergistic or an antagonistic interaction is predicted.

To overcome the limitations of the above empirical model, a pure competitive interaction model can be derived, assuming that effects after single administration of the interacting compounds can be characterized on the basis of the sigmoidal $E_{\text{max}}$ model (eq. 2). Competitive models are of an additive nature. The interaction between two drugs A and B is additive when the isobole, i.e., the curve of drug concentration at half-maximal effect of drugs A and B when present separately, and N is the Hill factor, a constant expressing the sigmoidicity of each drug’s concentration effect relationship. Several variations to the model were included in the evaluation, either assuming the $E_{\text{max}}$ or the Hill coefficient (N) of midazolam and α-OH-midazolam to be equal. A limitation of this model is that when the Hill factor is not exactly 1, either a synergistic or an antagonistic interaction is predicted.

The data were analyzed by a new mechanism-based model where effect is viewed to be a function of the benzodiazepine receptor binding-induced stimulus (S). This mechanism-based approach allows the event at the receptor level to be separated from the multitude of processes as result of the formation of the drug-receptor complex. This has the important advantage that empirical factors such as the Hill factor are omitted from the model. The details of the mechanism-based modeling approach are presented as an Appendix to this paper. When using this mechanism-based approach, the relationship between drug concentration and effect is characterized by the following equation:

$$E = f(S) = f \left( \frac{e_{PD} \cdot C}{C + K_{PD}} \right)$$

Mechanism-Based Interaction Modeling. Subsequently, the data were analyzed by a new mechanism-based model where effect is viewed to be a function of the benzodiazepine receptor binding-induced stimulus (S). This mechanism-based approach allows the event at the receptor level to be separated from the multitude of processes as result of the formation of the drug-receptor complex. This has the important advantage that empirical factors such as the Hill factor are omitted from the model. The details of the mechanism-based modeling approach are presented as an Appendix to this paper. When using this mechanism-based approach, the relationship between drug concentration and effect is characterized by the following equation:

$$E(C_{A}, C_{B}) = f(S) = f \left( \frac{e_{PD} \cdot C_{A}}{K_{PD,A} + C_{A}} \cdot \frac{C_{B}}{K_{PD,B} + C_{B}} \right)$$

where $K_{PD, A}$ and $K_{PD, B}$ are the in vivo estimated receptor affinity, $e_{PD, A}$ and $e_{PD, B}$ are the relative efficacy of the midazolam and α-OH-midazolam, where the $e_{PD}$ of the drug displaying the highest efficacy is set to one. The relationship $f$ based modeling approach are presented as an Appendix to this paper. When using this mechanism-based approach, the relationship between drug concentration and effect is characterized by the following equation:

$$E(C_{A}, C_{B}) = f(S) = f \left( \frac{e_{PD} \cdot C_{A}^{n_{A}}}{C_{A} + K_{PD,A}^{n_{A}}} \cdot \frac{C_{B}^{n_{B}}}{C_{B} + K_{PD,B}^{n_{B}}} \right)$$

where $K_{PD, A}$ and $K_{PD, B}$ are the in vivo estimated receptor affinity, $e_{PD, A}$ and $e_{PD, B}$ are the relative efficacy of the midazolam and α-OH-midazolam, where the $e_{PD}$ of the drug displaying the highest efficacy is set to one. The relationship $f$ is a function describing the relationship between receptor occupancy and response to drug concentration. After combined administration the competitive receptor interaction can be described by (Kenakin, 1993):

$$E(C_{A}, C_{B}) = f(S) = f \left( \frac{e_{PD} \cdot C_{A}^{n_{A}}}{C_{A} + K_{PD,A}^{n_{A}}} \cdot \frac{C_{B}^{n_{B}}}{C_{B} + K_{PD,B}^{n_{B}}} \right)$$
tionships after separate administration of midazolam and α-OH-midazolam were analyzed on basis of the sigmoidal Emax pharmacodynamic model (Fig. 3). The EC50 was 62 ± 10 ng/ml for midazolam and 406 ± 31 ng/ml for α-OH-midazolam (Table 2). Values (mean ± S.E.) for Emax and Hill factor were 191 ± 6% and 190 ± 4% and 0.87 ± 0.11 and 1.29 ± 0.12 for midazolam and α-OH-midazolam, respectively.

Empirical and Competitive Interaction Modeling. The performance of the empirical interaction model (eq. 3) and the competitive interaction model (eq. 8) was evaluated by comparing the by each model derived pharmacodynamic estimates with those as estimated upon separate administration. First, the data obtained upon administration of midazolam separately and upon administration of the combination were used to retrieve the pharmacodynamic parameters of midazolam and α-OH-midazolam by fitting the pharmacodynamic models to both data sets simultaneously. Next, the data obtained upon separate administration of α-OH-midazolam and the combination were used to estimate the pharmacodynamic parameters of both benzodiazepines. The results of this analysis are shown in Table 3. Interestingly, upon visual inspection of the fits no remarkable differences were observed. Comparison with the estimated values obtained upon separate administration of the compounds (Table 2) shows, however, that the empirical interaction model is unable to accurately estimate values of the pharmacodynamic parameters of midazolam and α-OH-midazolam. With the competitive interaction model, accurate estimates of the pharmacodynamic parameters of midazolam but not of α-OH-midazolam were obtained. Here again by visual inspection the fits appeared good, without accurately estimating pharmacodynamic parameters. The competitive interaction model was also tested in a slightly modified form by assuming the Emax values and Hill factors of midazolam and α-OH-midazolam to be equal (eq. 8). This simplified model also failed to accurately estimate the pharmacodynamic parameters of midazolam and α-OH-midazolam.

Mechanism-Based Modeling. The values of the pharmacodynamic parameters of midazolam and α-OH-midazolam according to the mechanism-based model are summarized in Table 3. Similar values of efficacy for midazolam and α-OH-midazolam were observed. The value of the KPD was 43 ± 5 ng/ml for midazolam and 258 ± 25 ng/ml for α-OH-midazolam. On the basis of the mechanism-based interaction model, realistic estimates of the pharmacodynamic parameters of both midazolam and α-OH-midazolam were obtained where using the data from the combined administration (Table 3).
Discussion

The fact that many (psychotropic) drugs are converted into active metabolites has major implications for pharmacokinetic/pharmacodynamic modeling. Typically in this situation the effect cannot be predicted by adding the effects of the parent drug and the metabolite separately, and rather complex interaction models are required. In this investigation it is shown that in the situation where a drug is converted into an active metabolite, accurate prediction of the pharmacological response is only feasible if a mechanism-based interaction model is used.

In the present study midazolam was used as a model drug. It is well established that in humans this drug is converted into an active metabolite, α-OH-midazolam, and that particularly upon oral administration this metabolite contributes significantly to the pharmacological response (Crevoisier et al., 1983; Mandema et al., 1992b). In the rat no α-OH-midia-

TABLE 2
Pharmacodynamic parameters of midazolam (mean ± S.E.) estimated on basis of separate administration by use of the sigmoidal E\text{max} model (eq. 2), by the empirical (eq. 3) and by the competitive model (eq. 7).

<table>
<thead>
<tr>
<th>Model</th>
<th>Separate Administration</th>
<th>Combined Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midazolam</td>
<td>Data sets</td>
<td>A</td>
</tr>
<tr>
<td>Data sets</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>EC\text{50}</td>
<td>620 ± 9.6</td>
<td>266 ± 122</td>
</tr>
<tr>
<td>E\text{max}</td>
<td>191 ± 6</td>
<td>377 ± 30</td>
</tr>
<tr>
<td>N</td>
<td>0.87 ± 0.11</td>
<td>0.45 ± 0.05</td>
</tr>
<tr>
<td>α-OH-midazolam</td>
<td>Data sets</td>
<td>B</td>
</tr>
<tr>
<td>Data sets</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>EC\text{50}</td>
<td>406 ± 31</td>
<td>1800 ± 1200</td>
</tr>
<tr>
<td>E\text{max}</td>
<td>190 ± 4</td>
<td>387 ± 43</td>
</tr>
<tr>
<td>N</td>
<td>1.29 ± 0.12</td>
<td>0.35 ± 0.03</td>
</tr>
</tbody>
</table>

\*Significantly different from pharmacodynamic parameters estimated after separate administration (p < .05, ANOVA).

\*Statistically significant different from Midazolam (p < .05, ANOVA).
vestigation can then be used to estimate the pharmacodynamic parameters of the metabolite.

The empirical pharmacodynamic interaction model (eq. 3) failed to accurately estimate the EC_{50} for both midazolam and α-OH-midazolam (Table 2). Interestingly in other interaction studies with benzodiazepines, this model performed quite well (Mandema et al., 1992a,c). This can be explained by the fact that in those investigations, the benzodiazepines differed significantly in their intrinsic activity, which allowed for a different study design. In these previous investigations the concentration of one of the benzodiazepines (typically the full agonist) was brought to steady state by administering a continuous infusion. Subsequently the second benzodiazepine (partial/-inverse agonist or competitive antagonist) was administered in a short infusion and the effect intensity was observed as it went from maximal to baseline (in the case of a competitive antagonist) or to the maximum effect of the second compound (in the case of a partial or inverse agonist), and subsequently, because of the decay in concentration, back to the maximal effect of the first compound. The model was then used to estimate the pharmacodynamic parameters of only the second compound. In the current investigation the empirical interaction model is used to characterize the pharmacodynamics of both compounds, which in addition have a very similar E_{max} value. Another important factor is that, on theoretical grounds, the model is not valid for compounds with a Hill factor that is different from 1. In that situation the model does not predict an additive interaction (as it should in the case of competition of two ligands for the same receptor) but, depending on the value of the Hill factor, either a synergistic or an antagonistic interaction. To further examine the role differences in the Hill factor between the compounds, the data were also analyzed assuming the values of either the E_{max} or the Hill factor to be identical for both compounds. These simplified forms of the empirical model however also failed to retrieve the accurate pharmacodynamic parameter estimates. This means that the empirical interaction model is of little value to characterize the pharmacodynamic interaction between two full agonists.

The data from the combined administration of midazolam and α-OH-midazolam were also analyzed according to the competitive interaction model on the basis of eq. 8, where it is assumed that both the values of E_{max} and the Hill factor are identical. This model has the advantage over the empirical interaction model in that it is correctly derived from the sigmoidal E_{max} model and that it predicts an additive interaction also when the value of the Hill factor is different than 1. This competitive interaction model was found to accurately estimate the pharmacodynamic parameters of midazolam, but failed to retrieve the pharmacodynamic parameters of α-OH-midazolam correctly (Table 2). Presumably this can be explained by the fact that the true values of the Hill factor are different for both compounds (Table 2). A more general form of the competitive interaction model (eq. 7) was also tested. This model differs from the previous model (eq. 8) in that it does not have the assumption of equal values of E_{max} and the Hill factor. This model also failed to accurately estimate the pharmacodynamic parameters of both midazolam and α-OH-midazolam. This is most likely due to the fact that this model, where both the E_{max} and the Hill factor are to be estimated for the two compounds, is overparameterized. This means that without the addition of extra information it is impossible to obtain realistic pharmacodynamic parameter estimates.

In the present investigation the mechanism-based interaction model was the only model that was able to retrieve accurate estimates of the pharmacodynamic parameters of both midazolam and α-OH-midazolam (Table 3). A unique feature of this mechanism-based model is that it does not need a Hill factor to account for a sigmoidal concentration-effect relationship as it incorporates sigmoidicity through the nonlinear stimulus-response relationship. As a result the interaction between the compounds at the receptor can be described by a theoretically correct competitive interaction model that is based on receptor theory. An interesting feature of this approach is that it in principle also allows characterization of the interaction between more than two drugs (Van den Brink, 1977). A limitation of the mechanism-based modeling approach is that it requires detailed knowledge with regard to the general shape of the stimulus-response relationship. This requires the simultaneous analysis of the concentration-effect relationships of at least two other compounds as is shown in the Appendix. This also implies that a considerable amount of extra information is incorporated in the interaction model. Another important feature is that the values of only two pharmacodynamic parameters are estimated (K_{Pr} and e_{Pr}) instead of three (EC_{50}, E_{max}, and Hill factor) in most other models. This explains why overparameterization is not a problem with this particular model. It seems unlikely, however, that the incorporation of additional information on the stimulus-effect relationship explains entirely the improved performance of the mechanism-based interaction model relative to the empirical and the competitive interaction model. The fact that the mechanism-based model utilizes a theoretically correct model to characterize the competitive interaction between midazolam and α-OH-midazolam at the receptor is probably the most important factor.

In conclusion, the results from the present investigation show that the pharmacodynamic interactions between two full agonists (i.e., a parent drug + an active metabolite) in vivo can only be characterized adequately on the basis of the mechanism-based pharmacodynamic interaction model.

Appendix

Previously published data on the concentration-EEG effect relationships of flunitrazepam, midazolam, clobazam, and oxazepam (Mandema et al., 1991) were re-analyzed according to a new model that is based on the receptor theory proposed by Stephenson (1956) and Furchgott (1966). The model combines two independent parts to describe drug action: a drug-specific part, reflecting the drug’s affinity to and intrinsic efficacy at the receptor and a system-specific part describing the relationship between receptor occupancy and effect. The drug-specific part is characterized by the following equation:

\[
R_e = \frac{R_t \cdot C}{C + K_D}
\]

where R_e is the total concentration of receptors, K_D is the equilibrium dissociation constant of the drug receptor complex, C is the drug concentration, and R_t is the total concen-
tation of occupied receptors at drug concentration $C$. According to receptor theory each unit of drug receptor complex presents a quantal unit of stimulus to the system, which is referred to as intrinsic efficacy ($\varepsilon$). The summation of these stimuli (which is equal to the product of intrinsic efficacy and the total amount of occupied receptors) is the stimulus ($S$) to system. This stimulus is propagated into the ultimate effect through a chain of postreceptor events. This system-specific part of drug action is characterized by an unknown, but monotonically increasing function $f$. According to the theory the drug concentration-effect relationship can then be described by the following equation:

$$E = f(S) = f\left(\frac{\varepsilon \cdot Rt \cdot C}{C + K_D}\right) \tag{A2}$$

where $E$ is the effect intensity.

To apply this model to in vivo drug concentration effect relationships, the following simplifications must be made. First, the total amount of functional receptors cannot be measured in vivo, which means that only the product of $\varepsilon$ and $R_i$ can be estimated. Second, the absolute value of the product of $\varepsilon$ and $R_i$ can also not be determined. Only the relative value can be estimated by setting the value of $\varepsilon R_i$ product of the drug reaching the highest maximum effect to one. The relationship between drug concentration and effect can then be characterized by the following equation (Kenakin, 1993b):

$$E = f(S) = f\left(\frac{C}{C + K_{PD}}\right) \tag{A3}$$

where $K_{PD}$ is the in vivo estimated receptor affinity and $\varepsilon R_i$ is the efficacy of the drug relative to the drug reaching the highest maximum effect for which $\varepsilon R_i$ equals 1.

Simultaneous analysis of data on the concentration-EEG effect relationships of flunitrazepam, midazolam, oxazepam, and clobazam (Mandema et al., 1991) allowed independent estimation of $K_{PD}$, $\varepsilon R_i$, and $f$. Thereby the relationship between initial stimulus and observed pharmacological effect was assumed to be identical for the four different benzodiazepines and characterized by a natural cubic spline (DeBoor, 1978). The knots of the spline were placed at equidistant intervals on the stimulus axis (i.e., equidistant intervals between 0 and 1). The number of knots was determined on the basis of the Log-Likelihood criterion ($p < .01$) and visual inspection of the fits. The data were analyzed using a pooled data approach within the NONMEM computer program (NONMEM project group).

The mean values of the $K_{PD}$ and $\varepsilon R_i$ of flunitrazepam, midazolam, oxazepam, and clobazam are given in Table 4. The values of the binding constant for the interaction with the benzodiazepine receptor in vitro are presented as well. Table 4 shows that the in vivo estimated $K_{PD}$ values based on free drug concentrations ($K_{PD, f}$) are close to the in vitro estimated receptor affinity $K_i$ and that the ratio between $K_{PD, f}$ and $K_i$ is reasonably constant. This indicates that on the basis of the mechanism-based model realistic estimates of the potency of the benzodiazepines are obtained. The relationship between stimulus and EEG effect is shown in Fig. 4.

It is nonlinear with relatively small increase in EEG effect at stimulus intensities less than 0.2 and an almost linear but steeper increase in EEG effect at stimulus intensities between 0.2 and 1.

### References


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