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Adaptive changes in the pharmacodynamics of midazolam in different experimental models of epilepsy: kindling, cortical stimulation and genetic absence epilepsy

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1 The objective of this investigation was to determine *quantitatively* whether experimental epilepsy is associated with a change in the pharmacodynamics of benzodiazepines *in vivo*. For that purpose the pharmacodynamics of midazolam were quantified by an integrated pharmacokinetic-pharmacodynamic approach in three different models of experimental epilepsy: amygdala kindling, cortical stimulation and genetic absence epilepsy.

2 The time course of the EEG effect was determined in conjunction with the decline of drug concentrations after intravenous administration of 10 mg kg^{-1} midazolam. The pharmacokinetics of midazolam were most adequately described by a bi-exponential equation. No influence of epilepsy on the pharmacokinetics of midazolam was observed.

3 The increase in β activity (11.5–30 Hz) of the EEG as derived by Fast Fourier Transformation analysis was used as pharmacodynamic endpoint. For each individual rat the increase in β activity was directly related to the concentration in blood on the basis of the sigmoidal E_{max} pharmacodynamic model. In all three models a significant reduction in the maximal effect was observed, in amygdala kindling 28%, in the cortical stimulation model 49% and in genetic absence epilepsy 37%. No differences in the other pharmacodynamic parameters, E_0, $EC_{50,u}$ and Hill factor, were observed.

4 It is inferred that in three different models of epilepsy there is a similar change in GABAergic functioning which is associated with a significant reduction in the intrinsic activity of midazolam *in vivo*. These models provide therefore a useful basis for further studies on the mechanism of epilepsy-induced changes in pharmacodynamics of anti-epileptic drugs.

Keywords: Kindling; epilepsy; γ-aminobutyric acid; benzodiazepines; pharmacokinetic-pharmacodynamic modelling

Introduction

The development of pharmacotherapeutic resistance for the anticonvulsive effects of benzodiazepines limits the therapeutic usefulness of this class of drugs in maintenance therapy of seizure disorders. Most studies have focussed on adaptive changes in the efficacy of benzodiazepines as a result of chronic treatment, indicating the ability of the GABAergic system to adapt to chronic occupation of the benzodiazepine site within the GABA-benzodiazepine receptor complex (Gallager & Primus, 1991).

Despite the major attention to the influence of chronic treatment on the therapeutic efficacy of benzodiazepines, it has to be realized that epilepsy itself can also be of major importance. It has been shown that the GABAergic system can adapt to the development of epilepsy, as is seen for example in kindling, an experimental animal model for epilepsy (Kamphuis & Lopes da Silva, 1990; Löscher & Schwark, 1987). Indeed, there is a significant decrease in the sensitivity to GABA in dorsal raphe neurons (Hernandez *et al*., 1990). Probably this is caused by a long-term increase of basal as well as depolarization-induced GABA release, as has been reported, for example, after hippocampus kindling (During *et al*., 1992; Kamphuis *et al*., 1990). Furthermore a significant decrease in GABA stimulated $^{36}Cl^-$ flux as well as $GABA_A$ receptor mediated paired-pulse inhibition has been reported (Sneddon *et al*., 1990; Tietz & Chiu, 1991; Wu & Stan Leung, 1997; Zhao & Stan Leung, 1991), indicating a decrease in the GABA mediated neurotransmission. It is generally accepted that the mechanism of action of the benzodiazepines involves the allosteric modulation of the specific GABA-benzodiazepine receptor complex, thereby enhancing GABAergic inhibition *via* increasing the opening probability of $GABA_A$ receptor operated chloride channels. Thus, it might be expected that the efficacy of benzodiazepines is reduced in epileptic patients.

The specific purpose of the present study was to establish quantitatively whether experimental epilepsy is associated with a change in the pharmacodynamics of midazolam *in vivo*. Therefore three different models for experimental epilepsy, i.e. the well-known model amygdala kindling (Goddard *et al*., 1969), the recently developed model of cortical stimulation (Voskuyl *et al*., 1989) and the genetic model of absence epilepsy (Wag/Rij) (Coenen & Van Luijtelaar, 1987), were used.

A number of years ago, Mandema *et al*. (1991b) have introduced quantitative EEG monitoring for the purpose of pharmacokinetic-pharmacodynamic modelling of the central nervous system effect of benzodiazepines. Estimates of the *in vivo* potency and intrinsic activity were obtained in individual animals by relating blood concentrations to the increase in β activity of the EEG. The observed *in vivo* potencies and intrinsic activities were in good agreement with the affinity to and intrinsic efficacy at the GABA-benzodiazepine receptor

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complex, confirming that the increase in β activity of the EEG reflects modulation at the GABA-benzodiazepine complex *in vivo* (Mandema *et al*., 1991a; Mandema *et al*., 1992). Moreover, a close correlation between the potency of different benzodiazepine agonists with respect to their EEG-effects and anticonvulsant effects as determined in the PTZ-model was found (Mandema *et al*., 1991a). The advantage of an integrated pharmacokinetic-pharmacodynamic modelling approach, in order to investigate disease induced alterations in the pharmacodynamics, is that potentially complicating pharmacokinetic factors are excluded or accounted for (Levy, 1985).

After intravenous administration of midazolam the time course of the EEG effect was monitored continuously, in conjunction with serial arterial blood sampling. The midazolam induced increase in β activity was quantified on the basis of a integrated pharmacokinetic-pharmacodynamic modelling approach. The pharmacokinetic and pharmacodynamic parameters were used to detect differences between 'epileptic' and control animals and thereby to quantify alterations in GABAergic inhibition.

Methods

Animals

Adult male SPF Wistar rats, weighing 200–225 g (Harlan C.P.B., Zeist, The Netherlands) were used in the kindling and cortical stimulation procedure. Eight months old Wag/Rij rats (University of Nijmegen, Nijmegen, The Netherlands) were used as genetic model for absence epilepsy. It has been shown that the epilepsy in Wag/Rij rats is strongly age-dependent and that Wag/Rij animals younger than 3 months are almost seizure-free (Coenen & van Luijtelaar, 1987). Age matched ACI rats were used as control animals for the Wag/Rij rats (University of Nijmegen, Nijmegen, The Netherlands). The rats were housed individually in plastic cages, at a constant temperature of 21°C, and with a normal 12-h light-dark cycle (lights on: 08.00 h. to 20.00). Food (Standard Laboratory Rat, Mouse and Hamster Diets, RMH-TM, Hope Farms, Woerden, The Netherlands) and tap water were available *ad libitum*.

Surgical procedure

In all animals cortical electrodes for EEG recording were implanted under anaesthesia with 0.8 ml kg^{-1} Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) and 0.25 ml kg^{-1} Nembutal (Sanofi Sante, Maassluis, The Netherlands) as described before (Mandema & Danhof, 1990).

One day before experimentation, indwelling cannulas were implanted, in the right jugular vein for drug administration, and the right femoral artery for the serial collection of arterial blood samples (Mathôt *et al*., 1994). The protocol of this study was approved by the Committee on Animal Experimentation of Leiden University.

Kindling

Simultaneously with the implantation of the EEG electrodes, a bipolar electrode was implanted for kindling in the right basolateral amygdala (2.5 mm posterior and 5.4 mm lateral from bregma and 7.8 ventral from the brain surface). The electrodes consisted of twisted, 150 μm insulated stainless steel wires (E363/3, Plastics One, Roanoke, Virginia, U.S.A.). The tips were separated by 500 μm. A skull screw (11 mm anterior, 2.5 mm lateral from lambda) served as the reference electrode. Following a 1 week post-surgical recovery period, the threshold for afterdischarges was determined in the following manner: the rats were stimulated at 5 min intervals, starting at 25 μA (2 s, 50 Hz, 2 msec bipolar pulse train) and increasing the intensity by 50 μA increments until an afterdischarge of at least 1 s occurred. Thereafter the animals were kindled twice daily at 150 μA above threshold until six sequential stage five seizures (fully kindled) had been reached. The control rats were handled identically, but not stimulated. On each day of the kindling acquisition, the seizures severity was classified according to Racine *et al*. (1972).

Cortical stimulation

Simultaneously with the EEG electrodes, two stimulation electrodes (stainless screws, 1.2 mm diameter, Jeveka BV, Amstelveen, The Netherlands) were implanted bilaterally for cortical stimulation (3.5 mm left and right of the midline, 1.0 mm posterior to bregma). After 1 week post-operative recovery, the rats were stimulated up to the threshold for convulsions twice daily for 2 weeks to establish a stable baseline threshold (Voskuyl *et al*., 1989).

Pharmacokinetic-pharmacodynamic experiment

The effect of the induction of experimental epilepsy on the concentration-effect relationship of midazolam was investigated 24 h after the last stimulation in the three different experimental models of epilepsy, i.e. amygdala kindling, cortical stimulation and genetic absence epilepsy. The relationship between EEG effect and blood concentrations of midazolam were determined after intravenous infusion of 10 mg kg^{-1} of midazolam over 2 min. Midazolam was dissolved in equimolar hydrochloric acid and administered in a volume of 250 μl using a syringe infusion pump (Syringe infusion pump 22, Harvard Apparatus, South Natick, MA, U.S.A.).

All experiments started between 8.30 and 9.30 a.m. to standardize a possible influence of diurnal rhythms on the measurements. EEG recordings were started at least 45 min before drug administration and lasted approximately 6 h. During the experiment the animals were conscious, freely moving and were allowed free access to water.

Arterial samples for the determination of midazolam blood concentrations were drawn at predefined time-points (15 blood samples (50, 100 or 200 μl)) during and after the infusion. The samples were hemolyzed immediately in glass tubes containing 0.5 ml millipore water and stored at −20°C. HPLC analysis was performed according to Mandema *et al*. (1991b). During the experiment the EEG signal was continuously recorded from the fronto-central lead and, after band-pass filtering (0.1–100 Hz), subjected to on-line Fast Fourier Transformation analysis. For each 5 s epoch, the amplitudes in the β frequency band (11.5–30 Hz) were calculated and used as a measure of drug effect intensity. Reduction of the EEG data was performed by averaging spectral parameter values over predetermined time intervals.

Twenty-four hours after drug administration, a 3 ml blood sample was spiked with midazolam to contain 0.4 mg ml^{-1}. In each individual rat the plasma-to-blood ratio (P/B) and the extent of plasma protein binding (f_u) of midazolam were determined according to standard procedures (Mathôt *et al*., 1994).

Data Analysis

The pharmacokinetics and pharmacodynamics of midazolam were quantified in individual rats. The blood concentration-time profiles of midazolam were described by a polyexponential equation for intravenous infusion (Gibaldi & Perrier, 1982):

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

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

where C(t) is the concentration at time t, T the infusion duration and C_i and λ_i are the coefficients and exponents of the equation, respectively. Total blood clearance (Cl), the elimination half-life ($t_{1/2}$) and the volume of distribution at steady-state (V_{dss}) were calculated by standard methods from the coefficients and the exponents of the fitted functions (Gibaldi & Perrier, 1982). The functions were fitted to the data with weight factor y^{-2} using the non-linear least squares regression program Siphar (Simed SA, Creteil, France). In each individual rat the fitted function of the concentration-time profile was used to calculate the concentrations at the measured effect-time points.

The relationships between the midazolam concentrations and the EEG effect was described on basis of the sigmoidal E_{max} model (Holford & Sheiner, 1981):

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

where E_C is the observed effect at blood concentration C, E_0 is the baseline EEG effect, Emax is the maximal effect, EC_{50} is the blood concentration at half maximal effect and n is a constant expressing the sigmoidicity of the concentration-effect relationship (Hill factor). Values of the baseline EEG effect were obtained by averaging the EEG effect over 30 minutes preceding drug administration. EC_{50} values based on free midazolam concentration ($EC_{50,u}$) were obtained after correction for binding of the compound to blood cells and plasma proteins. The equations were fitted to the data using the nonlinear least-square regression program Siphar version 3.0 (Simed Sa, Creteil, France).

The pharmacokinetic and pharmacodynamic parameter estimates of the different groups were statistically compared using the parametric one-way analysis of variance (ANOVA) or a non-parametric Kruskall-Wallis test, if more appropriate. A significance level of 5% was selected. All data are reported as the means $\pm$ s.e.mean, unless indicated otherwise.

Results

Kindling and stabilization procedure

Kindled animals reached stage five within on average eight sessions (range 4–13) as characterized by behavioural tonic/clonic convulsions (Racine *et al.*, 1972). Stimulation was continued until six generalized seizures had been elicited; this required a mean of 14 sessions (range 9–18). The observed rate of kindling is consistent with previously reported data (Löscher *et al.*, 1995; Wada *et al.*, 1997).

In the cortical stimulation model the susceptibility to seizures increased with repeated stimulation up to the threshold for localized seizure activity (TLS), as reflected in a significant, progressive reduction of the average TLS from 598 ± 18 μA to 370 ± 16 μA (paired *t*-test, $n = 6$, $P < 0.001$), fully consistent with previous results (Voskuyl *et al.*, 1989).

Pharmacokinetics

Individual blood midazolam concentration-time profiles after intravenous administration of midazolam are shown for each experimental model of epilepsy and their corresponding control animal in Figure 1. In all rats the concentration-time profiles were described by a bi-exponential function. The pharmacokinetic parameters are summarized in Table 1. No statistical differences were observed between the parameters for the 'epileptic' animals (i.e. amygdala kindling, cortical stimulation or Wag/Rij rats) and their corresponding controls.

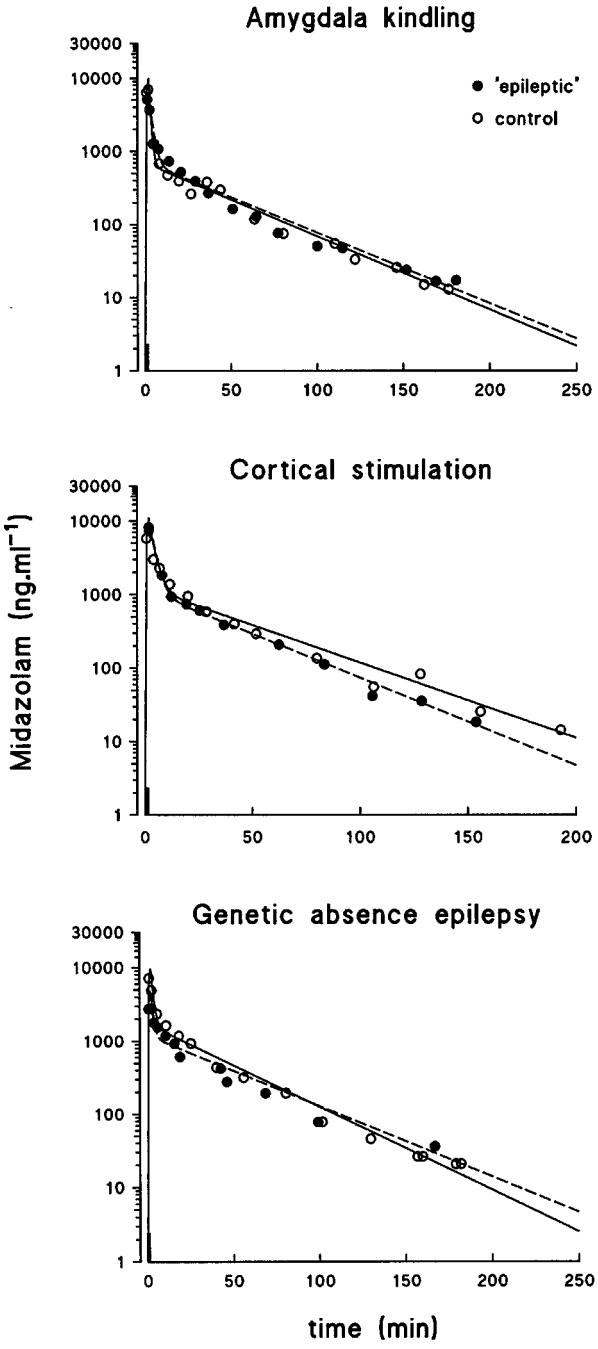

Figure 1 Individual concentration time profiles after intravenous administration of 10 mg kg^{-1} midazolam during 2 min. The solid (control) and dashed (experimental epilepsy) lines represent the predicted blood concentration on fitting a bi-exponential function to the concentration-time data.

Table 1 Influence of different experimental models of epilepsy on the pharmacokinetic parameters estimates of midazolam. The values are reported as means±s.e.mean, $n=6-7$

	Cl (ml.min^{-1}.kg^{-1})	$Vdss$ (l.kg^{-1})	$t_{½}$ (min)	P/B	f_u (%)
Kindling	111±5	3.0±0.1	35±2	1.4±0.1	8.4±1.1
Placebo	101±5	2.8±0.2	34±1	1.4±0.1	6.5±0.8
CSM	104±8	2.2±0.2	23±1	1.2±0.1	5.8±1.1
Placebo	98±6	2.2±0.2	25±2	1.3±0.3	7.4±2.1
Wag/Rij	84±6	2.2±0.1	31±1	1.2±0.1	2.8±0.3
ACI	69±5	2.1±0.2	29±3	1.1±0.1	4.3±0.6*

*$P<0.05$ *versus* Wag/Rij.

The plasma-to-blood ratio (P/B) and free fraction in plasma (f_u) of midazolam were determined *in vitro*. The averaged values are summarized in Table 1. The free fraction of midazolam in plasma was significantly higher in the ACI rats in comparison to the Wag/Rij rats.

EEG effects

To investigate alterations in drug efficacy of midazolam, the increase in β activity of the EEG was assessed in epileptic animals and their corresponding controls. The time course of the effect on EEG after administration of midazolam in the different animal models is depicted in Figure 2. The time course of the midazolam induced increase in the β activity was significantly different between each epileptic and control group (t-test; $P<0.05$). In the epileptic animals a smaller maximal effect in β activity was observed.

The sigmoidal E_{max} model adequately described the concentration–EEG effect relationship in each animal, as illustrated for the different models in Figure 3. The estimated pharmacodynamic parameters for the midazolam-induced increase in β activity are summarized in Table 2. In all three animal models a significant decrease in intrinsic activity was observed: the E_{max} values were 69±7, 54±6, 72±8 μV^2 for amygdala kindling, cortical stimulation and genetic absence epilepsy respectively *versus* 96±4, 105±6 and 114± 18 μV^2 for their corresponding controls. No significant differences were detected between the pharmacodynamic parameters E_0, $EC_{50,u}$ and Hill factor.

Discussion

The purpose of the present study was to investigate *quantitatively* the influence of epilepsy on the pharmacokinetics and pharmacodynamics after an acute dose of the benzodiazepine agonist midazolam. In order to exclude an animal model specific change in the pharmacokinetic-pharmacodynamic relationship of midazolam, three different experimental models were used: amygdala kindling, cortical stimulation and genetic absence epilepsy. The main result emerging from this study was that in all models experimental epilepsy is associated with a reduced maximal effect of midazolam. Although there were small differences in pharmacokinetics (clearance and volume of distribution), these appeared to be strain related rather than epilepsy induced.

For each individual rat the change in EEG amplitude was correlated to the concentration in blood, in each case yielding a sigmoidal relationship. The advantage of this approach is that the parameters characterizing this relationship can be used directly for comparison of different groups, irrespective of any difference in pharmacokinetics (Levy, 1985). The only

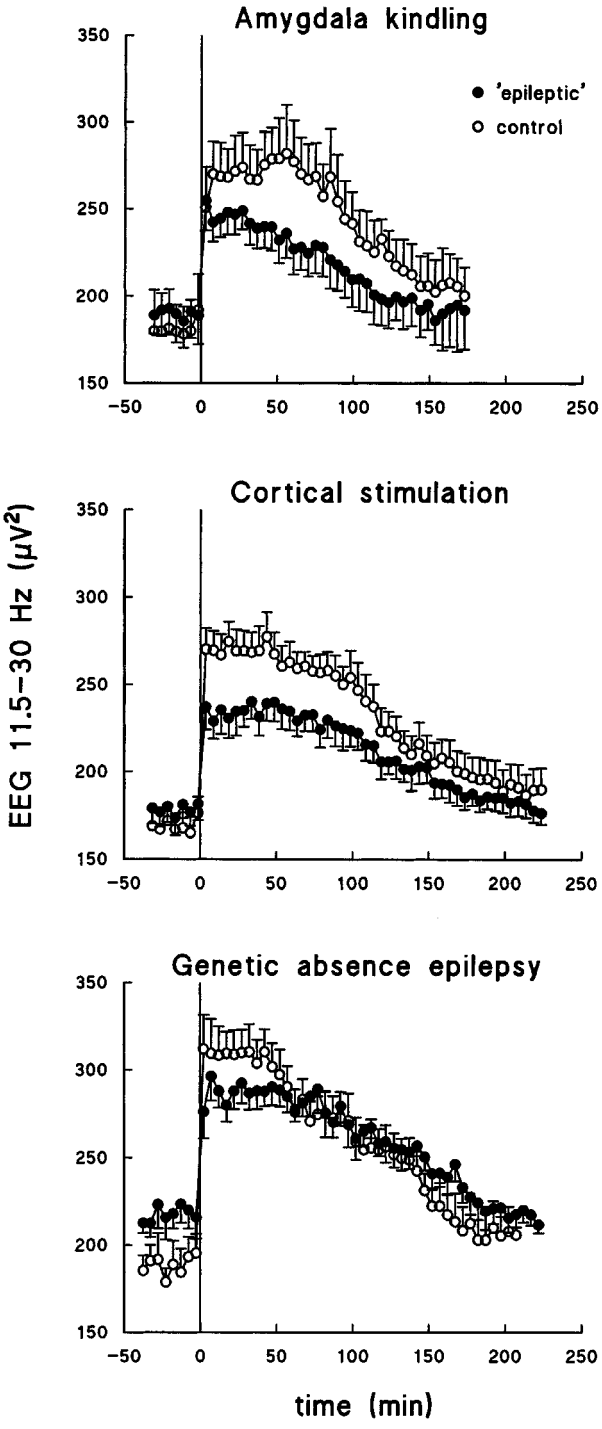

Figure 2 Time profile of the increase in β activity of the EEG after intravenous infusion of 10 mg kg^{-1} midazolam over 2 min; data are reported as means±s.e.mean, $n=6-7$.

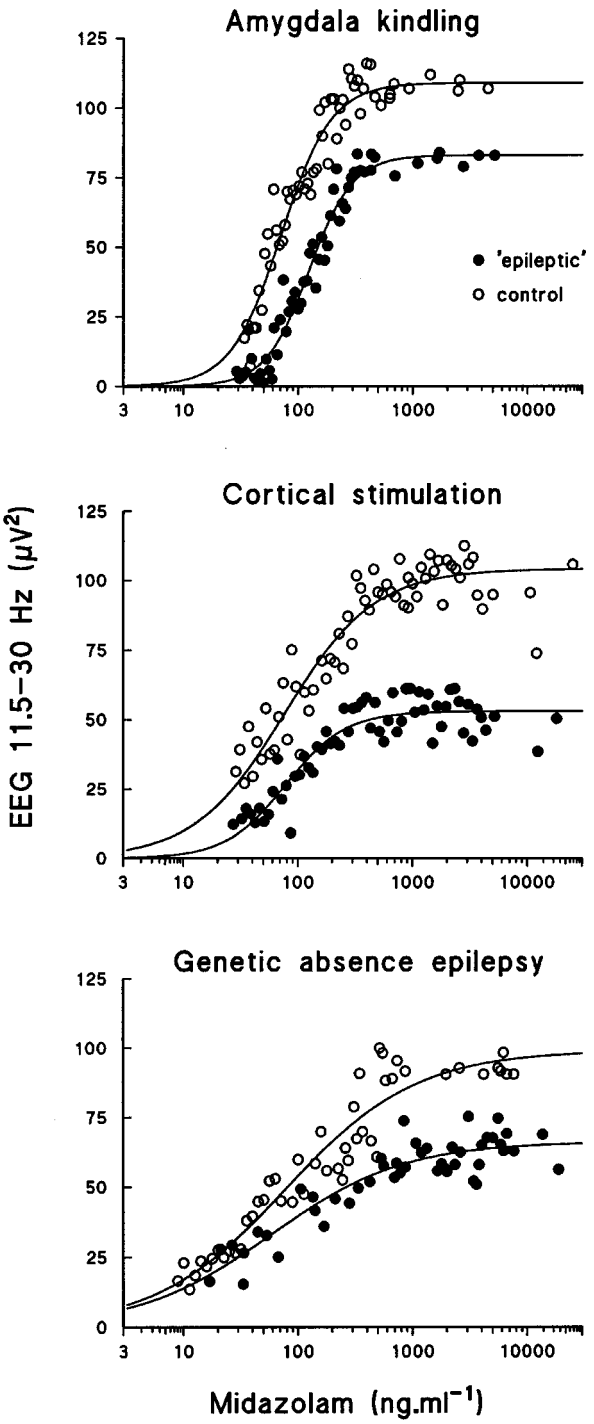

Figure 3 Concentration–EEG relationships in individual animals. The solid lines represent the best fit of the data according to the sigmoidal E_{max} model.

parameter that was changed was the maximal effect (intrinsic activity). Since it was demonstrated previously that this EEG effect reflects activation of the GABA-benzodiazepine receptor complex (Mandema *et al*., 1991a; Mandema *et al*., 1992), this suggests that in all three models enhancement of GABAergic inhibition is impaired by the same mechanism. Although this may seem remarkable in the light of the different modes of epileptogenesis in the three models, it may well be that reduced GABAergic inhibition is involved in all three models. For the genetic model of absence epilepsy and for kindling this has been demonstrated in several studies (e.g. Fueta *et al*., 1996; Luhmann *et al*., 1995). For the cortical stimulation model this has not been studied specifically. However, concerning the development of an epileptic state in the cortical stimulation model, this is achieved in a similar way as in kindling. In both animals models the brain becomes progressively more susceptible owing to repeated administration of low intensity, high frequency, electrical stimulation. Therefore, it is reasonable to assume that GABAergic inhibition is reduced in the cortical stimulation model as well.

On basis of the same arguments it might have been expected that the baseline EEG (E_0) would be decreased, but this was not the case. However, it has to be realized that only a small amount of receptor-activation contributes to baseline characteristics, and moreover a considerable variation (c.v. 14%) in baseline measurements is observed. So, it is possible that a small decrease in GABAergic inhibition under basal conditions is masked, due to variation in baseline characteristics. Finally, the $EC_{50,u}$ value tended to decrease both in the Wag/Rij and cortical stimulation model rats. The estimates of potency and intrinsic activity of midazolam for the EEG effect in control rats are in line with estimates in Wistar rats, previously reported (Mandema *et al*., 1991b). Considerable variation between the placebo $EC_{50,u}$ values in the kindling and cortical stimulation animals was observed. This variation in *in vivo* potency is in the same order of magnitude observed by others (Aarons *et al*., 1991). Therefore, this variation in $EC_{50,u}$ values, probably indicates the presence of a log-normal distribution for the $EC_{50,u}$ values, rather than effect of epileptic activity. Similar distributions were observed for acetylcholine and norepinephrine (Flemming *et al*., 1972). It is obvious that further studies are necessary to elucidate which mechanisms contribute to the decrease in intrinsic activity *in vivo*. Analysis of the concentration-effect data with the empirical Hill equation applied in this study only provides limited insights in the relation between receptor pharmacology and the *in vivo* pharmacodynamics, because the potency and intrinsic activity of a drug *in vivo* are not only dependent on compound related properties, intrinsic efficacy and affinity, but also on system related parameters such as receptor density and efficiency of the receptor-effect coupling system. Mechanism-based models have been proposed to characterize the pharmacodynamics of

Table 2 Pharmacodynamic parameters of midazolam in the different experimental models of epilepsy. The values are reported as means ± s.e.mean, $n = 6-7$

Treatment	E_0 (μV^2)	E_{max} (μV^2)	$EC_{50\ u}$ ($ng.ml^{-1}$)	*Hill slope*
Kindling	182 ± 14	69 ± 7**	9.3 ± 1.8	1.8 ± 0.3
Placebo	181 ± 16	96 ± 4	7.2 ± 1.3	2.7 ± 1.1
CSM	180 ± 9	54 ± 6**	3.4 ± 1.8	1.8 ± 0.3
placebo	168 ± 8	105 ± 6	3.9 ± 0.9	0.8 ± 0.2
Wag/Rij	218 ± 9	72 ± 8*	2.9 ± 1.4	1.5 ± 0.3
ACI	194 ± 10	114 ± 18	4.2 ± 1.0	0.8 ± 0.1

*$P < 0.05$ *versus* ACI, **$P < 0.01$ *versus* placebo.

drugs *in vivo* (Black & Leff, 1983; Van der Graaf *et al*., 1998). These models contain separate parameters for characterization of receptor binding and the multitude of post-receptor events. Such seperation of drug specific from tissue specific stimulus-effect propagation might be of value to understand the epilepsy-induced changes in the pharmacodynamics of midazolam in a mechanistic way. Furthermore, it is important to carry out *in vitro* studies receptor binding characteristics and $GABA_A$ receptor functioning, in order to reveal which process at the molecular level can explain the changes observed *in vivo*.

In conclusion, this is the first investigation where a change in the modulation of GABAergic function *in vivo* has been observed and *quantitatively* evaluated. Although the underlying mechanisms can not unambiguously be accounted for in the present study it is clear that the change in the pharmacodynamics of midazolam as observed in three different experimental models of epilepsy is of significant importance, with regard to treatment of epileptic disorders.

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References

AARONS, L., MANDEMA, J.W. & DANHOF, M. (1991). A population analysis of the pharmacokinetics and pharmacodynamics of midazolam in the rat. *J. Pharmacokinet. Biopharm.,* **19,** 485 – 496.

BLACK, J.W. & LEFF, P. (1983). Operational models of pharmacological agonism. *Proc. R. Soc. Lond. B.,* **220,** 141 – 162.

COENEN, A.M.L. & VAN LUIJTELAAR, E.L.J.M. (1987). The Wag/Rij rat model for absence epilepspy: Age and sex factors. *Epilepsy Res.,* **1,** 297 – 301.

DURING, M.J., CRAIG, J.S., HERNANDEZ, T.D., ANDERSON, G.M. & GALLAGER, D.W. (1992). Effect of amygdala kindling on the *in vivo* release of GABA and 5-HT in the dorsal raphe nucleus in freely moving rats. *Brain Res.,* **584,** 36 – 44.

FLEMMING, W.W., WESTFALL, D.P., DE LA LANDE, I.S. & JELLETT, L.B. (1972). Log-normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. *J. Pharmacol. Exp. Ther.,* **181,** 339 – 345.

FUETA, Y., ANDREASEN, M., MITA, T. & LAMBERT, J.D.C. (1996). Decrease in $GABA_A$-mediated inhibition in the hippocampal CA1 area of epileptic mice. *Epilepsia,* **37** (suppl.3), 97.

GALLAGER, D.W. & PRIMUS, R.J. (1991). Benzodiazepine tolerance and dependence: $GABA_A$ receptor complex locus change. *Biochem. Soc. Symp.,* **59,** 131 – 151.

GIBALDI, M. & PERRIER, D. (1982). Non-compartemental analyis based on statistical moment theory. In *Pharmacokinetics* (2nd edition), pp. 409 – 424. New York: Marcel Dekker.

GODDARD, G.V., MCINTYRE, D.C. & LEECH, C.K. (1969). A permanent change in brain function resulting from daily electrical stimulation. *Exp. Neurol.,* **25,** 295 – 330.

HERNANDEZ, T.D., ROSEN, J.B. & GALLAGER, D.W. (1990). Long-term changes in sensitivity to GABA in dorsal raphe neurons following amygdala kindling. *Brain Res.,* **517,** 294 – 300.

HOLFORD, N.H.G. & SHEINER, L.B. (1981). Understanding the dose-effect-relationship: Clinical application of pharmacokinetic-pharmacodynamic models. *Clin. Pharmacokinet.,* **6,** 429 – 453.

KAMPHUIS, W., HUISMAN, E., DREIJER, A.M.C., GHIJSEN, W.E.J.M., VERHAGE, M. & LOPES DA SILVA, F.H. (1990). Kindling increases the K^+-evoked and CA^{2+}-dependent release of endogenous GABA in area CA1 of rat hippocampus. *Brain Res.,* **511,** 63 – 70.

KAMPHUIS, W. & LOPES DA SILVA, F.H. (1990). The kindling model of epilepsy: The role of $GABA_{ergic}$ inhibition. *Neurosci. Res. Comm.,* **6,** 1 – 10.

LEVY, G. (1985). Variability in animal and human pharmacodynamic studies. In *Variability in Drug Therapy: Discription, Estimation and Control*, eds. Rowland, M., Sheiner, L.B. & Steimer. J.L. pp. 125 – 138. New York: Raven Press.

LÖSCHER, W. & SCHWARK, W.S. (1987). Further evidence for abnormal GABAergic circuits in amygdala-kindled rats. *Brain Res.,* **420,** 385 – 390.

LÖSCHER, W., WAHNSCHAFFE, U., HÖNACK, D. & RUNDFELDT, C. (1995). Does prolonged implantation of depth electrodes predispose the brain to kindling? *Brain Res.,* **697,** 197 – 204.

LUHMANN, H.J., MITTMAN, T., VAN LUIJTELAAR, G. & HEINEMANN, U. (1995). Impairment of intracortical GABAergic inhibition in a rat model of absence epilepsy. *Epilepsy Res.,* **22,** 43 – 51.

MANDEMA, J.W. & DANHOF, M. (1990). Pharmacokinetic-pharmacodynamic modelling of the central nervous system effects of heptabarbital using aperiodic EEG analysis. *J. Pharmacokinet. Biopharm.,* **5,** 459 – 481.

MANDEMA, J.W., KUCK, M.T. & DANHOF, M. (1992). Differences in intrinsic efficacy of benzodiazepines are reflected in their concentration-EEG effect relationship. *Br. J. Pharmacol.,* **105,** 164 – 170.

MANDEMA, J.W., SAMSON, L.N., DIOS-VIEITEZ, M.C., HOLLANDER-JANSEN, M. & DANHOF, M. (1991a). Pharmacokinetic-pharmacodynamic modeling of the electroencephalographic effects of benzodiazepines. Correlation with receptor binding and anticonvulsant activity. *J. Pharmacol. Exp. Ther.,* **257,** 472 – 478.

MANDEMA, J.W., TUKKER, E. & DANHOF, M. (1991b). Pharmacokinetic-pharmacodynamic modelling of the EEG effects of midazolam in individual rats: influence of rate and route of administration. *Br. J. Pharmacol.,* **102,** 663 – 668.

MATHÔT, R.A.A., VAN SCHAICK, E.A., LANGEMEIJER, M.W.E., SOUDIJN, W., BREIMER, D.D., IJZERMAN, A.P., DANHOF, M. (1994). Pharmacokinetic-pharmacodynamic relationship of the cardiovascular effects of adenosine A_1 receptor agonist N^6-cyclopentyladenosine in the rat. *J. Pharmacol. Exp. Ther.,* **268,** 616 – 624.

RACINE, R., OKUJAVA, V. & CHIPASHVILI, S. (1972). Modification of seizure activity by electrical stimulation. III. Mechanisms. *Electroencephalogr. Clin. Neurophysiol.,* **32,** 295 – 299.

SNEDDON, W.B., BURNHAM, W.M., PROWSE, S., CHENG, H.J. & KISH, S.H. (1990). Reduced GABA-mediated chloride flux in entorhinal cortex-kindled rat brains. *Epilepsy Res.,* **6,** 18 – 22.

TIETZ, E.I. & CHIU, T.H. (1991). Regional GABA-stimulated chloride uptake in amygdala kindled rats. *Neurosci. Lett.,* **123,** 269 – 272.

VAN DER GRAAF, P.H., VAN SCHAICK, E.A., MATHÔT, R.A.A., IJZERMAN, A.P. & DANHOF, M. (1998). Mechanism-based pharmacokinetic-pharmacodynamic modelling of the effect of N^6-cyclopentyladenosine (CPA) analogues on heart rate in rat: estimation of *in vivo* operational affinity and efficacy at adenosine A_1 receptors. *J. Pharmacol. Exp. Ther.,* **283,** 809 – 816.

VOSKUYL, R.A., DINGEMANSE, J. & DANHOF, M. (1989). Determination of the threshold for convulsions by direct cortical stimulation. *Epilepsy Res.,* **3,** 120 – 129.

WADA, Y., SHIRAISHI, J., NAKAMURA, M. & KOSHINO, Y. (1997). Role of serotonin receptor subtypes in the development of amygdaloid kindling in rats. *Brain Res.,* **747,** 338 – 342.

WU, C. & STAN LEUNG, L. (1997). Partial hippocampal kindling decreases efficacy of presynaptic $GABA_B$ autoreceptors in CA1. *J. Neurosci.,* **17,** 9261 – 9269.

ZHAO, D. & STAN LEUNG, L. (1991). Effects of hippocampal kindling on paired-pulse response in CA1 in vitro. *Brain Res.,* **564,** 220 – 229.

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