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Effect of amygdala kindling on the central nervous system effects of tiagabine: EEG effects *versus* brain GABA levels

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1 The objective of this investigation was to determine the influence of amygdala kindling on the pharmacodynamics of tiagabine *in vivo*, using quantitative EEG parameters and extracellular GABA concentrations as pharmacodynamic endpoints. In integrated pharmacokinetic/pharmacodynamic (PK/PD) studies the time course of these effects was determined in conjunction with plasma concentrations following intravenous administration of 10 mg kg^{-1}. An 'effect compartment' model was used to derive individual concentration–effect relationships.

2 Tiagabine produced an increase in the amplitude of the 11.5–30 Hz frequency band of the EEG. The relationship between concentration and EEG effect was non-linear and described by the Hill equation. In kindled rats the EC_{50} was reduced to 291 ng ml^{-1} from the original value of 521 ng ml^{-1} in controls. The values of all other parameters were unchanged.

3 In kindled rats the baseline extracellular GABA concentration was increased to 1.58 μM from 0.74 μM in controls. The relationships between tiagabine concentration and extracellular GABA concentration were again non-linear and described by the Hill equation. No differences were observed between kindled rats and controls. In the synaptoneurosmal preparation *in vitro* no changes in the functioning of the GABA transporter were observed.

4 It is concluded that unlike the situation with midazolam, there is no resistance to the EEG effect of tiagabine in the kindling model of experimental epilepsy. The observed shift in the concentration–EEG effect relationship to lower concentrations can presumably be explained by the increase in the baseline GABA levels.

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Abbreviations: Cl, clearance; GABA, γ-aminobutyric acid; PD, pharmacodynamics; PK, pharmacokinetics; $t_{½}$, terminal half-life; V_{dss}, volume of distribution at steady-state

Introduction

Benzodiazepines are highly effective anticonvulsants, but their use in the maintenance treatment of seizure disorders is limited by the development of functional tolerance (Haigh & Feely, 1988). Tolerance development is usually attributed to chronic treatment with benzodiazepines, but recently, it has been demonstrated that epileptic activity itself may also cause a reduced effect (Cleton *et al*., 1998). In three experimental models of epilepsy (amygdala kindling, direct cortical stimulation and genetic absence epilepsy) the maximum EEG effect of midazolam was significantly reduced. In a subsequent investigation it was shown by application of a mechanism-based pharmacokinetic/pharmacodynamic model that this reduced maximum EEG effect results from a decrease in the maximally achievable effect in the system, rather than the efficacy with which the drug–receptor interaction is translated into pharmacological effect. This indicates that the reduced maximal EEG effect is not caused by a change in benzodiazepine receptor function (i.e. the modulatory effect on the $GABA_A$ receptor). Subsequent studies in a brain synaptoneurosomal preparation *in vitro* have confirmed that benzodiazepine receptor function is indeed unchanged in amygdala kindled rats (Cleton *et al*., 1999c). A possible explanation for the reduced maximal EEG effect of midazolam is a change in the functioning of the $GABA_A$ receptor itself. In that case the pharmacodynamics of drugs that enhance GABAergic inhibition by different (indirect) mechanisms are expected to have changed as well.

Tiagabine provides an opportunity to investigate this. It is a relatively new, highly selective drug that enhances GABAergic neurotransmission by blockade of the re-uptake of GABA from the synaptic cleft (Krogsgard-Larsen *et al*., 1987). Recently we have developed an integrated pharmacokinetic/pharmacodynamic (PK/PD) model for the effect of tiagabine on the brain, using a quantitative EEG effect parameter (amplitude in the 11.5–30 Hz frequency band) as a pharmacodynamic endpoint (Cleton *et al*., 1999a,b). Integrated PK/PD modelling is a new approach to derive the concentration-effect relationship of tiagabine *in vivo*. Briefly, following a single intravenous administration, the time course of the drug effect is analysed in conjunction with the simultaneously obtained time course of the drug concentration in plasma. In this analysis a so called 'effect compartment' is postulated to account for the delay between drug concentration and effect (hysteresis). This allows derivation of the 'steady-state' drug concentration–effect relationship from the non steady-state data.

The objective of the present investigation was to determine the influence of experimental epilepsy on the pharmacodynamics of tiagabine. To this end the pharmacodynamics (i.e. 'steady-state' conjunction–effect relationships) was studied in

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amygdala kindled animals compared to controls, using quantitative EEG parameters as pharmacodynamic endpoint. In addition the effect on extracellular GABA-levels was used as a pharmacodynamic endpoint as this provides a more direct measure of the actions of tiagabine at the GABA-uptake transporter. Finally, the uptake carrier characteristics were determined *in vitro* in synaptosomal preparations of kindled and control animals.

Methods

Animals

Male SPF Wistar rats, weighing 200–250 g (Harlan C.P.B., Zeist, The Netherlands) were used. 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: 08.00–20.00 h). 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 (Jansen Pharmaceutica, Beerse, Belgium) and 0.25 ml kg^{-1} Nembutal (Sanofi Sante, Maassluis, The Netherlands) as described before (Mandema & Danhof, 1990). Two days before experimentation, indwelling canulas were implanted, in the right jugular vein for drug administration, and the right femoral artery for the serial collection of arterial blood samples. The Committee on Animal Experimentation of Leiden University approved the protocol of this study.

Different protocols were used for two groups of animals. One group of 13 animals (seven kindling, six control) was implanted with EEG and kindling electrodes and was used to investigate the effect of amygdala kindling on the EEG effect of tiagabine (EEG group). A second group of the same size was implanted with the same electrodes and in addition a microdialysis probe was implanted for the investigation of the extracellular GABA levels in the brain (microdialysis group). In the latter group no EEG recordings have been made.

Kindling

Simultaneously with the implantation of the EEG electrodes (11 mm anterior and −2.5 mm lateral (F_1), 3.0 mm anterior and −3.5 mm lateral (C_1) to lambda and a reference electrode 2.5 mm posterior to lambda), a bipolar electrode was implanted for amygdala kindling in the right basolateral amygdala (2.5 mm posterior and 5.4 mm lateral from bregma and 7.8 mm ventral from the brain surface). The electrodes consisted of twisted, 150 μm insulated stainless steel wires (E363/3, Plastics One, Roanoke, VA, 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 ms bipolar pulses at 50 Hz, train duration 2 s) 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) has been reached. The control rats were handled identically, but not stimulated. On each day of the kindling acquisition, the seizure severity was classified according to Racine *et al*. (1972).

Pharmacokinetic/pharmacodynamic experiment

The effect of the induction of experimental epilepsy on the concentration–effect relationship of tiagabine was investigated 24 h after the last stimulation in the kindling procedure. The relationship between plasma concentrations and EEG effect of tiagabine was determined after intravenous infusion of 10 mg kg^{-1} of tiagabine over 10 min. Tiagabine was dissolved in Millipore water and administered in a volume of 600 μl using a syringe infusion pump (Syringe infusion pump 22, Harvard Apparatus, South Natick, MA, U.S.A.).

All experiments started between 08.30 and 09.30 h to standardize a possible influence of diurnal rhythms on the measurements. EEG recordings were started at least 120 min before drug administration and lasted approximately 5 h. During the experiments the animals were conscious, freely moving and were allowed free access to water.

Arterial blood samples for the determination of tiagabine plasma concentrations were drawn at predefined time-points (15 blood samples (100 or 200 μl)) during and after the infusion. The blood samples were heparinized and kept on ice. After centrifugation (10,000 r.p.m., 10 min, 4°C) plasma was separated and stored in a clean tube at −20°C. The concentration of tiagabine was determined according to Gustavson & Chu (1992). During the experiment the EEG signal was continuously recorded from the fronto-central (F_1–C_1) lead and, after band-pass filtering (0.1–100 Hz), subjected to on-line Fast Fourier Transformation analysis for quantification. For each 5 s epoch, the amplitude in the β frequency band (11.5–30 Hz) was 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.

Microdialysis procedures

The microdialysis probes, CMA-12, were obtained from CMA/Microdialysis Ab (Stockholm, Sweden) (outer diameter 0.5 mm, membrane length 222, polycarbonate tubing). In three groups of rats an intracerebral guide cannula was implanted in the frontal cortex contralateral to the amygdala kindling electrodes (coordinates 3.7 mm posterior, 3.5 mm lateral from bregma and 1.7 mm from the brain surface in rostral direction at an angle of 30°C), simultaneously with the electrodes for kindling and EEG recording. In the afternoon of the day at which the animals had their sixth sequential stage five seizure, the probe was inserted through the guide cannula. The probes were constantly perfused with an artificial cerebrospinal fluid solution (in mM: NaCl 147, KCl 4.0, $CaCl_2.2H_2O$ 1.2, $MgCl_2.6H_2O$ 0.7) by means of a syringe infusion pump (Syringe infusion pump 22, Harvard Apparatus, South Natick, MA, U.S.A.). During the night the probe was perfused at a flow rate of 1.5 μl min^{-1}. Two hours before the administration of tiagabine the perfusion rate was increased to 3 μl min^{-1} and maintained at this flow rate for the rest of the experiment. At the end of the experiment the GABA recovery by the probe was determined *in vitro* at two different GABA concentrations. Three control animals received an equimolar bolus dose of sodium chloride (0.58 mg ml^{-1}) instead of tiagabine, in order to exclude an effect of the intravenous infusion on the GABA levels in the brain. During the 2 h before tiagabine administration, microdialysis samples were collected over 15 min intervals. During the first 90 min after tiagabine

dosing, samples were collected over 10 min intervals and the last samples up to 165 min after dosing were again collected at 15 min intervals.

Amino acid analysis

Stored microdialysis samples were mixed with 15 μl trichloroacetic acid (1% w v^{-1}), were centrifuged for 4 min ($12{,}000 \times g$) and the supernatant was used for analysis. Amino acid content was quantified by high-performance liquid chromatography after precolumn derivatization of a 25 μl sample with 50 μl *o*-phthaldehyde. The 125 mm long, 4.6 mm diameter column packed with Hypersil C-18 (3 μm particle size), was eluted isocratically with a buffer containing 0.1 M Na_2HPO_4, 1 mM Na_2-ethylenediaminetetraacetate (EDTA), 0.3% tetrahydrofuran and 35% (v v^{-1}) HPLC-grade methanol. The ω-phthaldehyde derivates were detected by a Jasco (model F821-FP) fluorimeter and evaluated on a Spectra Physics integrator. Amino acid levels were calculated by comparison with a standard mixture of 1.0 μM of GABA, with a day-to-day precision of 5.5% as described previously (Verhage *et al*., 1989).

Effect on [^{3}H]-GABA uptake in brain synaptosomes in vitro

Uptake of [^{3}H]-GABA into a synaptosomal preparation was assayed by a filtration assay (Fjalland, 1978). Cortical tissue was rapidly excised and homogenized in 10 ml of ice-cold 0.32 M sucrose with a Teflon-glass homogenizer (five strokes, Homogenisator Potter S, B. Braun, Melsungen, Germany). The homogenate was centrifuged for 10 min at 687 $\times g$ at 4°C, and the pellet was discarded. The supernatant was recentrifuged at 45,000 $\times g$ at 4°C. The pellet was resuspended in 5 ml of ice-cold buffer (in mM: NaCl 120, KCl 9.18, $CaCl_2$ 2.30, $MgSO_4$ 4.0, Na_2HPO_4 12.66, NaH_2PO_4 2.97 and glucose 10.0, pH 7.4).

Fifty microlitres of this synaptosomal suspension, diluted into 225 μl buffer and 100 μl testsubstance in water, was preincubated for 10 min at 37°C. Thirty microlitres of [^{3}H]-GABA (final concentration, 0.72 nM, spec. activity 79 Ci $mmol^{-1}$, Nen Life Sciences, Dupont de Nemours) and unlabelled GABA (final concentration, 20.5 nM) were then added and incubation was continued for another 10 min. Synaptosomes were recovered by rapid filtration through Whatmann GF/C glass fibre filters, pre-soaked with 0.5% polyethylimine, under vacuum. Filters were washed three times with 7.5 ml of ice-cold saline, and the tritium trapped on the filters was assessed by conventional scintillation counting in 5 ml of LSC Emulsifier-safe (Packard, Downers Grove, IL, U.S.A.). Non-carrier-mediated uptake was determined in the presence of nipecotic acid (180 μM) and was subtracted from total to give carrier-mediated [^{3}H]-GABA uptake.

Data analysis

In analogy to the previous studies a population approach was applied to quantify the pharmacokinetics and pharmacodynamics of tiagabine (Cleton *et al*., 1999a,b). In this approach the data from all individual rats for both treatments, kindling and control, were fitted simultaneously.

The statistical models used in this analysis were described in full detail in a previous study (Cleton *et al*., 1999c). In short, the models take into account both intra- and interindividual variation. For convenience, interindividual variability was always expressed as coefficient of variation (c.v.) in this study. An effect of chronic treatment on any of the parameters in the different equations is expressed as a difference δ from control. In case of a significant effect, δ will be significantly different from zero.

From the individual parameter estimates, values for total plasma clearance (Cl), volume of distribution at steady-state (V_{dss}) and elimination half-life ($t_{½}$) were calculated following standard procedures (Gibaldi & Perrier, 1982). Values of clearance and volume of distribution at steady-state were normalized for individual weight.

The relationship between tiagabine concentrations and the EEG effect or tiagabine concentrations and GABA levels were quantified by postulating an 'effect compartment' which represents the drug that actually is available at the synapses for inhibition of the GABA uptake transporters (Sheiner *et al*., 1979). Under this interpretation the 'effect compartment' model is linked to the plasma by a first-order process (k_{eo}) and achieves a drug concentration C_e. The equation is:

$$\frac{dCe}{dt} == k_{e0}\ (C_p - C_e) \qquad (1)$$

using C_e as a driving force of the inhibition of the GABA uptake transporter in the synaptic cleft. The 'effect' site concentration *versus* EEG or GABA effect relationship was characterized according to the sigmoidal E_{max} model, as previously described (Cleton *et al*., 1999a).

$$E = E_0 + \frac{\alpha \cdot C_e^{n_H}}{EC_{50}^{n_H} + C_e^{n_H}} \qquad (2)$$

where C_e is the effect-site concentration of tiagabine, E_0 the baseline activity, α the maximal effect, EC_{50} the tiagabine concentration at half-maximal effect and n_H the Hill factor.

Inhibition of [^{3}H]-GABA uptake in brain synaptosomes in vitro

In analogy to the previous study the *in vitro* inhibition of [^{3}H]-GABA uptake was fitted with the following equation using a population approach (Cleton *et al*., 1999b):

$$inhibition = E_0 - \frac{\alpha \cdot A^{n_H}}{IC_{50}^{n_H} + A^{n_H}} \qquad (3)$$

in which E_0 is the basal uptake, α the maximal inhibition of the uptake, IC_{50} the concentration at half-maximal inhibition of the uptake, n_H the Hill slope and A the concentration of tiagabine.

Statistics

All GABA concentrations are expressed as absolute concentrations after correction for the *in vitro* recovery which averaged $16.2 \pm 2.0\%$ ($n = 16$). The pharmacokinetic 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 significant level of 5% was selected.

Results

Kindling

Kindling progressed to stage five within on average seven sessions (range 4–8) as characterized by behavioural convulsions (Racine *et al*., 1972). Stimulation was continued until six

generalized seizures had been elicited; this required a mean of 12 sessions (range 10–14). The observed rate of kindling is consistent with previously reported data (Löscher *et al*., 1995; Cleton *et al*., 1998).

Pharmacokinetics

The time course of average drug concentrations following intravenous infusion of tiagabine over 10 min in the kindled *versus* control animals from the groups used for the EEG recordings and the microdialysis experiment respectively, is shown in Figure 1. The solid line represents the population mean best fits of the pharmacokinetic model to the data. A bi-exponential equation was used to describe the concentration–time profiles. Pharmacokinetic parameters estimates were derived for each individual animal on basis of Bayesian *post hoc* analysis. The averaged pharmacokinetic parameters are summarized in Table 1. A small, but significantly higher clearance ($P=0.021$) was observed in the kindled rats of the microdialysis group. No statistical differences in the other pharmacokinetic parameters were observed between the kindled animals and their corresponding controls. A trend towards a lower volume of distribution at steady-state was observed in the EEG group *versus* the microdialysis group.

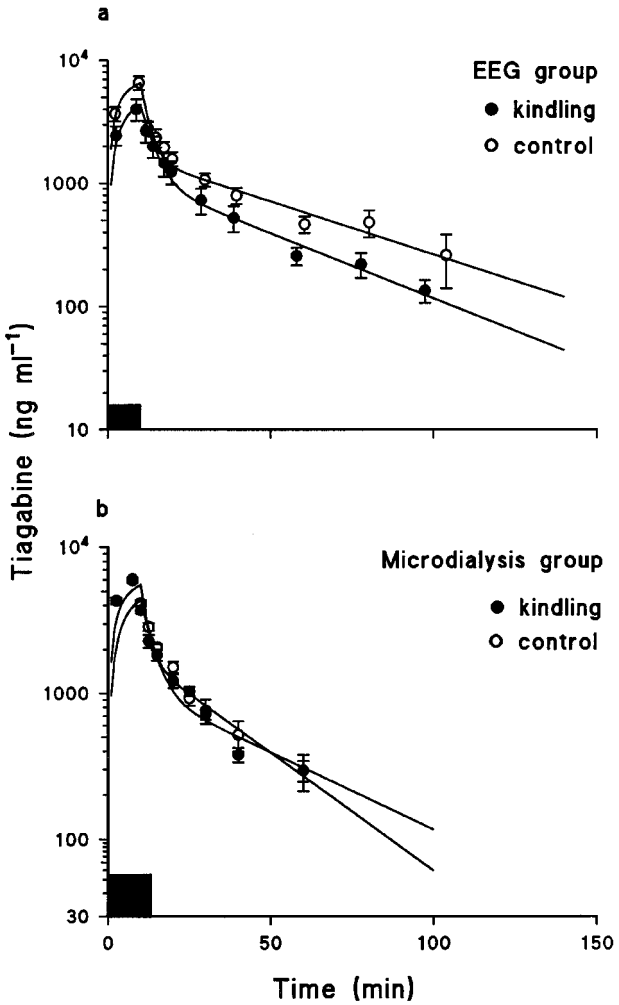

Figure 1 Mean plasma concentration *versus* time profiles after an i.v. infusion (black bar) of 10 mg kg^{-1} tiagabine over 10 min for the EEG group (a) or the microdialysis group (b); the solid lines represent the best fits to the data on basis of a population pharmacokinetic model (mean±s.e.mean, $n=6-7$).

EEG effects

To investigate alterations in drug efficacy of tiagabine, the increase in β activity (11.5–30 Hz) of the EEG was assessed in kindled and control animals. The time course of the effect on the EEG after administration of tiagabine is depicted in Figure 2. The effect compartment model was applied in order to describe the delay between plasma concentrations and EEG effect. The relationship between concentration and EEG effect was non-linear and described by the Hill equation (Figure 3). The estimated pharmacodynamic parameters for the tiagabine-induced increase in β activity are summarized in Table 2. Kindling caused a significant reduction δ of 230±21 ng ml^{-1} of the value of EC_{50}, from the population mean value of 521 ng ml^{-1} in control animals (95% C.I. is $188<\delta_{EC50}<272$; $\delta_{EC50}\neq 0$, $P<0.05$). The other population pharmacodynamic parameter estimates were (mean±s.e.mean of estimate) E_0 178 μV, α 90 μV, Hill factor 1.9 and $t_{1/2}$ k_{e0} 17 min. The pharmacodynamic parameter estimates are similar to those previously reported (Cleton *et al*., 1999a,b).

Brain GABA concentrations

Tiagabine-mediated inhibition of GABA uptake resulted in an increase in brain GABA concentrations. The time profiles of the GABA concentrations that were observed in kindled and control animals are depicted in Figure 4. Administration of vehicle produced no effect on GABA levels. A substantial increase δ of 0.84±0.04 μM in baseline GABA concentration from 0.74±0.02 μM to 1.58 μM was observed in kindled animals (95% C.I. is $0.76<\delta_{E0}<0.92$, $\delta_{E0}\neq 0$, $P<0.05$) (Table 3).

Table 1 Influence of amygdala kindling on the pharmacokinetic parameters estimates of tiagabine

	EEG group		*Microdialysis group*	
	Control	*Kindling*	*Control*	*Kindling*
Cl (ml min^{-1} kg^{-1})	72±4	119±6*	106±9	119±12
V_{dss} (l kg^{-1})	2.4±0.3	3.0±0.3	1.7±0.3	1.8±0.4
$t_{1/2}$ (min)	35±5	29±4	18±4	16±4

The values are represented as mean±s.e.mean, $n=6-7$, $P<0.05$.

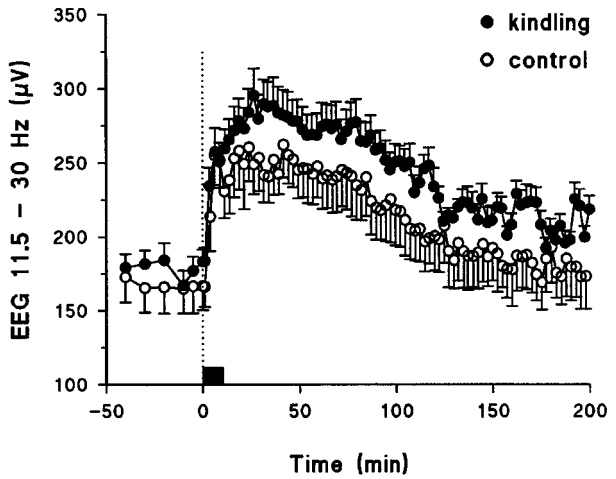

Figure 2 Time profile of the increase in β activity of the EEG after intravenous administration of 10 mg kg^{-1} tiagabine over 10 min; data are reported as mean±s.e.mean, $n=6$.

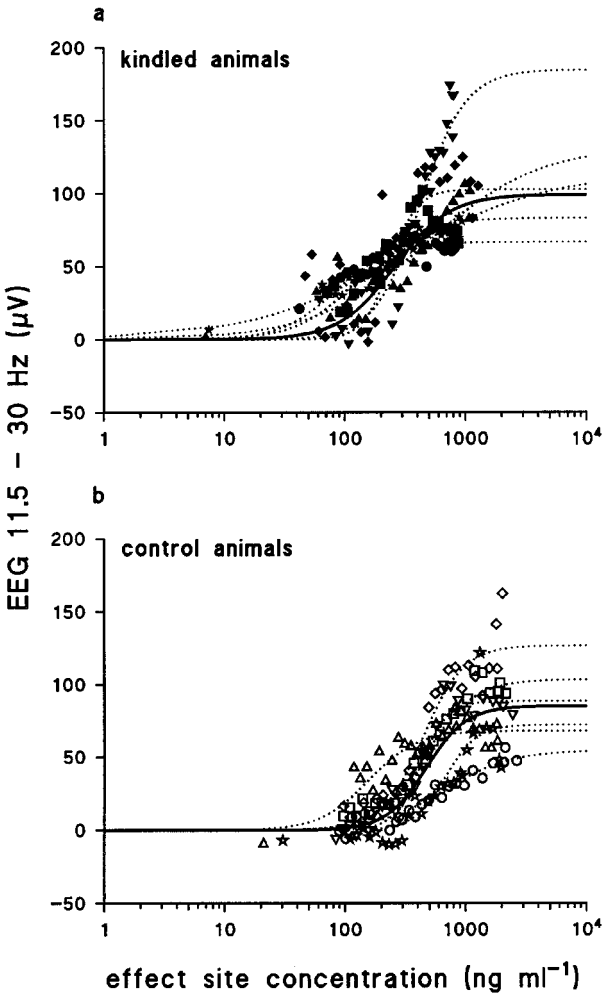

Figure 3 Plasma concentration–effect relationships for the EEG following intravenous infusion of 10 mg kg^{-1} for 10 min. The solid line was obtained by simultaneous fitting of the Hill equation to the data. The dashed lines represent the individual curves obtained by a Bayesian procedure.

Moreover, administration of tiagabine resulted in higher maximal GABA concentrations in the kindled animals. Typically, the GABA concentrations increased slowly and reached a maximum after approximately 30–40 min. GABA concentrations did return entirely to their original baseline values, within the time span of the experiment.

The individual Baylian estimates of the pharmacokinetic parameters were used to estimate tiagabine plasma concentrations at the time points of the GABA effect data. A profound delay (hysteresis) between plasma concentrations and brain GABA concentrations was observed. The effect compartment model was applied to account for the observed time delay. After solving the hysteresis, the observed relationship between tiagabine concentration and the increase in GABA concentration was non-linear and described by the Hill equation. The estimated pharmacodynamic parameters are shown in Table 3.

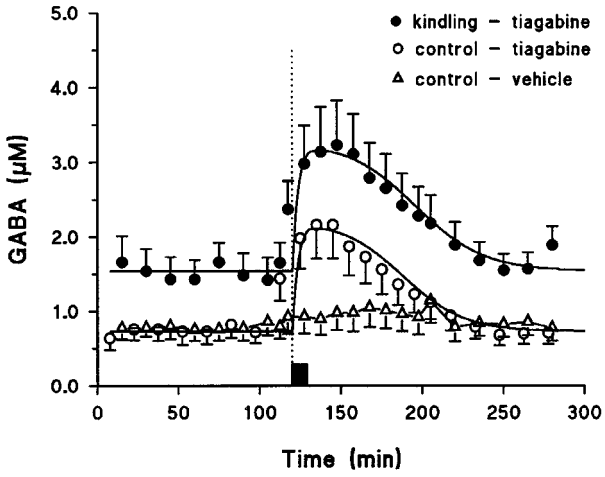

Figure 4 Time profiles of the GABA concentrations after intravenous infusion of 10 mg kg^{-1} tiagabine over 10 min (black bar) in the control and kindled animals and the placebo group that received an infusion of saline; data are illustrated as mean ± s.e.mean. The solid line represents the predicted time profile on basis of the effect compartment model with the sigmoidal E_{max} model.

Table 2 Hill equation parameter estimates for the effect of amygdala kindling on the EEG effect

	Population mean	*δ (amygdala kindling)*
k_{e0} (min^{-1})	0.042 ± 0.008 (51%)	–
α (μV)	90 ± 8 (31%)	–
EC_{50} (ng ml^{-1})	521 ± 90 (42%)	230 ± 21 ($188 < \delta_{EC50} < 272$)
n_H	1.9 ± 0.3 (60%)	–
E_0 (μV)	178 (3%)	–

A possible difference (expressed as δ) between any of the parameters of the two treatments was determined in a stepwise procedure. In the first run a difference was postulated for all parameters, except E_0. If they did not differ significantly from zero, they were fixed to zero. This table shows only the results of the final run (mean ± s.e.mean estimate, $n = 6$). Numbers in parentheses are either c.v. (population mean), describing the interindividual variation, or the 95% confidence interval.

Table 3 Hill equation parameter estimates for the effect of amygdala kindling on the GABA concentrations

	Population mean	*δ (amygdala kindling)*
k_{e0} (min^{-1})	0.035 ± 0.003 (32%)	–
α (μM)	1.7 ± 0.2 (46%)	–
EC_{50} (ng ml^{-1})	550 ± 92 (26%)	–
n_H	1.8 ± 0.4 (81%)	–
E_0 (μM)	0.74 ± 0.02 (36%)	0.84 ± 0.04* ($0.76 < \delta_{E0} < 0.92$)

A possible difference (expressed as δ) between any of the parameters of the two treatments was determined in a stepwise procedure. In the first run a difference was postulated for all parameters, including E_0. If they did not differ significantly from zero, they were fixed to zero. This table shows only the results of the final run (mean ± s.e.mean estimate, $n = 7$; $P < 0.05$ $\delta \neq 0$). Numbers in parentheses are either c.v. (population mean), describing the interindividual variation, or the 95% confidence interval.

Beside the difference in baseline values, no other differences in the pharmacodynamic parameters were observed.

Inhibition of [^{3}H]-GABA uptake in vitro

Tiagabine inhibited the uptake of [^{3}H]-GABA into rat forebrain synaptosomal preparations in a concentration-dependent way (Figure 5). No statistically significant differences in the uptake characteristics between the treatment groups were detected (Table 4).

Discussion

In this study we applied an integrated pharmacokinetic/pharmacodynamic modelling approach to determine quantitatively the influence of experimentally induced epilepsy (amygdala kindling) on the pharmacodynamics of tiagabine in rats. The findings show that experimental epilepsy is associated with a shift in the concentration–EEG effect relationship of tiagabine to lower concentrations while the maximum EEG effect remains the same. In addition, it is shown that although the baseline extracellular GABA concentration is increased, the functioning of the GABA-uptake transport is unaffected in kindled rats. The observation of an increased brain sensitivity is in contrast with findings in a similar study with midazolam, where a reduced maximum EEG effect was observed in kindled rats (Cleton *et al*., 1998; 1999c).

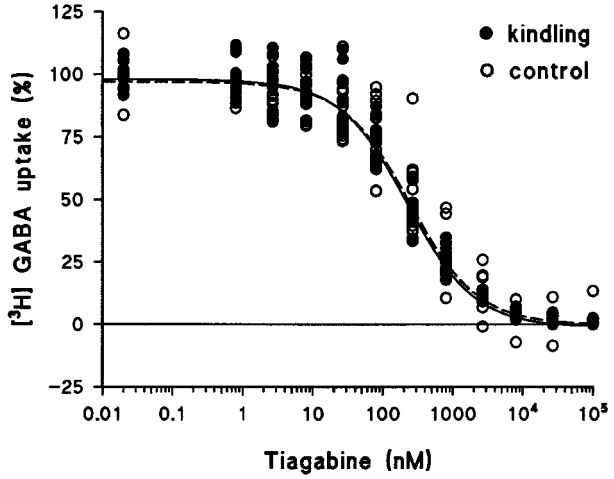

Figure 5 Inhibition of [^{3}H]-GABA uptake into rat forebrain synaptosomal preparations. The solid line and the dashed line represent the population fit of all individual data for control and kindled animals respectively.

Table 4 Hill equation parameter estimates for *in vitro* difference in [^{3}H]-GABA uptake

	Population mean	*δ (amygdala kindling)*
α (%)	96 + 2 (0%)	
IC_{50} (nM)	237 + 28 (41%)	
n_H	0.92 + 0.1 (17%)	
E_0 (%)	98 (3%)	–

A possible difference (expressed as δ) between any of the parameters of the two treatments was determined in a stepwise procedure. In the first run a difference was postulated for all parameters, E_0. If they did not differ significantly from zero, they were fixed to zero. This table shows only the results of the final run (mean ± s.e.mean of estimate, $n = 6$). Numbers in parentheses are either c.v. (population mean), describing the interindividual variation.

The pharmacokinetic parameters of tiagabine were similar to those observed in two previous studies (Cleton *et al*., 1999a,b). A moderate effect of kindling on the pharmacokinetics of tiagabine was observed. The values of the various parameters differed substantially, however, between the EEG and the microdialysis group. At present we have no explanation for these differences. In previous studies however also wide differences in individual pharmacokinetic parameters have been reported with values of the clearance varying between 35–150 ml min^{-1} kg^{-1} and of the volume of distribution between 0.5 and 2.1 l kg^{-1} (Cleton *et al*., 1999a,b). This suggests that the observed pharmacokinetic parameters are within the normal range.

The hysteresis between the plasma concentration of tiagabine and the effect on both the EEG effect and the extracellular GABA concentration was characterized by application of the effect compartment model (Sheiner *et al*, 1979). This model was validated in two previous investigations (Cleton *et al*., 1999a,b). Interestingly, the values of the rate constant for equilibration with the effect compartment were identical for both pharmacodynamic endpoints, i.e. EEG and GABA concentration. In a previous investigation it has been demonstrated that hysteresis for the EEG effect is primarily caused by slow transport of tiagabine across the blood-brain barrier rather than by rate-limiting steps related to the turnover of GABA (Cleton *et al*., 1999a). The observations of the present investigation are entirely consistent with this conclusion. Furthermore, the observation that the values of the K_{e0} were the same in kindled rats *versus* controls, indicates that no changes in the equilibration with the effect compartment have occurred. Thus a change in biophase equilibration is unlikely to be a confounding factor in the analysis of the concentration–effect relationships.

The values of the pharmacodynamic parameter estimates showed a relatively large interindividual variability, but are in line with previously reported data (Cleton *et al*., 1999a,b). The maximum EEG effect was similar in kindled rats and controls. However, a leftward shift was observed in the concentration–effect relationship. The latter is in contrast with observations for midazolam, where in experimental epilepsy a consistent reduction in maximum EEG effect is observed (Cleton *et al*., 1998; 1999c). These findings indicate that different factors determine the maximum effect of these two classes of drugs, which enhance GABAergic inhibition by different mechanisms. For benzodiazepines it has been demonstrated that the reduction in maximum EEG effect is not caused by changes in the allosteric modulation of GABAergic inhibition (Cleton *et al*., 1999c), indicating that some other factor is involved, possibly changes in $GABA_A$ receptor properties. The observation that in the kindling model the maximum EEG effect of tiagabine is unchanged is of interest. It indicates that compared to benzodiazepines the enhancement of GABAergic inhibition by tiagabine is less sensitive to functional adaptation.

Changes in extracellular brain GABA concentration are a more direct reflection of uptake blockade and were therefore determined by microdialysis. No differences were observed in the pharmacodynamic parameters, indicating that the GABA uptake transporter characteristics are not affected by kindling. The maximal increase (188%) in GABA levels in control

animals is in line with previously reported values, after subcutaneous and intraperitonial administration of tiagabine and other GABA uptake inhibitors (Fink-Jensen *et al*., 1992; Richards & Bowery, 1996). The observed parallelism between the GABA and EEG effect suggests that the increase in the β EEG effect indeed reflects blockade of the GABA uptake inhibitor.

The absence of changes in the tiagabine mediated inhibition of GABA uptake characteristics is in line with our own *in vitro* assay, which demonstrate absence of changes in the GABA uptake carriers, but in contrast to other studies. A significant decrease in the GABA uptake carrier density after amygdala kindling has been reported (During *et al*., 1995), associated with changes in the mRNA encoding for the GABA uptake carrier type I (Hirao *et al*., 1998). One reason may be that alterations are regionally specific. Alternatively, methodological differences may play a role. In our system the functional carrier was investigated in a freshly prepared brain homogenate, whereas During *et al*. (1995) used the classical approach of frozen and washed brain homogenates. An important factor is also that in the present study *in vitro* uptake carrier characteristics were determined in the same strain of rats and under identical experimental circumstances as the *in vivo* investigations.

The microdialysis experiments furthermore revealed an important effect of the kindling procedure that was not directly evident from the EEG measurements. There was a substantial increase in baseline GABA concentration, presumably reflecting elevated GABA release. The baseline EEG, however, showed only a small upward trend. Considering the considerable variation in baseline EEG (S.D. 28 μV), it is possible that the change in basal GABA concentrations and the consequent enhancement of activation of the $GABA_A$ receptor is masked in the EEG by the natural fluctuations in the baseline.

The observed increase in baseline GABA level in kindled animals is in agreement with previous studies in rats and humans, which reported an up to 4 fold increase in basal values (Ueda & Tsura 1995; During & Spencer, 1993). Presumably, the increase is a compensatory mechanism to the increased seizure susceptibility. Possibly this is caused by downregulation of $GABA_B$ receptors after seizures (Haas *et al*., 1989; Wu & Leung, 1997), which in turn may prevent motor seizures by diminishing autoinhibition and thereby increasing extracellular GABA concentrations.

The increased baseline GABA concentration may be relevant for an explanation of the leftward shift of the tiagabine concentration–EEG effect relationship. Since tiagabine administration in kindled animals resulted at every time-point in higher concentrations of GABA than in controls, the increase in baseline GABA level apparently adds to or amplifies the effect of blockade of the uptake carrier. In other words it may make the action of tiagabine so efficient that this fully compensates the functional adaptation of the $GABA_A$ receptor.

In summary, this is the first investigation in which the effect of amygdala kindling on the pharmacodynamics of tiagabine has been studied and quantitatively evaluated. It is inferred that tiagabine-induced enhancement of GABAergic inhibition is less sensitive to functional adaptation in response to epileptic activity than modulation of GABAergic inhibition by benzodiazepines.

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