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# Stereoselective central nervous system effects of the R- and S-isomers of the GABA uptake blocker *N*-(4,4-di-(3-methylthien-2-yl)but-3-enyl) nipecotic acid in the rat

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**1** The 'effect compartment' model was applied to characterize the pharmacodynamics of the R- and S-isomers of tiagabine in conscious rats *in vivo* using increase in the $\beta$ activity of the EEG as a pharmacodynamic endpoint.

**2** No pharmacokinetic differences in plasma were observed between R- and S-tiagabine. The values for clearance and volume of distribution at steady-state were $103 \pm 10$ *versus* $90 \pm 6$ ml min$^{-1}$ kg$^{-1}$ and $1.8 \pm 0.2$ *versus* $1.6 \pm 0.2$ l kg$^{-1}$ for the R- and S-isomer, respectively. In contrast, plasma protein binding showed a statistically significant difference with values of the free fraction of $5.7 \pm 0.5$ and $11.4 \pm 0.6$%. In addition the rate constant for transport to the effect compartment was also different with values of 0.027 *versus* 0.067 min$^{-1}$.

**3** For both isomers the relationship between concentration and EEG effect was non-linear and successfully characterized on basis of the Hill equation. A statistically significant difference in the value of $EC_{50}$ of $328 \pm 11$ *versus* $604 \pm 18$ ng ml$^{-1}$ was observed for R- and S-tiagabine respectively. The values of the other pharmacodynamic parameters were identical.

**4** It is concluded that the differences in *in vivo* pharmacodynamics of R- and S-tiagabine can be explained by stereoselective differences in both the affinity to the GABA-uptake transporter and the degree of non-specific protein binding in plasma and at the effect site.

**Keywords:** Pharmacokinetics; pharmacodynamics; effect compartment; tiagabine; GABA uptake inhibitor; GABA uptake transporter

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

## Introduction

$\gamma$-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (Krogsgaard-Larsen, 1988). Impaired GABAergic control has been shown to play a pivotal role in neurologic disorders, such as epilepsy or anxiety. Therefore, there is a continuous interest in development of drugs that can enhance GABAergic inhibition (Löscher & Schwark, 1987; Olsen & Avoli, 1997). Since GABA is removed from the synaptic cleft by uptake into the presynaptic terminal and into astrocytes (Krogsgaard-Larsen, 1988), drugs that inhibit this process strengthen the inhibitory action of GABA and can therefore be used for the prevention and treatment of seizures (Krogsgaard-Larsen *et al*., 1987).

From a therapeutic point of view, inhibition of uptake has several attractive features. For example, since it involves enhancement of the natural transmitter action, it ensures that physiological specificity and selectivity of transmitter action is retained (Nielsen *et al*., 1991). This is in contrast to a directly acting receptor agonist, which produces a continuous and, perhaps, non-physiological pattern of receptor stimulation.

Recently, a new series of potent GABA uptake inhibitors has been synthesized in a search for compounds, which are active *in vivo* (Andersen *et al*., 1993). R-tiagabine, (R)-*N*-(4,4-di-(3-methylthien-2-yl)but-3-enyl) nipecotic acid (Figure 1), has a high affinity for the GABA uptake carrier ($K_D = 30$ nM) and is not a substrate for the GABA uptake transporter (Braestrup *et al*., 1990).

The relative potency of GABA uptake inhibitors is typically determined by the *in vitro* inhibition of synaptosomal [$^3$H]-GABA uptake or receptor binding. In radioligand binding studies R-tiagabine has been reported to exhibit a 4 fold higher affinity for the GABA uptake carrier than the S-diastereomer (Braestrup *et al*., 1990). This difference in potency has also been observed in a functional assay for the GABA uptake carrier, in which the inhibition of [$^3$H]-GABA uptake in synaptosomes has been quantified. So far however, no studies have been published on the *in vivo* potency of the enantiomers of tiagabine. In this respect it is important that there can be substantial differences in pharmacokinetics between enantiomers. Such differences may include renal and metabolic clearance, plasma protein binding and tissue binding (Drayer, 1986; Crom, 1992). Thus in studies on the *in vivo* potency of enantiomers, differences in pharmacokinetics should be taken into consideration (Levy, 1994).

The primary objective of the present investigation was to study stereoselectivity in the pharmacodynamics of the diastereomers of tiagabine in individual rats *in vivo*. To this

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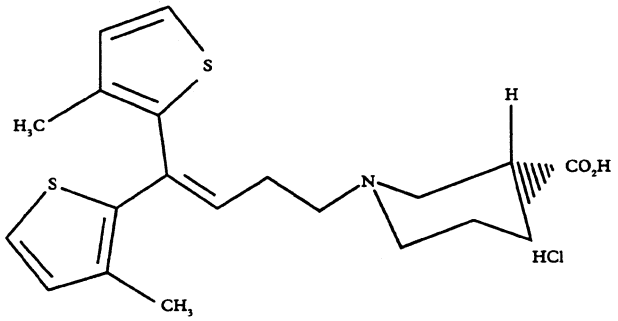


**Figure 1** Chemical structure of the R-isomer of *N*-(4,4-di-(3-methylthien-2-yl)but-3-enyl)nipecotic acid (tiagabine).

end pharmacokinetic-pharmacodynamic experiments were performed according to a crossover design, in which conscious rats received on two different occasions a single administration of R- or S-tiagabine. The tiagabine-induced increase in $\beta$ activity of the EEG (11.5–30 Hz) was used as pharmacodynamic endpoint. A previously developed population PK/PD model was applied to derive the pharmacodynamic parameter estimates of the diastereomers (Cleton *et al*., 1999). The secondary objective of the present investigation was to determine to what extent the difference in *in vivo* potency of tiagabine diastereomers could be explained by the difference in affinity for the GABA uptake transporter. Therefore, GABA uptake transporter affinity was also determiend *in vitro*, on the basis of inhibition of [$^3$H]-GABA uptake in synaptosomes.

## Methods

### *Animals and surgery*

The pharmacokinetic-pharmacodynamic experiments were performed in a group of seven rats, weighing 200–250 g, according to a randomized crossover design. The animals were assigned to two groups, which received initially either 10 mg kg$^{-1}$ R-tiagabine or 20 mg kg$^{-1}$ S-tiagabine during an intravenous infusion of 10 min. One week later the other diastereomer was administered. The compounds were administered in a volume of 750 $\mu$l distilled water using a programmable Harvard Apparatus Pump 22 (Harvard Apparatus, South Antic, MA, U.S.A.). The pharmacodynamics of tiagabine were characterized on the basis of quantitative EEG monitoring as has been described previously (Mandema & Danhof, 1990). Briefly, 1 week before the start of the experiments cortical electrodes for EEG recording were implanted under anaesthesia with 0.8 ml kg$^{-1}$ of Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) and 0.25 ml kg$^{-1}$ Nembutal (Sanofi, Maassluis, The Netherlands). One day before the pharmacokinetic-pharmacodynamic experiment, a cannula was implanted in the right femoral artery and jugular vein, tunnelled subcutaneously to the back of the neck and exteriorized. During the pharmacokinetic-pharmacodynamic experiments, the output of the fronto-central lead on the left hemisphere was continuously recorded using a DAM 50 differential amplifier (WPI, Sarato, U.S.A.) and subjected to on-line Fast Fourier Analysis for quantification. During the recordings the low pass filter was set at 0.1 Hz and the high pass filter at 100 Hz. For each 5 s epoch, the amplitudes in the $\beta$ frequency band (11.5–30 Hz) of the fronto-central lead 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. In order to minimize spontaneous fluctuations in the level of vigilance, the rats were forced to walk in a slowly rotating drum. During the experiment serial arterial blood samples (100–200 $\mu$l) were taken at 0, 2.5, 5, 7.5, 10, 12.5, 15, 20, 25, 30, 45, 60, 80 and 100 min relative to the start of the infusion. The blood samples were heparinized and kept on ice. Plasma was separated by centrifugation and stored in a clean tube at $-20^\circ$C until HPLC analysis (Gustavson & Chu, 1992). After sampling the arterial line was flushed with a few microliter of saline containing 20 I.U. ml$^{-1}$ heparin to prevent clotting. At the end of the first experiment the cannulas were filled with 40% g v$^{-1}$ polyvinylpyrolidine (P-0930, Sigma Aldrich, Zwijndrecht, The Netherlands) containing 40 I.U. ml$^{-1}$ heparin to prevent clotting.

### *Protein binding*

The extent of plasma protein binding ($f_u$, free fraction) of R- and S-tiagabine was determiend in each individual animal, as described previously for R-tiagabine (Cleton *et al*., 1999). In short, a 750 $\mu$l plasma sample was spiked with one of the diastereomers to a concentration of 1000, 2000 or 10,000 ng ml$^{-1}$. A sample of 100 $\mu$l was retained for HPLC analysis and the remaining plasma was subjected to ultrafiltration. Free compound was separated from plasma protein bound compound by filtration at $1090 \times g$ at 37°C using an YMT ultrafiltration membrane (Amicon Divisions, Danvers, MA, U.S.A.). Unbound tiagabine concentrations were determined in 100 $\mu$l of the ultrafiltrate. Corresponding tiagabine concentrations in plasma and ultrafiltrate were used to calculate the free fraction.

### *Drug assay*

The biological samples were prepared for HPLC analysis according to Gustavson & Chu (1992), with some modifications. Briefly, 500 ng of internal standard (desmethyl-tiagabine) in 100 $\mu$l acetonitrile:water (15 : 85 v v$^{-1}$) was added to a 50 or 100 $\mu$l plasma sample and further diluted with an equal amount of water. After mixing, the samples were subjected to liquid-solid extraction on C8 solid phase extraction columns (50 mg, Analytichem International, Harbor City, CA, U.S.A.). Following preconditioning with $2 \times 1$ ml methanol and $2 \times 1$ ml water, the cartridges were loaded with the sample. After the sample was drawn through the column under gentle suction, the extraction column was rinsed with $2 \times 1$ ml water. The compounds of interest were eluted with $2 \times 0.2$ ml methanol and 0.4 ml water. One hundred microlitres of the eluate was injected into the chromatographic system. The HPLC system consisted of a Spectrasystem P4000 solvent delivery system (Thermo Separation Products Inc., San Jose, CA, U.S.A.), an ISS 100 autosampler (Perkin-Elmer, Norwalk, Connecticut, U.S.A.) and a Focus fast scanning UV detector operating at 300 nm (Thermo Sep. Products Inc.). A home-made Spherisorb 3ODS2 column (C18, 100 mm, 4.6 mm I.D., 3 $\mu$m particle size, Phase Separations Ltd., Deeside, U.K.) was used. Data processing was performed with a Spectrasystem PC1000 reporting integrator. The mobile phase consisted of a mixture of methanol, acetonitrile and 20 mM citrate buffer containing 0.05% triethylamine (pH 2.1), in a ratio of 25/35/40 (v v$^{-1}$). At a flow-rate of 0.8 ml min$^{-1}$ the retention times of tiagabine and the internal standard (desmethyl-tiagabine) were 4.1 and 4.8 min, respectively. Tiagabine concentrations were calculated using the tiagabine/internal standard (desmethyl-tiaga-

bine) peak-height ratio. The limit of quantification, defined as the lowest concentration that can be determined with a coefficient of variation of 10%, was 50 ng ml$^{-1}$ in a 50 $\mu$l sample. Linear ($r > 0.9996$) calibration curves were obtained in the concentration range of 0–28 $\mu$g ml$^{-1}$. The recovery of tiagabine was 93% at the concentration of 5150 ng ml$^{-1}$ ($n = 5$). The recovery of the internal standard was 89% ($n = 5$). Accuracy (percentage of spiked value) and within-day precision (c.v.) in four different spiked plasma samples with tiagabine concentrations in the range between 0.44–9.5 $\mu$g ml$^{-1}$ ranged between 97.6%–94.6% and 0.6–5.5% ($n = 16$) respectively. The accuracy, measured over a period of 1 week ($n = 4$) for concentrations of 440 ng ml$^{-1}$ and 9.5 $\mu$g ml$^{-1}$, was 95.6% and 98.5%, the day-to-day precision 4.8 and 1.6% respectively.

### *Effect on [$^3$H]-GABA uptake in brain synaptosomes* in vitro

Uptake of [$^3$H]-GABA uptake 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 (5 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 (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 $\mu$l buffer and 100 $\mu$l test substance in water was preincubated for 10 min at 37°C. Then 30 $\mu$l 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 added, and incubation was continued for another 10 min. Synaptosomes were then recovered by rapid filtration through Whatmann GF/C glass fibre filters, presoaked with 0.5% polyethylimine, under vacuum. Filters were washed three times, each time 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 $\mu$M) and was subtracted from total to give carrier-mediated [$^3$H]-GABA uptake.

### *Data analysis*

A population approach was applied to quantify the pharmacokinetics and pharmacodynamics of tiagabine. In this approach the data from all individual rats for the administration of both isomers could be fitted simultaneously while explicitly taking into account both inter-individual variability in the model parameters as well as interindividual residual error (Schoemaker & Cohen, 1996). The modelling was performed with the non-linear mixed effects modelling software NONMEM (NONMEM project group, University of California, San Francisco, U.S.A.).

To allow a stepwise building of the final integrated pharmacokinetic-pharmacodynamic model, the pharmacokinetic model has been set up as a set of differential equations, each describing the drug amounts in the different compartments. A two-compartment model was selected on the basis of the Akaike information criterion (Akaike, 1974). The plasma concentration *versus* time data were modelled according to the following differential equations:

$$\frac{dA_1}{dt} = -k_{10}A_1 - k_{12}A_1 + k_{21}A_2 + m \quad (1)$$

$$\frac{dA_2}{dt} = -k_{21}A_2 + k_{12}A_1 \quad (2)$$

in which $m = R_0$ for $t < T$ and $m = 0$ otherwise, where $R_0$ and $T$ are the rate and duration of infusion. $A_1$ and $A_2$ represent the amount of tiagabine in the central compartment ($A_1$) and peripheral compartment ($A_2$). $K_{10}$ is the elimination constant from the central compartment, $k_{12}$ the distribution constant from the central compartment to the peripheral compartment and $k_{21}$ the distribution constant from the peripheral compartment to the central compartment. Plasma concentrations ($C_p$) of tiagabine were calculated from the amount in the central compartment:

$$C_p = \frac{A_1}{V_c} \quad (3)$$

Thus, the estimated parameters for this model are the rate constants $k_{10}$, $k_{12}$, $k_{21}$ and the central volume of distribution $V_c$. Interindividual variability on these parameters was modelled according to an exponential equation. Thus it is assumed that the parameters are log-normally distributed:

$$P_i = \theta \bullet \exp(\eta_i) \quad (4)$$

Where $\theta$ is the population value for parameter $P$, $P_i$ is the individual value and $\eta_i$ is the random deviation of $P_i$ from $P$. The values for $\eta$ are assumed to be independently normally distributed with mean zero and variance $\omega^2$. Residual error was characterized by a combination of a proportional and an additive error model:

$$C_{m\,ij} = Cp_{ij} \bullet (1 + \varepsilon_{1\,ij}) + \varepsilon_{2\,ij} \quad (5)$$

Where $Cp_{ij}$ is the $j^{th}$ plasma concentration for the $i^{th}$ individual predicted by the model, $Cm_{ij}$ is the measured concentration, and $\varepsilon_{1ij}$ and the $\varepsilon_{2ij}$ account for the residual deviance of the model predicted value from the observed concentration. The values for $\varepsilon$ are assumed to be independently normally distributed with mean zero and variance $\sigma^2$.

The values for the population parameters $\theta$, $\omega^2$ and $\sigma^2$ are estimated with use of the first order method in NONMEM. Individual parameter estimates were obtained in a Bayesian *post hoc* step. 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 was quantified by postulating an 'effect compartment' which represents the drug that actually is available at the synapses for inhibition of the GABA uptake transporters. Under this interpretation the effect compartment model is linked to the plasma by a first-order process ($k_{e0}$) (Verotta *et al*., 1989) and achieves a drug concentration $C_e$. The equation is:

$$\frac{dC_e}{dt} = k_{e0}(C_p - C_e) \quad (6)$$

using $C_e$ as driving force of the inhibition of the GABA uptake transporter in the synaptic cleft. Instead of the GABA levels, the EEG has been postulated as a direct response to the

inhibition of the elimination of GABA. The effect site concentration–EEG effect relationship was characterized according to the sigmoidal $E_{max}$ model, as previously described (Cleton *et al*., 1999):

$$E = E_o + \frac{\alpha \bullet C_e^{n_H}}{EC_{50}^{n_H} + C_e^{n_H}} \quad (7)$$

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

Interindividual variability was modelled on all pharmacodynamic parameters. The interindividual variability in the pharmacodynamic parameters, which was assumed to be log-normally distributed, was modelled according to the following exponential equation:

$$P_i = (\theta + \delta) \bullet \exp(\eta_i) \quad (8)$$

However, the parameters $E_0$ and $\alpha$, which were assumed to exhibit a normal distribution of the individual values, were modelled according to an additional equation:

$$P_i = (\theta + \delta) + \eta_i \quad (9)$$

In both equations (8,9) $\theta$ is the population value for parameter $P$, $P_i$ is the individual value, $\eta_i$ is the random deviation of $P_i$ from $P$. $\delta$ is the difference in the parameter $\theta$ of *S*-tiagabine, compared to R-tiagabine (in this approach S-tiagabine is considered a 'treatment' that may cause a change in a parameter). The values for $\eta_i$ are assumed to be independently normally distributed with mean zero and variance $\omega^2$, the interindividual variation, which relates to biological and exponential errors. Interindividual variability was always expressed as coefficient of variation (c.v.). In case of a significant difference between the R- and S-isomer in the pharmacodynamic parameter estimates ($\theta$), $\delta$ is significantly different from zero. As for the pharmacokinetics, the residual variability in the pharmacodynamics has been captured in a combined proportional and additive error model (eqn. 5).

Although the above equations constitute an integrated pharmacokinetic-pharmacodynamic model, the pharmacodynamic data set has been fitted separately from the pharmacokinetic data. A two-step approach has been applied in which first individual estimates were obtained for the pharmacokinetic parameters. In the second step the pharmacokinetics were set fixed to these values and the pharmacodynamic parameter estimates were obtained. In this way it was avoided that biases in the pharmacokinetic data could interfere with the estimation of the pharmacodynamic parameters and vice versa.

### *Inhibition of [$^3$H]-GABA uptake in brain synaptoneurosomes* in vitro

In analogy to the pharmacokinetic-pharmacodynamic relationships, the *in vitro* inhibition of [$^3$H]-GABA uptake was fitted with the following equation using a population approach with the same additive normal error model for the interindividual residual variations as described above:

$$\text{inhibition} = E_0 - \frac{\alpha \bullet A^{n_H}}{IC_{50}^{n_H} + A^{n_H}} \quad (10)$$

in which $E_0$ is the basal uptake, $\alpha$ 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. The difference between both isomers was characterized by the term $\delta$, reflecting the difference in either $\alpha$ or $IC_{50}$, as explained before.

### *Statistical analysis*

The pharmacokinetic parameter estimates for both isomers, as obtained according to standard procedures, were statistically evaluated using a paired *t*-test. A significance level of 5% was selected. All data are reported as mean ± s.e.mean, unless indicated otherwise. In case of the pharmacodynamic parameter estimates there is a significant difference between the two isomers when $\delta$ is significantly different from zero (see explanation below equation 9).

## Results

### *Pharmacokinetics*

The time course of the concentration of R- and S-tiagabine during and after intravenous infusion in 10 min is shown in Figure 2. The solid lines represent the population fit of the pharmacokinetic model for each treatment. A two-compartment pharmacokinetic model was found to best describe the concentration-time profiles of both isomers. The averaged pharmacokinetic parameters are summarized in Table 1. The pharmacokinetic parameters for both isomers were identical.

The degree of plasma protein binding was determined in the same individual animals using ultrafiltration. The free fraction of tiagabine was determined at three concentrations, 11610 ± 466, 2141 ± 47, 1670 ± 51 ng ml$^{-1}$ for R-tiagabine and 11985 ± 1574, 2149 ± 61 ng ml$^{-1}$ and 1867 ± 61 ng ml$^{-1}$ for S-tiagabine. The average values of the free fraction in plasma

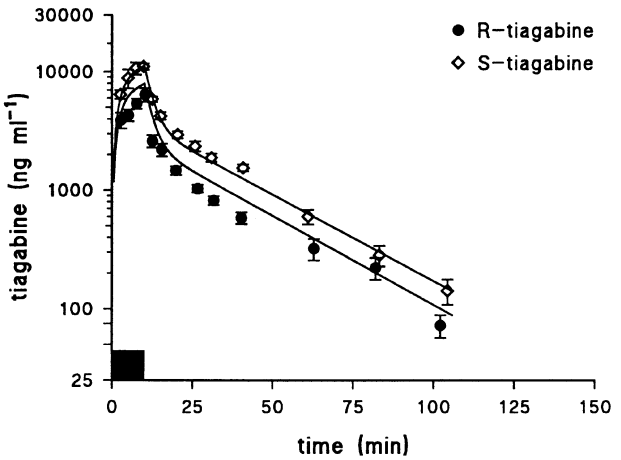


**Figure 2** Plasma concentration time profiles after intravenous administration of R- and S-tiagabine over 10 min (Black bar); data are presented as mean ± s.e.mean ($n = 7$) and the solid lines represent

**Table 1** Pharmacokinetic parameter estimates of R- and S-tiagabine after i.v. infusion in rats

| *Compound* | $t_{½}$ (min) | $Cl$ (ml min$^{-1}$ kg$^{-1}$) | $V_{dss}$ (l kg$^{-1}$) | $f_u$ (%) |
|---|---|---|---|---|
| R-tiagabine | 20 ± 1 | 103 ± 10 | 1.8 ± 0.2 | 5.7 ± 0.5 |
| S-tiagabine | 23 ± 3 | 90 ± 6 | 1.6 ± 0.2 | 11.4 ± 0.6* |

The pharmacokinetic parameters were obtained on basis of Bayesian *post-hoc* analysis of the population data (mean ± s.e.mean, $n = 7$). *$P < 0.01$ paired t-test.

**Table 2** Hill equation parameter estimated for *in vivo* difference in EEG effect between R- and S-tiagabine

| | *Population mean* | $\delta$ *(S-tiagabine* versus *R-tiagabine)* |
|---|---|---|
| $k_{e0}$ | 0.031 $\pm$0.007 | 0.040$\pm$0.019* |
| ($min^{-1}$) | (15%) | ($0.002 < \delta_{ke0} < 0.078$) |
| $\alpha$ | 73$\pm$6 | – |
| ($\mu$V) | (25%) | |
| $EC_{50}$ | 361$\pm$26 | 243$\pm$104* |
| (ng $ml^{-1}$) | (19%) | ($35 < \delta_{EC50} < 451$) |
| $n_H$ | 2.5$\pm$0.6 | – |
| | (42%) | |
| $E_0$ | 199 | – |
| ($\mu$V) | (11%) | |

A possible difference (expressed as $\delta$) between any of the parameters of the two diastereomers was determined in a stepwise procedure. In the first run a difference was postulated for all paremeters, 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$\pm$s.e.mean of estimate, $n=7$; $^*P<0.05$ $\delta \neq 0$). Numbers in parentheses are either c.v. (population mean), describing the interindividual variation, of the 95% confidence interval.

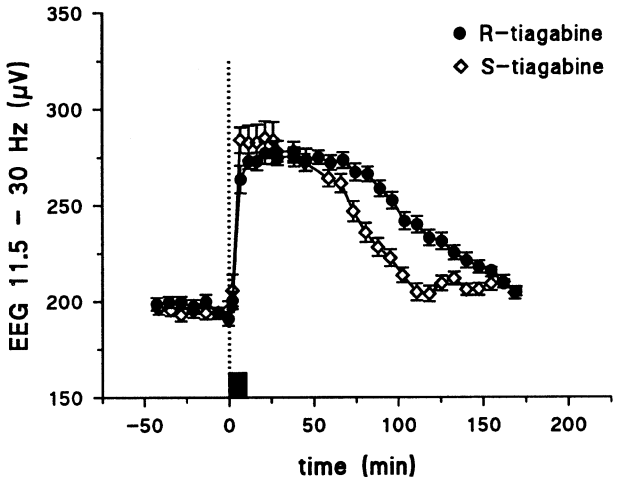


**Figure 3** EEG effect time profiles after intravenous administration of R- and S-tiagabine over 10 min (black bar); data are presented as mean$\pm$s.e.mean of estimate, $n=7$.

**Table 3** Hill equation parameter estimates for *in vitro* difference in [$^3$H]-GABA uptake between R- and S-tiagabine

| | *Population mean* | $\delta$ *(S-tiagabine* versus *R-tiagabine)* |
|---|---|---|
| $\alpha$ | 91$\pm$3 | – |
| (%) | (4%) | – |
| $IC_{50}$ | 140$\pm$80 | 233$\pm$91* |
| (nM) | (50%) | ($51 < \delta_{IC50} < 415$) |
| $n_H$ | 0.84$\pm$0.1 | – |
| | (0%) | |
| $E_0$ | 102 | – |
| (%) | (3%) | |

A possible difference (expressed as $\delta$) between any of the parameters of the two diastereomers 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$\pm$s.e.mean of 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.

($f_u$ are summarized in Table 1. The $f_u$ was shown to be concentration-independent and averaged 5.7$\pm$0.5% for R-tiagabine *versus* 11.4$\pm$0.6% for S-tiagabine ($P<0.01$, paired *t*-test).

## *Pharmacodynamics*

The time profiles of the EEG effects after intravenous administration of both isomers are shown in Figure 3. After the start of the infusion, the increase in $\beta$ activity showed a delay relative to the increase in plasma concentrations. After reaching maximal effect, the effect returned slowly to baseline values after termination of the infusion. Although a 2 fold higher dose of S-tiagabine was administered, the duration of the EEG effect was shorter than for R-tiagabine.

A profound hysteresis loop was observed between plasma concentrations and EEG effect (Figure 4A). After minimization of the hysteresis on basis of the effect compartment model, all individual concentration-effect data were fitted simultaneously to the Hill equation to provide estimates for the concentration-effect curve parameters, the asymptote $\alpha$, the midpoint location $EC_{50}$ and the slope $n_H$ (Table 2). The no-drug response value ($E_0$) was fixed in the modelling procedure to the averaged EEG effect values over the 30 min period

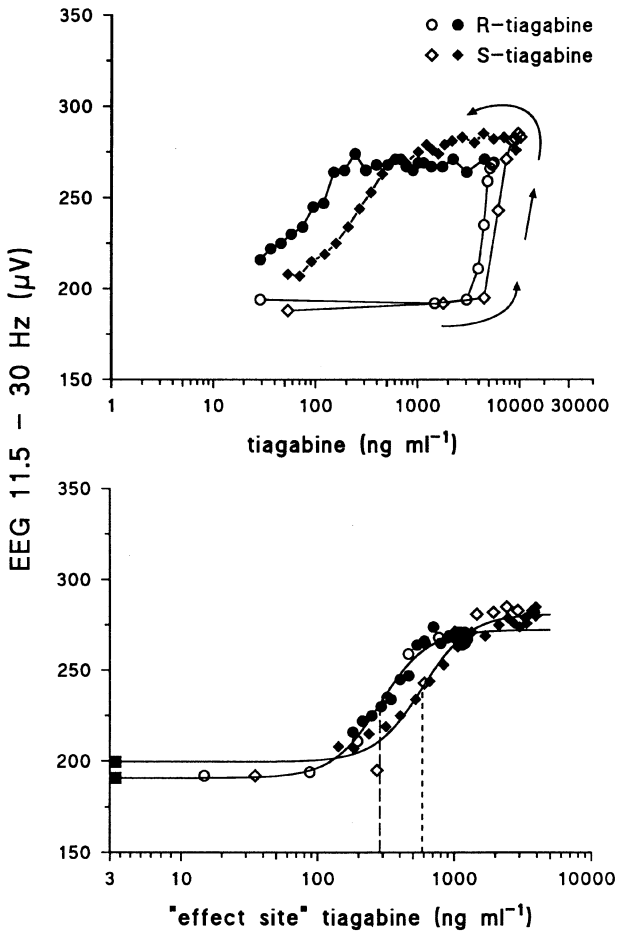


**Figure 4** Upper panel EEG effect *versus* plasma tiagabine concentrations for two individual animals. The concentration-EEG effect data are plotted with open *versus* solid symbols for the rising and falling phases of tiagabine concentrations, respectively. A profound hysteresis loop is observed. Lower panel shows the resulting hypothetical effect compartment concentration *versus* effect curve after collapsing the two limbs of the hysteresis plot, according to the 'effect compartment' model.

preceding drug administration. S-tiagabine, which is considered a 'treatment' in this approach, produced an increase $\delta_{EC50}$ of $243 \pm 104$ ng ml$^{-1}$ (mean $\pm$ s.e. of estimate), which was significantly different from zero (95% C.I. $35 < \delta_{EC50} < 451$). This resulted in an $EC_{50}$ of $604 \pm 18$ ng ml$^{-1}$ for S-tiagabine, compared to $328 \pm 11$ ng ml$^{-1}$ for the R-isomer. Correction of the $EC_{50}$ values for protein binding, to yield the $EC_{50}$ based on free drug concentrations ($EC_{50,u}$) gave values, which were still significantly different: an $EC_{50,u}$ of $68.9 \pm 4.2$ ng ml$^{-1}$ for S-tiagabine and $18.6 \pm 1.7$ ng ml$^{-1}$ for R-tiagabine ($P < 0.01$, paired $t$-test). The averaged concentration – EEG relationships for both isomeres are shown in Figure 4B. Furthermore, S-tiagabine produced a change in the distribution to the site of action $\delta_{ke0}$ of $0.040 \pm 0.019$ (mean $\pm$ s.e. of estimate; 95% C.I.: $0.002 < \delta_{ke0} < 0.078$), resulting in a $k_{ke0}$ value $0.067 \pm 0.014$ min$^{-1}$ for S-tiagabine, compared to $0.027 \pm 0.004$ min$^{-1}$ for the R-isomer. Correction of the $k_{e0}$ values for protein binding ($k_{e0}/f_u$) yielded values of $0.47 \pm 0.08$ *versus* $0.58 \pm 0.12$ min$^{-1}$ for the R- and S-isomer, respectively.

### *Inhibition of [$^3$H]-GABA uptake*

Both isomers inhibited the uptake of [$^3$H]-GABA into rat forebrain synaptosomal preparations in a concentration dependent way. However, tiagabine did not cause complete inhibition. At a concentration of 10 $\mu$M a small but reproducible uptake remained, i.e. 8% for R-tiagabine and 18% for S-tiagabine, respectively (Figure 5). Table 3 shows the difference in *in vitro* inhibition of [$^3$H]-GABA uptake characteristics for both isomers. S-tiagabine produced a significant difference $\delta_{IC50}$ of $233 \pm 91$ nM (mean $\pm$ s.e. of estimate; 95% C.I. $51 < \delta_{IC50} < 415$), resulting in $IC_{50}$ values of 392 nM for S-tiagabine, compared to 136 nM for R-tiagabine. The other uptake characteristics were not significantly different between the diastereomers.

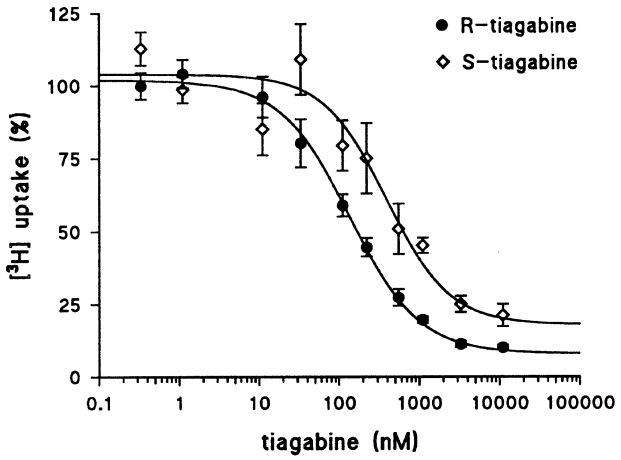


**Figure 5** Inhibiton of [$^3$H]-GABA uptake into rat forebrain synaptosomal preparations. The solid lines represent the population fits of all individual data. Data are represented as mean $\pm$ s.e.mean of estimate.

Comparison of the $EC_{50}$, $IC_{50}$ and $\alpha$ values obtained in the *in vivo* and *in vitro* experiments are shown in Table 4. A close correlation between the potency of the isomers in the EEG model and their affinity for the GABA uptake carrier is reflected by the nearly equal ratios of 0.36 – 0.46.

## Discussion

In receptor bindings as well as functional assay studies *in vitro* it has been shown that R-tiagabine is a 4 fold more effective blocker of the GABA uptake transporter than the S-isomer (Braestrup *et al*., 1990). However, it has to be noted that *in vivo* the potency is not only determined by affinity to the receptor but also by pharmacokinetic factors, like plasma protein binding and clearance (Danhof, 1989). Thus far, this aspect had not been studied for tiagabine. In the present study, the relationship between central nervous system effects and the pharmacokinetics of the diasteromers of tiagabine has been determined and compared in individual rats in an integrated pharmacokinetic-pharmacodynamic study. A previously developed population PK/PD model was applied to estimate the pharmacokinetic and pharmacodynamic parameter of both diastereomers (Cleton *et al*., 1999). This approach offers the advantage that it is possible to compare different treatments regardless of the distribution of the data (i.e. normal *versus* log normal distribution) or the variation in the data (Schoemaker & Cohen, 1996). In the present study the population model was used to obtain Bayesian estimates of the various pharmacokinetic and pharmacodynamic parameters of R- and S-tiagabine in individual rats. Separately, the data were analysed also by the standard two-stage approach (data not shown). The parameter estimates obtained in this way were very similar to those obtained with the population model. This confirms the validity of the population approach for the analysis of these 'rich' data sets.

The plasma pharmacokinetics of R- and S-tiagabine after intravenous administration were most adequately described by a bi-exponential function. No differences in the pharmacokinetics in plasma between both isomers were observed, indicating that the elimination of the diastereomers is of no importance to the observed differences in the time-effect profiles. The values of the pharmacokinetic parameter estimates of R-tiagabine were in the same range as those derived after a single administration of the R-isomer (Cleton *et al*., 1999). A difference in protein binding of both diastereomers was observed (Table 1), resulting in a 2 fold higher value of the free fraction of the S-isomer.

The tiagabine assay used in this study was not enantioselective. Therefore, it cannot be entirely excluded that racemic inversion has occurred. However, it is very unlikely that this has been a confounding factor. In case of significant interconversion from the R- to the S-enantiomer, the values of the $EC_{50}$ would be dependent on the administered dose. In a previous investigation in rats, no dose-dependency of the

**Table 4** Comparison of the pharmacodynamic parameters of R- and S-tiagabine *in vivo* and *in vitro*

| | $EC_{50}$ *or* $IC_{50}$ | | | $\alpha$ | | |
|---|---|---|---|---|---|---|
| | *EEG* (ng ml$^{-1}$) | *[$^3$H]-GAGA upt.* (ng ml$^{-1}$) | *Ratio* | *EEG* ($\mu$V) | *[$^3$H]-GAGA upt.* (%) | *Ratio* |
| R-tiagabine | 18.6 | 51.5 | 0.36 | 72 | 94 | 0.77 |
| S-tiagabine | 68.9 | 148.3 | 0.46 | 83 | 86 | 0.97 |

pharmacodynamic parameters of R-tiagabine was observed in the dose range of 3–30 mg $kg^{-1}$ (Cleton *et al*., 1999).

The lack of influence of the different protein binding on the plasma clearance can be explained by a high extraction ratio of both diastereomers (Rowland *et al*., 1973). Comparison of the values of the total plasma clearance with the typical hepatic blood flow in rats (80–100 ml $min^{-1}$ $kg^{-1}$), indicates that both R- and S-tiagabine are drugs with a high hepatic extraction ratio. Tiagabine is extensively metabolized and excreted as oxothiolene metabolites. In bile two different glutathione conjugates have been identified (Ostergaard *et al*., 1995). No information is available with regard to the pharmacological activity of these metabolites.

Between the two isomers an almost 3 fold difference in the rate constant for equilibration with the effect compartment ($k_{e0}$) was observed (Table 2). The equilibration between plasma concentrations and EEG effects was rather slow, with values of on average 26 *versus* 10 min to reach 50% equilibration for R- and S-tiagabine, respectively. There are two alternative explanations for the difference in rate of transport to the site of action. First, if passive diffusion is the sole mechanism of transport of tiagabine into the brain (effect site), the differences in $k_{e0}$ can be explained by stereoselective protein binding at the plasma or effect site. Indeed, stereoselective binding of tiagabine to the plasma proteins was observed and correction of the $k_{e0}$ for the protein binding results in identical results in identical values for the transport to the site of action. Another explanation for the observed differences may be the existence of carrier-mediated transport of tiagabine across the blood-brain-barrier, with the transport of the S-isomer being more rapid. Although the GABA uptake transporters are indeed widely distributed through the rat brain (Suzdak *et al*., 1994), active transport of tiagabine across the blood-brain-barrier seems to be unlikely. It has been convincingly demonstrated that, although tiagabine binds to transporter with high affinity, this carrier (Braestrup *et al*., 1990) does not actively transport it.

The pharmacodynamic differences between the two enantiomers are shown in Figure 4. The steady-state tiagabine plasma concentration–EEG effect relationship of the individual isomers could be described by the sigmoidal $E_{max}$ model. As the curves differ only by a shift along the concentration axis, the difference in affinity for the GABA uptake transporter may explain the difference in *in vivo* pharmacodynamics. After correction for the free fraction, the *in vivo* $EC_{50,u}$ and the *in vitro* affinity as established by inhibition of [$^3$H]-GABA uptake, can be compared. The potency based on free R- and S-tiagabine concentrations, 50 and 183 nM respectively, is in the same order of magnitude as the $IC_{50}$ for the inhibition of [$^3$H]-GABA uptake *in vitro* of 136 and 392 nM, respectively. Furthermore, the difference in *in vivo* potency between the two isomers ($EC_{50,u}$ ratio of S/R = 3.7) is similar to the values observed *in vitro* in the present investigation ($IC_{50,u}$ ratio = 2.9) and as reported in the literature ($IC_{50,u}$ ratio = 4.0; Braestrup *et al*., 1990). This clearly shows that the observed difference in *in vivo* potency can be explained entirely by the difference in affinity to the GABA uptake transporter.

Comparison of the absolute values shows that the *in vivo* $IC_{50,u}$ is approximately 60% lower than the value observed *in vitro*. This suggests that *in vivo*, GABA uptake carriers need to be only partially occupied by the transport inhibitor to exert a maximal EEG effect. This is consistent with findings of a previous study, in which it was shown that 32% inhibition of the GABA uptake transporter *in vivo* can explain the maximal EEG effect (Cleton *et al*., 1999). It is of interest to note that the diastereomers do not completely inhibit the [$^3$H]-GABA uptake at a concentration of 10 $\mu$M *in vitro*, which may be explained by the existence of different GABA uptake transporters, type 1, 2 and 3 in rats (Borden, 1996). Tiagabine binds with high selectivity to the GABA uptake transporter type 1, the predominant transporter in rat brain, accounting for approximately 85% of GABA transport (Borden *et al*., 1994; Borden, 1996). The $IC_{50}$ values of R-tiagabine for the type 2 and 3 transporter are 1410 and 2040 $\mu$M, respectively (Borden *et al*., 1994). The much lower affinities indicate that at the maximal concentration (10 $\mu$M) in our *in vitro* study, these transporters are still active. The observation that for S-tiagabine the residual uptake is 18% indicates that the selectivity of this enantiomer to the type 1 transporter is similar to that of R-tiagabine.

In conclusion, stereoselective differences in *in vivo* potency as well as transport to the site of action between the two enantiomers of tiagabine were observed. The findings of the present study can be explained by both a difference in affinity to the GABA transporter and a difference in non-specific protein binding in plasma.

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