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Effect of the GABA uptake inhibitor tiagabine on sleep and EEG power spectra in the rat

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1 The sleep profiles induced by agonists and agonistic modulators of γ-aminobutyric acid$_A$ ($GABA_A$) receptors differ markedly. With regard to $GABA_A$ agonists, the effects may be due to the fact that these agents are poor substrates for uptake and are therefore likely to activate $GABA_A$ receptors tonically. To investigate this possibility, we assessed the sleep effects of two doses (2 and 10 mg kg^{-1}) of the GABA re-uptake inhibitor tiagabine, administered intraperitoneally at light onset in 8 rats. Electroencephalogram (EEG) and electromyogram were recorded during the first 8 h after the injection.

2 Compared with vehicle, tiagabine had minimal effects on the temporal pattern of non-rapid eye movement sleep (non-REMS) and on the total time spent therein. However, tiagabine dose-dependently elevated EEG activity during non-REMs, most prominently in the lower frequencies (1–8 Hz) and least pronounced in the frequencies between 11 and 16 Hz. During the first 2 h after the injection, 10 mg kg^{-1} tiagabine elicited repetitive episodes of hypersynchronous EEG waves during wakefulness and slightly suppressed REMS. Except for these effects, tiagabine hardly influenced the time spent in and EEG activity during wakefulness and REMS.

3 The effects of tiagabine on state-specific EEG activity were qualitatively very similar to those elicited by $GABA_A$ agonists. These findings support the hypothesis that the influence of $GABA_A$ agonists on EEG signals may be caused by tonic stimulation of $GABA_A$ receptors.

Keywords: GABA; GABA uptake inhibitor; sleep; EEG spectral analysis

Introduction

A wealth of evidence firmly established the hypnotic effects of the benzodiazepines. Benzodiazepines are well known to reduce sleep onset latency, to increase the time spent in non-rapid eye movement sleep (non-REMS) and to suppress REMS in various species, including man and rats (Gaillard *et al*., 1973; Johnson *et al*., 1976; Borbély *et al*., 1985; Dijk *et al*., 1989; Mendelson & Martin, 1990; Lancel *et al*., 1996). Spectral analysis of the electroencephalogram (EEG) revealed that these compounds consistently decrease EEG activity in the lower frequencies ($\leqslant$10 Hz) and enhance EEG activity in the spindle frequency range ($\approx$11–16 Hz) during non-REMS (Borbély *et al*., 1985; Dijk *et al*., 1989; Lancel *et al*., 1996; 1997). If the selective enhancement of γ-aminobutyric acid$_A$ ($GABA_A$) receptor-mediated events by benzodiazepines is considered, these data indicate the involvement of $GABA_A$ receptors in the sculpturing of sleep-related EEG signals. However, systemic administration of the selective $GABA_A$ agonists muscimol or 4,5,6,7-tetrahydroisoxazolo (5,4-c)pyridin-3-ol (THIP) has been shown to exert no effect on sleep latency, to promote non-REMS and to increase low-frequency EEG activity during non-REMS in the rat (Lancel *et al*., 1996; Lancel & Faulhaber, 1996; Lancel, 1997). In agreement with these findings, THIP administered orally to man has been found to increase slow wave sleep (stages 3 and 4), to elevate low-frequency EEG activity, while decreasing the activity in the spindle frequencies during non-REMS (Faulhaber *et al*., 1997). Furthermore, recent studies in the rat demonstrated that co-administration of muscimol has minimal effects on the changes in sleep architecture evoked by benzodiazepines (Mendelson & Martin, 1990; Mendelson & Monti, 1993). Interestingly, muscimol effectively antagonizes the benzodiazepine-induced alterations in EEG activity during non-REMS (Lancel *et al*., 1997). These distinct differences between the sleep effects of agonistic modulators and agonists of $GABA_A$ receptors raise the possibility that subsets of the effects may be mediated by separate mechanisms related to GABAergic inhibition.

Synaptically released GABA binds to two types of receptors, termed $GABA_A$ and $GABA_B$ receptors. $GABA_A$ receptors, which form membrane-bound ligand-gated anion channels, are endowed with recognition sites for the neurotransmitter GABA and with various modulatory binding sites. Binding of GABA or an appropriate agonist to the GABA recognition site increases the membrane permeability to anions, primarily chloride (Thompson *et al*., 1988), usually resulting in a slight, short-lasting hyperpolarization. Agonistic modulators allosterically facilitate the GABA-induced chloride ion flux (for review, see Sieghart, 1995). $GABA_B$ receptors are coupled to Ca^{2+} or K^+ channels via second-messenger systems and their activation by GABA or $GABA_B$ agonists produces a much slower hyperpolarization (for review, see Deisz, 1997). The liberated GABA is eventually removed by high-affinity uptake into neurones and glial cells (Iversen & Kelly, 1975).

The divergent sleep effects of agonists and agonistic modulators of $GABA_A$ receptors are difficult to reconcile with the findings that $GABA_A$ agonists solely mimic the effects of endogenous GABA and benzodiazepines potentiate the action of endogenous GABA at $GABA_A$ receptors. The notable difference between endogenous GABA and GABA analogues is that, under normal conditions, GABA exerts a spatio-temporally confined action, whereas the latter, being poor substrates for uptake, cause more tonic and widespread effects. Possibly, such a tonic action of $GABA_A$ agonists accounts for their distinct effects on sleep parameters.

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In the present study, we assessed the influence of two doses of the potent and specific GABA uptake inhibitor tiagabine on sleep in the rat. Tiagabine, which readily crosses the blood-brain barrier, has been shown to inhibit the re-uptake of synaptic GABA into neurones and glia (for review, see Suzdak & Jansen, 1995). Inherently, tiagabine would have a dual effect: on the one hand elevating the steady state level of GABA and on the other hand perhaps prolonging and enhancing the action of endogenous GABA. Thus, tiagabine may provide additional information which of the various effects of GABAergic drugs governs the specific sleep related changes.

Methods

The experiments were approved by the local commission for animal welfare. The methods for recording and data analysis were as described previously (Lancel *et al*., 1997). Briefly, eight adult male Wistar rats (Charles River Laboratories; Sulzfeld, Germany), chronically implanted with EEG and electromyogram (EMG) electrodes, were housed individually in a ventilated, sound-attenuated Faraday room under a 12 h light:dark schedule (lights on at 8 h 30 min, 50–120 lux) at an ambient temperature of 20–21°C, with free access to food and water.

All animals were subjected to two experimental conditions, which were run in random order and were separated by $\geqslant 3$ days. Each condition consisted of 2 consecutive days. On the first day vehicle (pyrogen-free saline) was administered and on the second day 2 or 10 mg kg^{-1} tiagabine. All injections were given intraperitoneally at light onset. Tiagabine ((**R**)-N-(4,4-di-(3-methylthien-2-yl)but-3-enyl) nipecotic acid hydrochloride) was kindly provided by Novo Nordisk (Mainz, Germany). EEG and EMG were continuously recorded during the first 8 h after the injection.

The EEG signal was subjected to spectral analysis and average EEG power densities were computed for the frequencies between 0.5 and 25.5 Hz (0.5 Hz bins for the frequency bands $\leqslant$4.5 Hz and 1 Hz bins for the higher frequencies) for 10 s epochs. An offline programme displayed the EEG and EMG signals of these 10 s epochs on screen for the visual scoring of the states wakefulness, non-REMS, pre-REMS and REMS (see Neckelmann & Ursin, 1993 for scoring criteria and Figure 1 for representative EEG traces). Pre-REMS, also called intermediate state (Gandolfo *et al*., 1994) or transition type sleep (Neckelmann & Ursin, 1993), precedes REMS.

For each 8 h after the injection period the latency to non-REMS and REMS and the number and average duration of the non-REMS (including epochs of pre-REMS) and REMS episodes were determined and for each 2 h interval the time spent in each vigilance state was computed. With a 1 or 2 factor repeated-measures analysis of variance (ANOVA; Greenhouse Geisser correction; factors: condition and time), we tested whether the data of the two vehicle conditions differed significantly from one another. As this was not the case for any of the vigilance state parameters, mean values were computed over the two vehicle conditions for each animal. Differences between vehicle and the two doses of tiagabine were tested with a 1 or 2 factor repeated-measures ANOVA, with the factors treatment and time (2 h intervals). For each 2 h interval average EEG power densities during wakefulness, non-REMS (excluding epochs of pre-REMS) and REMS were computed. Since the amplitude of EEG signals decreases as a function of time, EEG power densities were standardized by expressing them as a percentage of the average EEG power density in the same frequency band and vigilance state during the corresponding 8 h vehicle recording, and were then log transformed. To analyse the dynamics of δ activity (0.5–4 Hz) during non-REMS episodes, all non-REMS episodes that were preceded by at least two 10 s epochs of wakefulness and lasted ten or more epochs were selected from the first 4 h after the injection. Average δ activity was computed for the last epoch of wakefulness and the first 9 epochs of non-REMS. Thereafter, the data were expressed as a percentage of average δ activity in non-REMS during the entire corresponding vehicle recording period and log transformed. Statistical analysis of the differences between vehicle and each dose of tiagabine was performed with a 2 factor repeated-measures ANOVA, with the factors treatment (vehicle vs tiagabine) and time (2 h interval or 10 s epoch). ANOVA was followed by a two-sided, paired t test where appropriate.

Results

Vigilance states

Tiagabine did not significantly alter the latency to non-REMS, or the number and average duration of the non-REMS and REMS episodes (Table 1). ANOVA revealed a significant treatment effect on the latency to REMS ($F(2,14)=9.2$, $P<0.005$). Compared with vehicle, 2 mg kg^{-1} tiagabine did not affect REMS latency, whereas 10 mg kg^{-1} tiagabine significantly increased REMS latency by about 25 min.

ANOVA performed on the time spent in each vigilance state yielded a significant treatment effect for pre-REMS ($F(2,14)=12.4$, $P<0.001$). Over the 8 h recording period 2 mg kg^{-1} tiagabine significantly ($P<0.03$) increased pre-REMS from $4.8\pm1.3\%$ (mean$\pm$s.d.) during vehicle to $5.5\pm1.8\%$. *Post hoc* analysis per 2 h interval revealed that

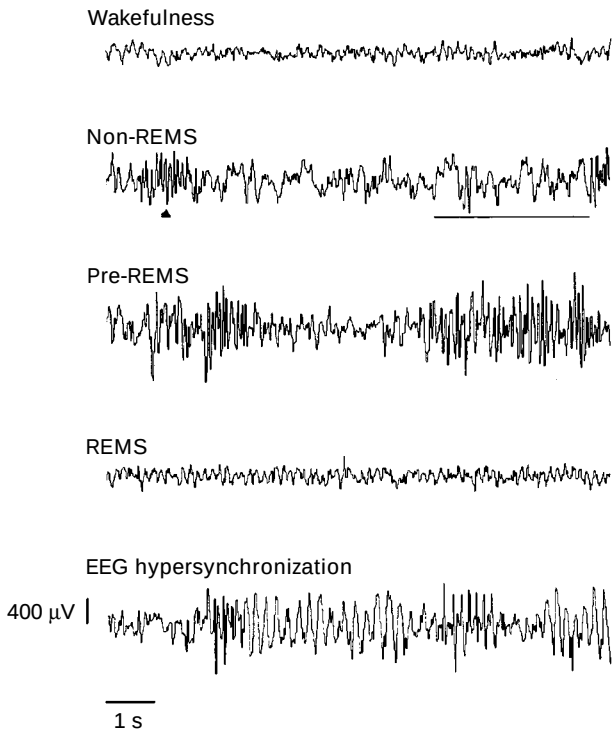

Figure 1 Typical 10 s EEG recordings for wakefulness, non-REMS, pre-REMS and REMS as well as for hypersynchronous EEG activity produced after administration of 10 mg kg^{-1} tiagabine. During non-REMS a spindle is marked by an arrowhead and δ waves by a bar.

pre-REMS was significantly increased during intervals 3–4 and 7–8 (Table 2). In contrast, 10 mg kg^{-1} tiagabine slightly, but significantly ($P<0.05$) decreased pre-REMS to 4.3±1.6% of the 8 h recording period, caused by a significant reduction during the first 2 h interval. For the other states studied no significant effects were found.

EEG power densities during wakefulness, non-REMS and REMS

In accordance with previous studies (Walton *et al*., 1994; Coenen *et al*., 1995), tiagabine transiently evoked repetitive episodes of hypersynchronous 4–7 Hz EEG signals (see Figure 1 for an example), with a duration of between 1 and 10 s and which were accompanied by low muscle activity. Such episodes were observed during 1.7±2.7% and 33.9±14.6% of the 10 s epochs during the first 2 h following 2 and 10 mg kg^{-1} tiagabine, respectively, and mainly occurred in wakefulness (≈85%). These hypersynchronous EEG waves appeared very rapidly, on average 6.2±7.3 min after administration of 10 mg kg^{-1} tiagabine. Epochs containing such hypersynchronous signals were omitted from the EEG analysis.

ANOVA performed on the EEG power densities after the administration of vehicle and 2 or 10 mg kg^{-1} tiagabine did not reveal any significant effect of tiagabine on EEG activity in wakefulness (Figure 2a), indicating that, apart from the induction of trains of hypersynchronous EEG waves, tiagabine does not alter EEG signals during wakefulness.

Analysis of the EEG power densities in non-REMS during vehicle and 2 mg kg^{-1} tiagabine yielded a significant treatment effect for the frequencies between 1.5 and 11 Hz and for most frequencies ⩾19 Hz. Compared to vehicle, this dose of tiagabine enhanced EEG activity in the respective frequencies over the 8 h recording period (Figure 2b). *Post hoc* analysis per 2 h interval showed that these effects were present during the first two 2 h intervals (Figure 3a). ANOVA also revealed a significant interaction effect between the factors treatment and time for all frequencies ⩽13 Hz and for the frequencies from 15 up to 23 Hz, reflecting that the tiagabine-induced transient elevations changed the temporal development of EEG activity in these frequencies. For 10 mg kg^{-1} tiagabine, ANOVA yielded significant treatment and interaction effects for all frequencies ⩾1 Hz. Over the 8 h recording period, tiagabine elevated EEG activity during non-REMS in all frequencies, most prominently in the δ and θ (6–9 Hz) bands and only marginally in the spindle frequency range (Figure 2b). The enhancements were maximal during the first 2 h interval and gradually decayed thereafter (Figure 3b).

Analysis of the EEG power densities during REMS did not yield significant effects of 2 mg kg^{-1} tiagabine. For

Table 1 Non-REMS and REMS latency, episode frequency and duration

	Vehicle		*Tiagabine 2 mg kg^{-1}*		*Tiagabine 10 mg kg^{-1}*	
Non-REMS						
Latency	25.5	(10.4)	23.5	(12.4)	31.2	(14.6)
Episode frequency	85.4	(21.3)	86.8	(17.1)	81.7	(16.9)
Episode duration	3.57	(0.87)	3.58	(0.79)	3.79	(0.79)
REMS						
Latency	56.7	(17.6)	56.0	(21.4)	83.7	(25.3)*
Episode frequency	37.4	(11.5)	36.8	(11.1)	35.6	(12.1)
Episode duration	1.47	(0.37)	1.49	(0.32)	1.51	(0.38)

Values are means±s.d. ($n=8$). Latency and episode duration are expressed in min. * Significant difference from vehicle ($P<0.02$, 2-sided, paired t test).

Table 2 Vigilance states (% of recording time)

State	*Time* (h)	*Vehicle*		*Tiagabine 2 mg kg^{-1}*		*Tiagabine 10 mg kg^{-1}*	
Wakefulness	1–2	34.4	(10.2)	31.1	(10.4)	45.3	(13.2)
	3–4	36.6	(12.2)	27.2	(12.0)	20.1	(9.4)
	5–6	22.9	(8.7)	28.2	(13.5)	29.2	(12.4)
	7–8	25.9	(9.2)	22.4	(12.7)	17.7	(7.4)
Non-REMS	1–2	55.3	(7.9)	59.4	(8.0)	49.7	(11.5)
	3–4	49.2	(9.1)	54.9	(9.3)	64.0	(6.5)
	5–6	59.0	(7.7)	53.7	(10.3)	53.3	(9.7)
	7–8	54.4	(9.0)	57.2	(11.5)	61.6	(7.0)
Pre-REMS	1–2	3.6	(1.7)	3.1	(1.7)	1.5	(0.8)**
	3–4	4.1	(2.1)	5.3	(1.1)*	5.1	(2.5)
	5–6	5.7	(1.2)	6.1	(2.9)	4.7	(2.0)
	7–8	5.8	(1.9)	7.4	(2.4)**	5.9	(2.7)
REMS	1–2	6.5	(2.0)	6.5	(3.7)	3.6	(2.4)
	3–4	10.1	(2.8)	12.6	(4.0)	10.6	(3.7)
	5–6	12.3	(1.9)	11.9	(2.7)	12.8	(3.1)
	7–8	13.9	(1.3)	13.1	(3.1)	14.9	(2.6)

Values are means±s.d. ($n=8$). Significant differences from vehicle are indicated by * $P<0.05$ and ** $P<0.01$, 2-sided, paired t test.

10 mg kg^{-1} tiagabine, ANOVA revealed a significant treatment and interaction effect for the frequencies between 12 and 14 Hz and from 16 up to 20 Hz. The enhancement of EEG activity in these frequencies during the 8 h recording period (Figure 2c) was mainly due to elevations during the first 4 h after the injection (not shown).

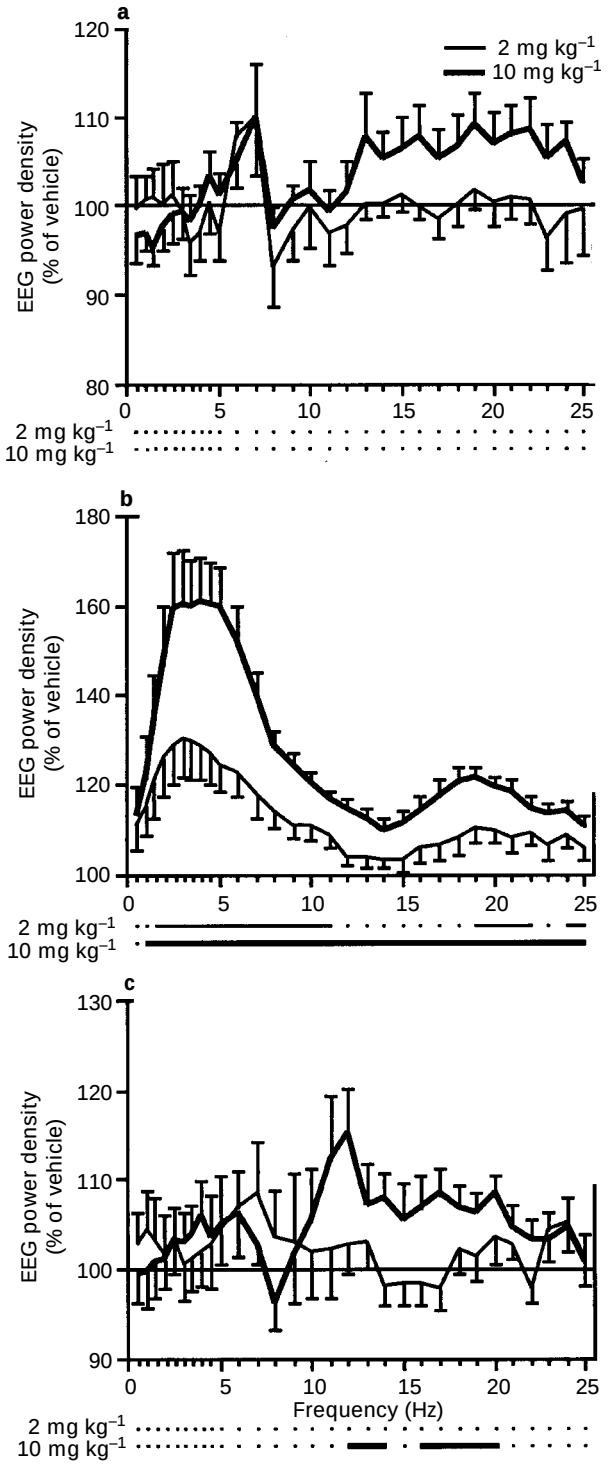

Figure 2 EEG power densities during (a) wakefulness, (b) non-REMS and (c) REMS over the 8 h recording period after administration of 2 and 10 mg kg^{-1} tiagabine. Curves connect mean values and vertical lines show s.e.mean ($n=8$). For plotting purposes, the data were expressed as percentage of the corresponding vehicle values. Dots below the graphs denote frequency bands. Lines through the dots indicate frequencies for which ANOVA revealed significant treatment effects ($P<0.05$, run on standardized and log transformed 2 h values).

Dynamics of δ activity during non-REMS

ANOVA yielded a significant treatment effect ($F(1,7)=13.6$, $P<0.008$) of 2 mg kg^{-1} tiagabine on δ activity during the initial phase of the non-REMS episodes. Tiagabine significantly enhanced δ activity from the second epoch of non-REMS onwards (Figure 4a). The interaction between the factors treatment and time was also significant ($F(9,63)=4.5$, $P<0.003$). To analyse the differences in time course in more detail, changes in δ activity over consecutive 10 s epochs were computed and compared between the treatments (two-sided, paired t test run on log transformed values). After 2 mg kg^{-1} tiagabine, δ activity rose more rapidly during the transition from wakefulness to non-REMS (vehicle: $33.6\pm8.1\%$/10 s; tiagabine: $46.3\pm18.7\%$/10 s, $P<0.01$).

Similar, though more pronounced effects were observed after administration of 10 mg kg^{-1} tiagabine (treatment: $F(1,7)=28.3$, $P<0.001$; treatment × time: $F(9,63)=7.2$, $P<0.003$). It induced an overall enhancement of δ activity during non-REMS (Figure 4b) and markedly increased the

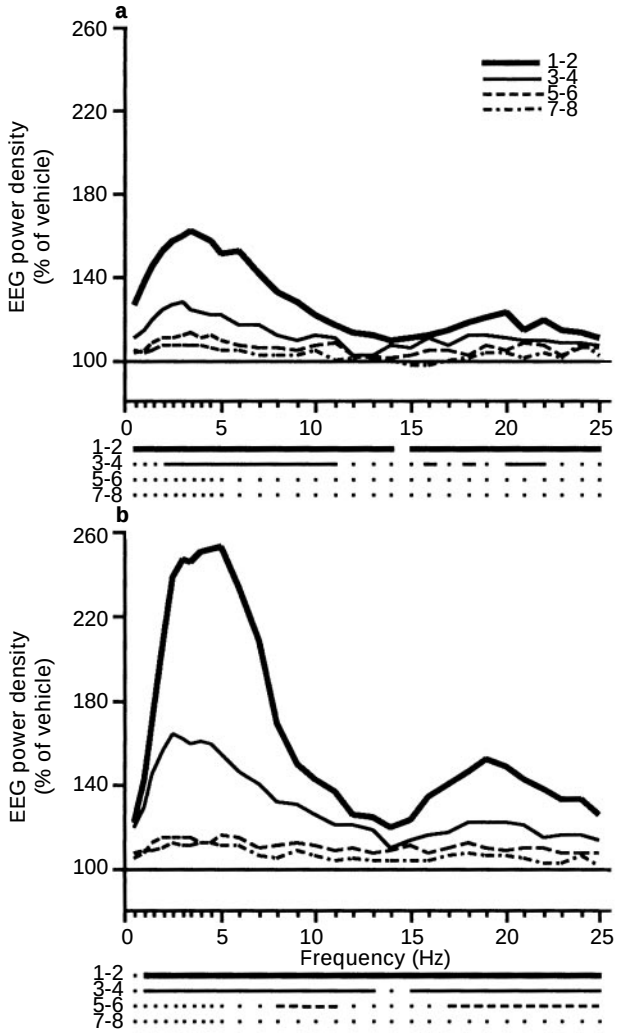

Figure 3 Time course of EEG power densities in non-REMS over 2 h intervals after administration of 2 (a) and 10 mg kg^{-1} (b) tiagabine. Curves connect mean values ($n=8$). For plotting purposes, the data were expressed as percentage of the corresponding vehicle values. Dots below the graphs denote frequency bands. Lines through the dots indicate frequencies for which significant differences from vehicle were found ($P<0.05$, 2-sided, paired t test run on standardized and log transformed values).

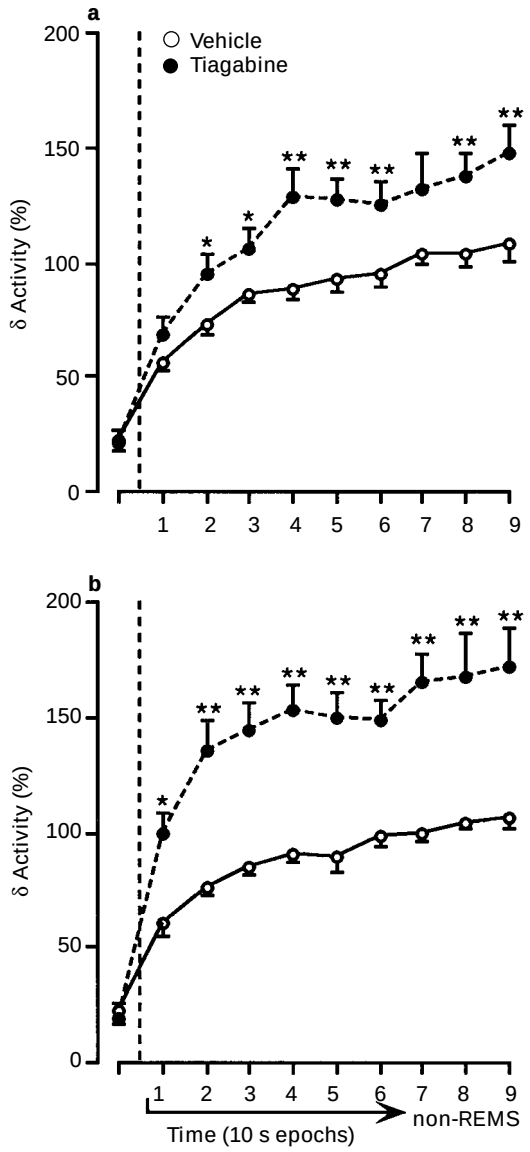

Figure 4 Time course of δ activity across the last 10 s epoch of wakefulness and the first 9 epochs of non-REMS in the non-REMS episodes during the first 4 h after injection. Curves connect values and vertical lines show s.e.mean ($n=8$). The data are expressed as percentage of the average δ activity in non-REMS during the entire corresponding vehicle period. Significant differences from vehicle are indicated by $^{*}P<0.05$ and $^{**}P<0.01$, 2-sided, paired t test run on standardized and log transformed values.

rate of rise of δ activity during the transition from wakefulness to non-REMS from $38.1\pm14.6\%/10$ s to $80.9\pm23.6\%/10$ s ($P<0.003$).

Discussion

The data presented reveal that tiagabine causes a marked and dose-dependent enhancement of EEG power density in all frequency bands during non-REMS, which indicates an overall increase in the synchronization of EEG signals. In contrast, tiagabine had minimal effects on EEG activity during wakefulness and REMS.

Tiagabine had little effect on time in wakefulness (Table 2) and on the EEG activity during wakefulness (Figure 2a). However, during the first 2 h after the administration of 10 mg kg^{-1} tiagabine, and to a lesser extent after 2 mg kg^{-1}, normal EEG activity was interrupted by periods of hypersynchronous EEG waves in the frequency range from 4 to 7 Hz (Figure 1). Previous studies showed that tiagabine enhances the duration and frequency of occurrence of absence-like EEG activity (Walton *et al*., 1994; Coenen *et al*, 1995), in line with the view that enhanced GABAergic transmission exacerbates non-convulsant epilepsy (for references, see Liu *et al*., 1991). Therefore, the hypersynchronous EEG signals may represent episodes of absence type activity.

The timing of non-REMS was unaffected by tiagabine (Tables 1 and 2). Nevertheless, it produced pronounced changes in the EEG during non-REMS: it dose-dependently enhanced EEG power density, most prominently in the δ and θ frequency bands and least pronounced in the spindle frequencies (Figure 2b). The elevation of δ activity was associated with a steeper rise during the transition from wakefulness to non-REMS and with an increase in the maximal level reached in the course of the non-REMS episodes (Figure 4). Since δ activity is generally thought to reflect the intensity of non-REMS, the present data suggest that tiagabine markedly increases non-REMS intensity in a dose-related manner.

Tiagabine had minor effects on time in REMS (Table 2), on the number and duration of the REMS episode (Table 1) and on the EEG activity during REMS (Figure 2c). However, 10 mg kg^{-1} tiagabine significantly increased REMS latency (Table 1) and decreased the time spent in pre-REMS during the first 2 h post injection (Table 2). These effects may be caused by a direct, short-lasting inhibition of pre-REMS and REMS, but may also be a consequence of the phenomenon that during the first 2 h postinjection part of the non-REMS episodes ($\approx25\%$) ended in hypersynchronous EEG activity.

The effects of tiagabine on the time spent in each vigilance state and on state-specific EEG activity differ substantially from that of agonistic modulators of $GABA_A$ receptors. In contrast to tiagabine, benzodiazepines suppress absence epileptic seizures (Marescaux *et al*., 1985) and markedly enhance high-frequency EEG activity during both wakefulness and REMS in the rat (Mandema *et al*., 1991; Lancel *et al*., 1996). Benzodiazepines are well known to shorten non-REMS latency, to decrease low-frequency EEG activity, while increasing spindling in the EEG during non-REMS, to increase prominently pre-REMS and decrease REMS (Mendelson & Martin, 1990; Mendelson & Monti, 1993; Gandolfo *et al*., 1994; Lancel *et al*., 1996). If the effects of benzodiazepines on sleep and on EEG signals are mainly mediated by an enhancement of $GABA_A$-mediated inhibition, the differences between the influence of benzodiazepines and tiagabine on these parameters indicate that tiagabine is unlikely to enhance the amplitude and duration of physiological inhibitory postsynaptic potentials (i.p.s.ps). Indeed, small spontaneous i.p.s.ps in hippocampal neurones are unaffected by a nipecotic acid derivative, despite a considerable prolongation of evoked i.p.s.ps (Isaacson *et al*., 1993; Draguhn & Heinemann, 1996).

More importantly, the effects of tiagabine on EEG signals are comparable with those induced by selective $GABA_A$ agonists in the rat. Similar to tiagabine, both muscimol and THIP elicit hypersynchronous EEG waves (Vergnes *et al*., 1984), do not influence the latency to non-REMS, enhance EEG activity, especially δ activity, during non-REMS and increase the rate of rise as well as the maximal level of δ activity attained during the non-REMS episodes (Lancel & Faulhaber, 1996; Lancel *et al*., 1996; Lancel, 1997). Furthermore, similar to the effects of tiagabine, THIP has been shown to have minimal effects on the EEG during wakefulness (Lancel, 1997),

THIP and muscimol have been found to increase slightly EEG activity in the higher frequencies during REMS (Lancel *et al*., 1996; Lancel, 1997) and a high dose of THIP has been shown to increase the latency to REMS (Lancel & Faulhaber, 1996). Since tiagabine markedly elevates steady state extracellular GABA levels (Fink-Jensen *et al*., 1992), these similarities support the postulate that the EEG effects of selective $GABA_A$ agonists may be due to a more tonic activation of $GABA_A$ receptors than usually achieved by GABA. However, the sleep responses to tiagabine and $GABA_A$ agonists differed in some respects. While both muscimol and THIP promote non-REMS and tend to lengthen the duration of the non-REMS episodes (Lancel *et al*., 1996; Lancel & Faulhaber, 1996; Lancel 1997), tiagabine was devoid of such effects. Furthermore, the enhancement of δ activity during non-REMS induced by 10 mg kg^{-1} tiagabine clearly exceeded that observed previously after administration of muscimol and THIP. These findings may be accounted for by differences in the doses employed, but may also be due to differences in the site and mechanisms of action of these substances. Whereas muscimol and THIP massively and probably tonically activate $GABA_A$ receptors, tiagabine inhibits the uptake of endogenously released GABA, thus maintaining its physiological specificity, which may be responsible for the absence of an increase in non-REMS time and maintenance. Moreover, a reduction of GABA uptake also enhances $GABA_B$ receptor-mediated inhibition, both temporally and spatially (Isaacson *et al*., 1993). The relative enhancement of $GABA_B$ inhibition by tiagabine, compared to an exclusive $GABA_A$ activation by muscimol and THIP, may add to the stronger non-REMS-specific EEG effects of tiagabine. The $GABA_B$ agonist baclofen was shown to promote non-REMS and REMS in man (Guilleminault & Flagg, 1984; Finnimore *et al*., 1995). Conversely, systemic administration of the $GABA_B$ antagonist CGP 35348 decreases non-REMS and REMS and reduces δ activity during non-REMS in the rat (Wiarderna *et al*., 1996) and intrathalamic administration of various $GABA_B$ antagonists consistently decreases low-frequency EEG activity during non-REMS in cats (Juhász *et al*., 1994). Thus, the large elevation of δ activity during non-REMS after administration of tiagabine may be due to an enhanced activation of $GABA_B$ receptors, in addition to the changes in the $GABA_A$ system.

Which mechanisms may be responsible for the pronounced effects of tiagabine on non-REMS-specific EEG activity? During non-REMS, tiagabine dose-dependently enhanced EEG activity in all frequency bands, which indicates an overall increase in synchronization of the EEG signals. Recent electrophysiological research revealed the presence of a slow rhythm (<1 Hz), which organizes and synchronizes episodes of higher frequencies (δ, spindle and fast-frequencies $\geqslant 20$ Hz) (Steriade *et al*., 1993b, c; 1996). The cortically-generated slow oscillations generally consist of prolonged depolarizations, interrupted by long-lasting hyperpolarizations, and are reflected in and modulated by thalamic neurones (Steriade *et al*., 1993a). If tiagabine enhances $GABA_B$ i.p.s.ps in thalamic and/or cortical synapses, like in hippocampal neurones (Isaacson *et al*., 1993), it may effectively contribute to the phase locking capacity of slow oscillations. Tiagabine did not augment EEG activity of all frequencies to the same extent: strikingly large elevations occurred in the δ bands and small elevations in the spindle frequencies. Thalamic neurones exhibit rhythmic activity of several frequencies, near resting V_m they display spindle oscillations. These are triggered in the GABAergic reticular thalamic nucleus, which imposes rhythmic i.p.s.ps in target thalamic cells, where they lead to postinhibitory rebound bursts that are transferred to the cortex (Steriade *et al*., 1991; Nuñez *et al*., 1992). The fact that agonistic modulators of $GABA_A$ receptors, such as benzodiazepines, promote spindling and the finding that a $GABA_A$ antagonist, but not a $GABA_B$ antagonist, blocks spindle oscillations in thalamic neurones (von Krosigk *et al*., 1993), indicate that $GABA_A$ receptors are critically involved in the generation of spindles. At a more negative Vm thalamic neurones display δ oscillations (Steriade *et al*., 1991; Nuñez *et al*., 1992) and they are enhanced by the progressive hyperpolarization during non-REMS (Hirsch *et al*., 1983). δ Oscillations result from the interplay between two voltage-gated currents: a hyperpolarization-activated inward Na^+ and K^+ current (McCormick & Pape, 1990) and a low-threshold Ca^{2+} current (Jahnsen & Llinas, 1984) and synaptic inhibition (Crunelli & Leresche, 1991). Earlier studies showed that benzodiazepine-agonistic modulators of $GABA_A$ receptors (Borbély *et al*., 1985; Dijk *et al*., 1989; Lancel *et al*., 1996; 1997) and $GABA_B$ antagonists (Juhász *et al*., 1994) suppress δ activity, indicating that $GABA_B$ receptors, but not $GABA_A$ receptors, are positively involved in the genesis of δ oscillations. However, the previous observation that both THIP and muscimol augment δ activity in man and/or rats (Lancel *et al*., 1996; Lancel & Faulhaber, 1996; Faulhaber *et al*., 1997; Lancel, 1997), suggests that tonic stimulation of $GABA_A$ receptors does effectively contribute to the generation of δ oscillations. Indeed, it has recently been demonstrated that tiagabine elevates extracellular GABA levels in the thalamus (Richards & Bowery, 1996). Therefore, the relatively large enhancement of δ activity may be brought about by a dual effect of tiagabine, a tonic elevation of GABA, particularly in the thalamic circuits, leading to an increased V_m, together with spatially increased action of GABA recruiting more $GABA_B$ receptors.

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