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Effects of the GABA_B Antagonist CGP 35348 on Sleep–Wake States, Behaviour, and Spike–Wave Discharges in Old Rats

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ABSTRACT: The GABA_B antagonist CGP 35348 was intraperitoneally given in doses of 100, 300, and 900 mg/kg to old rats. These rats were earlier chronically provided with EEG and EMG electrodes. Sleep recordings based on visual inspection of EEG and EMG recordings were made for 3 h post injection, and spontaneous behaviour in the recording cage was additionally observed. With 100 and 300 mg/kg, the drug produced an increase in the duration of REM sleep compared to the saline-injected control group. The REM sleep latency was correspondingly reduced. Non-REM sleep and total sleep duration increased and an s-shaped dose–response relationship was found. Explorative behaviour was diminished after injections with 100 and 300 mg/kg CGP 35348. The number and duration of spike–wave discharges were reduced after all doses of CGP 35348 and during all 3 recording hours. The latter outcomes confirm the strong suppressive action of this drug on spike–wave discharges; these effects have also been reported in models of absence epilepsy. The hypnotic properties and especially the increase in REM sleep after the administration of CGP 35348 deserve attention considering the paucity of drugs which facilitate REM sleep. The discovery of drugs promoting REM sleep might have theoretical as well as clinical consequences.

KEY WORDS: GABA_B antagonist, CGP 35348, Sleep, REM sleep, Old rats, Spike-wave discharges.

INTRODUCTION

GABA is a main inhibitory neurotransmitter in the mammalian brain. In several studies, different agents modulating GABA-ergic neurotransmission have been evaluated, and none of them found significant effects on sleep [20,21]. This is rather surprising, considering the fact that GABA is an important inhibitory neurotransmitter and that benzodiazepines, the hypnotic drugs of choice, are assumed to exert their action on the GABA-receptor complex. Mendelson and Martin [20] and Mendelson and Monti [21] studied the effects on sleep in rats of the benzodiazepine flurazepam, the GABA_A agonist muscimol, and antagonist bicuculline. They found that only flurazepam facilitated sleep, but that both GABA-ergic

compounds had only minimal effects. Apparently, that study does not support the hypothesis that the hypnotic actions of benzodiazepines are mediated by GABA-ergic mechanisms.

However, GABA interacts with at least two subtypes of GABA receptors, designated as GABA_A and GABA_B receptors, which show a heterogeneous distribution in the brain. These two subtypes seem to be endowed with distinct neural functions [2,3,7]. Little is known about the role of GABA_B in the regulation of sleep–wake states, and the role of GABA_B agonists and antagonists on sleep–wake states should be investigated. Recently, a centrally active GABA_B antagonist, CGP 35348, has been described [2,7,9,23]. This drug is more potent than phaclofen, a weak GABA_B antagonist, which is unable to pass the blood–brain barrier. It is already known that CGP 35348 acts as a potent anti-absence drug in various rat and mouse models [11,16,17,18,28].

Also, considering the fact that various anti-epileptic drugs, such as barbiturates and benzodiazepines, have hypnotic effects, it was tentatively argued that CGP 35348 may alter sleep–wake states. In this context, the purpose of the present study was to examine the effects of the GABA_B antagonist CGP 35348 on sleep–wake states as well as on spike–wave discharges in the electroencephalogram (EEG). To establish the profile of this drug more extensively, the effects on behaviour were also studied. Old rats showing spike–wave discharges in their EEG were used so that putative hypnotic and anti-epileptic effects of CGP 35348 could be investigated at the same time.

METHODS

Eight male Wistar rats, kindly donated by Troponwerke (Cologne, Germany) with body weights between 389 and 460 g and ages of about 24 months, were used. Animals lived under a 12–12 LD cycle with white lights on at 2000 h. Red light was switched on during the dark period. Rats were implanted with a permanent tripolar EEG electrode set for recording the fronto-occipital EEG, and a bipolar electrode set for recording the nuchal electromyogram (EMG) (for details, see [31]).

Rats were randomly assigned to one of four order groups of two subjects. All animals received saline, 100, 300, or 900 mg/kg CGP

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TABLE 1
MEANS AND SEMS OF DURATION OF WAKEFULNESS (WAKE), NON-REM SLEEP, REM SLEEP, MEAN REM PERIOD LENGTH IN SECONDS, AND % REM SLEEP/TST ON THE FIRST, SECOND, AND THIRD HOUR AFTER INJECTION OF VARIOUS DOSES OF CGP 35348

Hour	Doses CGP 35348 in mg/kg			
	0	100	300	900
1				
Wake	2691 ± 202	1950 ± 119*†	2062 ± 138*‡	2730 ± 144
non-REM	805 ± 162	1334 ± 112*†	1289 ± 123*‡	842 ± 128
REM	84 ± 44	325 ± 56*†	237 ± 51*‡	39 ± 19
Mean REM	43.7 ± 10.9	49.0 ± 6.3	68.6 ± 19.1	50.3 ± 24.4
% REM/TST	6.5 ± 2.7	20.1 ± 3.6*†	15.7 ± 3.2*‡	3.1 ± 1.5
2				
Wake	2253 ± 269	2004 ± 145	1545 ± 180*	1362 ± 192*‡
non-REM	1084 ± 234	1308 ± 162†	1516 ± 71	1900 ± 163*
REM	226 ± 55	324 ± 61	556 ± 71*‡†	341 ± 82
Mean REM	63.2 ± 10.9	53.8 ± 6.8	71.9 ± 7.3	49.4 ± 7.2
% REM/TST	16.8 ± 4.5	20.5 ± 3.5	27.5 ± 3.6†	14.5 ± 2.9
3				
Wake	2346 ± 294	2318 ± 174	1915 ± 176*	1800 ± 210*‡
non-REM	952 ± 192	973 ± 127	1211 ± 122	1479 ± 157*
REM	193 ± 78	315 ± 80	484 ± 86	360 ± 93
Mean REM	52.5 ± 9.5	76.3 ± 17.3	52.0 ± 10.4	46.6 ± 4.9
% REM/TST	12.5 ± 3.2	23.7 ± 4.3	27.9 ± 3.8*	18.5 ± 3.8

* Different from 0 mg/kg, † different from 900 mg/kg, ‡ different from 100 mg/kg.

35348 in four different sequences according to a Latin square design to control for order effects, so each rat received all three drug doses and the control injection. CGP 35348, solved in saline, was administered IP at a volume of 2 ml/kg. The drug was kindly donated by Ciba Geigy (Basel, Switzerland). The intersession interval was at least 48 h. Pharmacokinetic studies of CGP 35348 have not been performed in vivo; pharmacological data show that the EEG effects of CGP 35348 disappear in 2 to 3 h after administration [28]. Although kinetics might be different in old subjects, it is not likely that it will largely exceed the half-life values of commonly used rats. Therefore, it seems safe to state that the drug effects are not likely due to the 48-h wash-out period. Moreover, in a Latin square design, order effects are counterbalanced.

After a recovery period of 10 days, rats were adapted to the perspex recording cage (30 × 25 × 35 cm) and to the recording leads for 16 h immediately prior injection. Drugs were administered at 1000 h. The study was performed in the dark period in order to allow putative hypnotic effects to be more easily demonstrable. A ceiling effect in the amount of sleep including REM sleep could be present in young adult rats, when measured during the light period [33]. EEG and EMG were recorded for 3 h after injection. The behaviour of the animals was recorded through a window from an adjacent room for a period of 30 min, from 30 to 60 min after injection.

Three behavioural categories were distinguished: exploratory behaviour, such as walking, sniffing, and rearing; automatic behaviour, such as grooming, eating, and drinking; and immobile behaviour [5].

Sleep and wake states were visually scored according to conventional EEG and EMG criteria. Wakefulness is characterised by a small amplitude, fast frequency EEG together with a high amplitude and/or a rapidly changing EMG; non-REM sleep by a large amplitude, low frequency EEG together with a moderate and relatively constant EMG; whereas REM sleep is characterised by a low voltage, high frequency EEG with predominant

theta activity, and a low amplitude EMG. Variables, all of which were determined for each and for the total of the 3 post-drug hours and all of which were scored with an accuracy of 1 sec, were: the duration of wakefulness (W), of non-REM sleep, of REM sleep, of total sleep time (TST), of the percentage REM/TST, the number of sleep periods and the mean duration and, finally, the sleep latency (the time elapsing from injection until the first period of 30 sec of uninterrupted non-REM) and REM sleep latency (time from injection until the first period of 30 sec of uninterrupted REM sleep). The EEG analyst was blind to the experimental conditions.

Spike-wave discharges were visually analysed per hour according to criteria reported elsewhere [32]. Briefly, trains of spike-waves with a spike (peak) amplitude of at least twice the background EEG and a duration of at least 1 sec were assigned as spike-wave discharges.

All sleep parameters and spike-wave discharges were analysed with an ANOVA with hour (three levels) and dose (four levels) as "within subject" factors. Dose was analysed with subject × dose as the error term; hour was tested with hour × subject as the error term; and dose × hour was analysed with dose × hour × subject as error term. If appropriate, post hoc tests according to Duncan were performed, and here a *p*-value smaller than .05 was considered to reflect significance. Behavioural data, overall (3 h) sleep data, non-REM sleep latency, and REM sleep latency were statistically evaluated with dose as "within subject" factor and eventually followed by the same post hoc tests.

RESULTS

Sleep-Wake Parameters

The duration of wakefulness, non-REM sleep, REM sleep, TST, and percentage REM/TST are presented in Table 1 for each hour separately and in Figure 1 for the total 3 h (overall). With

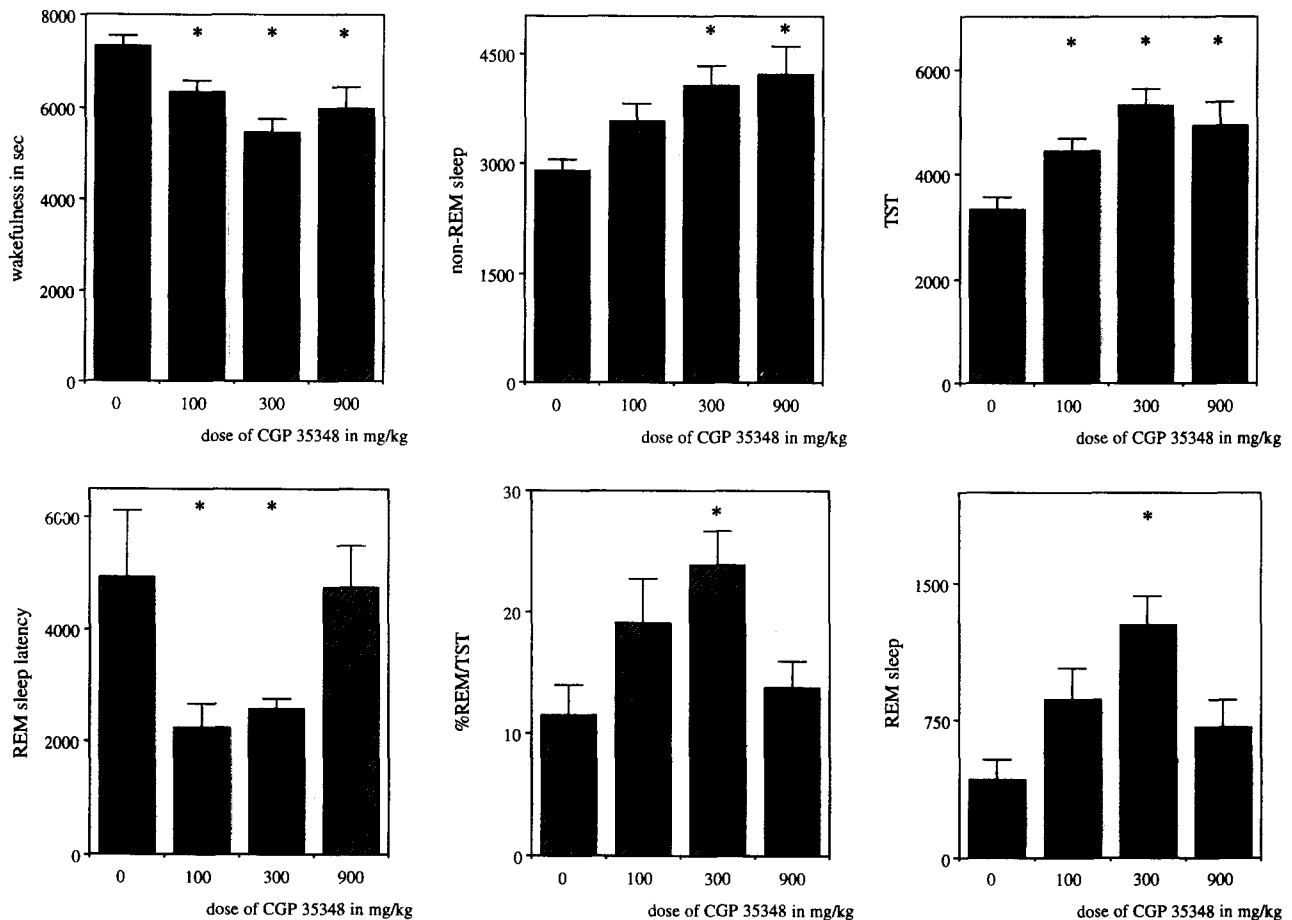


FIG. 1. The effects of various doses (0, 100, 300, and 900 mg/kg) of the GABA_B antagonist CGP 35348 on wakefulness, non-REM, REM, Total Sleep, percentage REM/TST, and REM sleep latency in the 3-h recording time. Means and SEMs in seconds (except for %REM/TST) are depicted.

respect to *wakefulness*, significant dose ($F = 5.88$, df 3,21, $p < .01$), hour ($F = 6.68$, df 2,14, $p < .01$) and dose \times hour interaction ($F = 3.29$, df 6,41, $p < .05$) effects were found. The post hoc tests were done separately for each hour since an interaction effect was found. These post hoc tests on the data from the first hour revealed that, compared to saline, there was less wakefulness after 300 mg/kg CGP 35348. There was also less wakefulness in the second hour after 300 and 900 mg/kg. In the third hour, the differences between the groups were no longer present. In the overall data, waking duration was decreased ($F = 5.84$, df 3,28, $p < .01$) after 100, 300, and 900 mg/kg CGP 35348, as well as the mean duration of the waking periods ($F = 13.48$, df 3,28, $p < .0001$; the means and SEMs were 310 ± 28 , 185 ± 16 , 141 ± 13 , and 178 ± 21 for the saline, 100, 300, and 900 mg/kg CGP 35348, respectively). The number of waking periods was higher ($F = 6.76$, df 3,28, $p < .001$) after 100 (35.9 ± 3.0), 300 (39.4 ± 2.3), and 900 mg/kg (35 ± 2.4) CGP 35348 than after saline (24.8 ± 1.8).

Significant dose ($F = 5.08$, df 3,21, $p < .01$), hour ($F = 6.09$, df 2,14, $p < .05$) and interaction effect between dose and hour ($F = 2.81$, df 6,41, $p < .05$) were found for the duration of *non-REM sleep*. The post hoc tests for the first hour showed that there was more non-REM sleep after 100 and 300 mg/kg CGP 35348 than after saline. In the second and third hour, there was more non-REM sleep after 900 mg/kg compared to saline and 100 mg/kg CGP 35348. Overall,

there was a significant dose effect ($F = 4.57$, df 3,28, $p < .01$), and there was more non-REM sleep after 300 and 900 mg/kg CGP 35348 than after saline. The number of non-REM sleep periods was enhanced after all doses CGP 35348 ($F = 8.41$, df 3,28, $p < .001$; the means and SEMs were 27.1 ± 2.6 , 38.1 ± 2.7 , 42.3 ± 1.7 , and 38.0 ± 1.7 for the saline, 100, 300, and 900 mg/kg groups respectively). The amplitude of the slow waves was reduced after the highest dose and, therefore, scoring of non-REM sleep was awkward. An illustration of the reduced amplitude of slow wave sleep after 900 mg/kg CGP 35348 is presented in Figure 2.

Significant dose ($F = 4.47$, df 3,21, $p < .05$), hour ($F = 12.13$, df 2,21, $p < .001$), and interaction ($F = 2.78$, df 6,41, $p < .05$) effects were present for the duration of *REM sleep*. The post hoc tests showed that there was more REM sleep in the first and second hour after 300 mg/kg compared to saline. Other groups which showed an increase in REM duration were the 100 mg/kg in the first hour. The 300 mg/kg group also showed more REM sleep in the second hour than the 900 and 100 mg/kg groups. In the overall scores, REM sleep duration was affected by CGP 35348 ($F = 5.55$, df 3,28, $p < .01$) and this was due to an increase after 300 mg/kg compared to saline and 900 mg/kg CGP 35348. The number of REM periods was enhanced only in the first hour after administration ($F = 8.14$, df 3,28, $p < .0001$) due to an increase after 100 and 300 mg/kg CGP 35348; the means and SEMs were 1.6 ± 0.7 , 6.5 ± 1.0 , 4.1 ± 0.8 , and 1.3

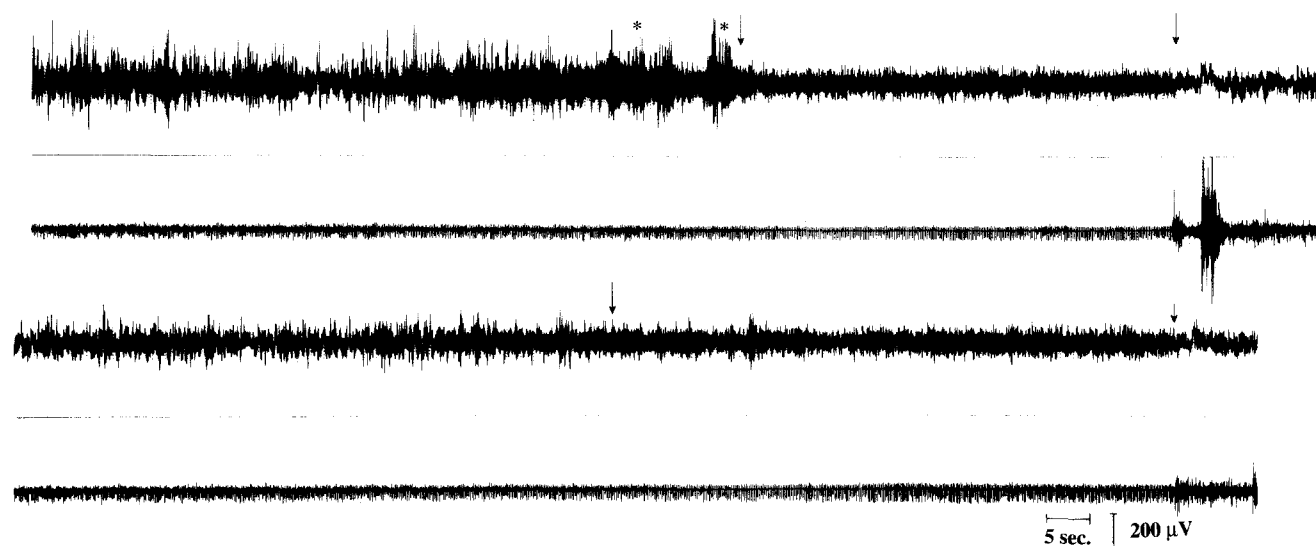


FIG. 2. The cortical EEG and the nuchal EMG on which heart activity is superimposed are shown. In the upper two traces, recorded after saline injection, a transition from non-REM sleep, via spindles belonging to the intermediate stage [10], to REM sleep and ending with an awakening is presented. The beginning and end of the REM sleep period is indicated with arrows. The spindles of intermediate stage are indicated with a star. In the lower two traces, a representative example of diminished amplitude of non-REM sleep waves after 900 mg/kg CGP 35348 is given. Here, also, a transition from non-REM to REM and wakefulness is presented. Again, the beginning and end of an REM sleep period is indicated with arrows. Note the small amplitude during non-REM sleep and the lack of intermediate sleep spindles at the transition from non-REM sleep to REM sleep. The sample is taken one and a half hour after drug administration.

± 0.6 for the saline, 100, 300, and 900 mg/kg CGP 35348 groups, respectively).

Also, significant dose ($F = 5.22$, df 3,21, $p < .01$) and hour ($F = 22.60$, df 2,24, $p < .0001$) effects were found for the *percentage REM/TST*. It was affected in the first ($F = 7.5$, df 3,28, $p < .001$) and third ($F = 3.06$, df 3,38, $p < .05$) hour and in the overall scores ($F = 3.98$, df 3,28, $p < .05$). In the first hour, percentage REM/TST was enhanced after 100 and 300 mg/kg, compared to saline and 900 mg/kg CGP 35348; in the third hour, the 300 mg/kg group had a higher percentage REM/TST than the saline group. The analyses of the overall scores showed a higher percentage for the 300 mg/kg group than the saline and the 900 mg/kg group.

There were no significant differences dose effects on *non-REM sleep latency* the means and SEM's were 1748 ± 348 , 1110 ± 22 g, 1313 ± 122 and 1784 ± 266 for saline, 100, 300, and 900 mg/kg CGP35348, respectively. However, *REM sleep la-*

tency showed a significant dose effect ($F = 3.60$, df 3,28, $p < .05$). The post hoc test showed that the REM-sleep latency was shorter after 100 and 300 mg/kg CGP 35348 compared to saline. Data are also presented in Figure 1.

Behaviour

The behavioural data are presented in Table 2. A significant dose effect was found for the duration of explorative behaviour ($F = 5.76$, df 3,21, $p < .01$). The duration of explorative behaviour was reduced after 100 and 300 mg/kg CGP 35348 compared to saline and 900 mg/kg CGP 35348. Also, the number of bouts of explorative behaviour were dependent on the dose CGP 35348 ($F = 3.96$, df 3,21, $p < .05$). After 300 mg/kg, there were fewer bouts of explorative behaviour compared to saline. As can be inferred from Table 2, CGP 35348 induced sedative effects as expressed by an increase of immobile behaviour; how-

TABLE 2
MEANS AND SEMS OF DURATION IN SECONDS, AND NUMBER OF BOUTS OF EXPLORATIVE, AUTOMATIC, AND IMMOBILE BEHAVIOUR RECORDED FROM THE 30TH TILL THE 60TH MINUTE AFTER INJECTION OF VARIOUS DOSES OF CGP 35348

Dose CGP 35348	Behaviour					
	Exploratory		Automatic		Immobile	
	Duration	Bouts	Duration	Bouts	Duration	Bouts
0	293 \pm 82	14.1 \pm 2.9	436 \pm 96	10.4 \pm 1.8	1034 \pm 162	10.6 \pm 2.1
100	86 \pm 26*	5.8 \pm 1.5*	241 \pm 103	5.9 \pm 1.3	1450 \pm 121	6.6 \pm 0.7
300	60 \pm 26*†	5.3 \pm 1.3*	161 \pm 87	5.6 \pm 1.4	1490 \pm 108	7.1 \pm 0.7
900	223 \pm 46	9.8 \pm 2.4	316 \pm 85	6.9 \pm 1.8	1220 \pm 102	8.4 \pm 1.6

* Different ($p < .05$) from 0 mg/kg, † different ($p < .05$) from 900 mg/kg CGP 35348.

TABLE 3

MEANS AND SEMS OF NUMBER AND TOTAL DURATION IN SECONDS OF SPIKE-WAVE DISCHARGES IN FIRST (1), SECOND (2), AND THIRD (3) HOUR AFTER INJECTION OF VARIOUS DOSES OF CGP 35348

Dose CGP 35348 in mg/kg	Hour 1		Hour 2		Hour 3		Total	
	Number	Duration	Number	Duration	Number	Duration	Number	Duration
0	25.3 ± 8.0	329 ± 79	28.0 ± 6.7	408 ± 118	27.5 ± 7.3	330 ± 78	26.9 ± 4.9	356 ± 52.8
100	9.5 ± 4.3*	130 ± 98	3.1 ± 2.2*	31 ± 17*	8.5 ± 2.0*	73 ± 35*	7.0 ± 2.1*†	86.1 ± 33.3*†
300	6.0 ± 2.0*	43 ± 22	0.5 ± 0.3*	3 ± 1*	0.0 ± 0.0*	0 ± 0*	2.2 ± 0.9*†	29.8 ± 10.5*†
900	1.3 ± 1.1*	17 ± 13*	0.0 ± 0.0*	0 ± 0*	0.0 ± 0.0*	0 ± 0*	0.4 ± 0.4*†	17.0 ± 3.1*†

* Different ($p < .05$) from 0 mg/kg, † different ($p < .05$) from all other groups.

ever, the effects just failed to reach significance ($F = 2.86$, df 3,21, $p < .06$).

Spike-Wave Discharges

A significant dose effect was found ($F = 17.56$, df 3,21, $p < .0001$) for the number of spike-wave discharges. The data are presented in Table 3. The reduction in number of spike-wave discharges was significant after all three doses of CGP 35348 in all 3 h, and all four groups differed from each other according to the post hoc tests ($p < .05$). For the total duration of spike-wave discharges also, a significant dose effect was found ($F = 38.20$, df 3,14, $p < .0001$). The post hoc tests confirmed the results obtained for the number of spike-wave discharges: all three doses significantly reduced the duration of the spike-wave discharges in all 3 h.

DISCUSSION

The first outcome of the present study was that CGP 35348 increased REM sleep. This increase in the duration of REM sleep could partly be ascribed to an increase in the number of REM periods in the first hour after injection and to tendencies in more and longer bouts of REM sleep in the other hours. The highest dose of CGP 35348 did not further enhance REM sleep compared to the middle dose: the increase in REM sleep duration was more moderate than what has been found for the middle dose. An inverted u-shaped curve was obtained if REM sleep duration was plotted against the dose of CGP 35348. The lack of clear REM sleep enhancing effects by the high dose was confirmed by the results on the REM sleep latency: this was reduced after 100 and 300 mg/kg but not after 900 mg/kg of CGP 35348. Upon the mechanisms underlying the inverted differences between the middle and the highest dose can only be speculated in terms of a nonspecific central or peripheral effect or modulation of another receptor which inhibits REM sleep. REM-enhancing effects are rare considering that almost all psychoactive drugs reduce REM sleep; until now only a few drugs have been known to promote REM sleep: cholinergic agonists such as carbachol [27], kainate and AMPA agonists [24], and a alpha-2 antagonist [1].

Non-REM sleep was enhanced by CGP 35348, and an S-shaped dose-response relationship was suggested by the data. Although a frequency spectrum of the EEG was not made, it was evident from Figure 2 that the amplitude of the delta waves during non-REM sleep was smaller after CGP 35348, especially after the highest dose. This impression is backed up by the work of Juhász et al., who found that GABA_B blockers shifted the frequency spectrum belonging to deep slow-wave sleep towards that of light slow-wave sleep, while the frequency spectra of REM sleep and wakefulness were not altered [13]. The sponta-

neous behaviour of the rats was altered by the drug at doses which reduced wakefulness and induced sleep.

It is well known that sleep and behavioural patterns in aged rats are markedly different from that of juvenile and adult rats, and that REM sleep, next to deep slow-wave sleep, is one of the parameters most affected by age [25,29]. REM sleep is decreased in old rats [25,29,30]. It cannot be excluded that the effects of CGP 35348 can be more easily found in old rats, who show a low amount of REM sleep. Perhaps, old rats are a better and more suitable model for the study of putative hypnotic drugs than young adult rats, which are most often used [33].

As previously reported, old Wistar rats show spike-wave discharges [34,35], and the morphology of the spike-wave discharges does not appear to be different from the ones we frequently study in WAG/Rij rats, a genetic model of human absence epilepsy [5,6,32]. The spike-wave suppressant effects of CGP 35348 are strong and long-lasting, and the effects are dose-dependent in the whole dose range and in all 3 h. The efficacy of GABA_B antagonists against spike-wave discharges is unchallenged, as is evident from recent studies in various models for generalised absence epilepsy [11,16,17,18,28]. It is uncertain whether and how the hypnotic and the spike-wave discharges' suppressant effects relate. It is possible that the hundreds of spike-wave discharges per day, covering a considerable time, prevent the occurrence of normal amounts of non-REM and REM sleep. If spike-wave discharges are inhibited by a drug, sleep might then be normalised and an increase in non-REM and REM sleep might be the consequence. This is not unlikely since Gandolfo et al. [10] described more abortive REM sleep periods and less REM sleep in spontaneous epileptic than in control rats. Therefore, it is interesting to compare the present results of CGP 35348 in old rats with data obtained in nonepileptic controls.

Considering the hypnotic properties of the benzodiazepines, which are generally seen as acting as GABA agonists, the evidence for a role for the neurotransmitter GABA in sleep is not unequivocal [19]. Myslobodsky and Mansour [22] found that gamma-acetylenic GABA and gamma-vanylic GABA, which both inhibit GABA transaminase, do not induce EEG-defined sleep. The GABA_A agonist muscimol produces an increase in waking and a decrease in sleep, especially in REM sleep [4]. However, the administration of bicuculline, a GABA_A antagonist, produced an even greater sleep suppression, which confounds a clear interpretation of the role of GABA_A in sleep-wake regulation [4]. In other studies, muscimol did not induce sleep and did not potentiate the effects of flurazepam [20,21]. It was even suggested that the hypnotic effects of the benzodiazepines are due to non-GABA-ergic mechanisms [21]. Besides the negative or controversial results with respect to the role of GABA and sleep, some positive effects have to be mentioned as well.

Sallanon et al. [26] injected muscimol in the hypothalamus in insomniac cats and found that the GABA agonist restored non-REM and REM sleep. Perfusion of GABA in the retrospontolateral thalamic nuclei produced both an increase in non-REM sleep as well in REM sleep [12], whereas an increase in GABA could be established during slow-wave sleep [14]. Friess et al. [8] described how administration of dehydro-epiandrosterone (DHEA) in humans increases rather selectively REM sleep. DHEA is regarded as a drug with mixed GABA_A agonistic-antagonistic properties. Lancel and co-workers [15] used higher doses of muscimol than used by Mendelson and Martin [20] and found an increase in both non-REM and REM sleep in rats. Little is known about the role of GABA_B receptors in sleep, and only Juhász et al. [13] described results on non-REM sleep. They found a shift in EEG spectra from deep delta to spindling and a reduction of deep non-REM sleep and an increase in light non-REM sleep in cats. In this experiment, the GABA_B antagonists saclofen and CGP 35348 were administered in the thalamus through dialysis probes.

In conclusion, this paper reports that the GABA_B antagonist CGP 35348 enhances non-REM sleep and REM sleep and reduces strongly spike-wave discharges. Both behavioural and EEG parameters contributed to the establishment of a hypnotic effect. The highest dose of CGP 35348 was not effective in promoting REM sleep. The REM-sleep-enhancing effects might have theoretical and clinical relevance.

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